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Active specific immunotherapy of Dukes B_2 and C colorectal carcinoma: Comparison of two doses of the vaccine*

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Summary. The ability of active specific immunotherapy to enhance immune responses to autologous tumor-associated antigens (TAA) and to prolong the disease-free interval was evaluated in patients with Dukes B_2 and C colorectal carcinoma who had undergone potentially curative resections. Patients were sensitized in the early postoperative period with irradiated autologous adenocarcinoma cells mixed with bacillus Calmette-Guérin (BCG) to yield either a low-dose vaccine (3×10^6 tumor cells) or a high-dose vaccine (1×10^7 tumor cells). Six of seven patients who received the low-dose vaccine developed delayed-type hypersensitivity (DTH) responses to autologous tumor cells upon completion of the vaccination, whereas all four patients receiving high-dose vaccine displayed a positive DTH response. However, DTH responses to autologous TAA waned within 3 months in all patients receiving the low-dose vaccine; DTH responses persisted for 3 months in three of the four high-dose vaccine patients. In vitro lymphoproliferative responses to TAA correlated with DTH responses to autologous tumor cells. Active specific immunotherapy appeared to induce specific immune responses either in vitro or in vivo to autologous TAA because it did not induce responses to autologous mucosa cells. There were no complications caused by BCG or tumor cells. This series demonstrates that active specific immunotherapy is a nontoxic treatment that augments immunity to autologous TAA.

Introduction

Adenocarcinoma of the colon and rectum is the second most prevalent cancer in the United States [14]. Surgery alone may cure 50% of the patients with this type of cancer. Unfortunately, chemotherapy has not been successful as an adjuvant therapy in those patients whose tumors have been resected successfully but who have a high risk for recurrence (i.e., the Dukes B_2 and C patients). Thus, new agents and different approaches to adjuvant treatment need to be developed. Hanna et al. [6, 7] have shown that active specific immunotherapy with irradiated autologous tumor cells mixed with bacillus Calmette-Guérin (BCG) may cure inbred strain 2 guinea pigs of pulmonary metastases established by the i.v. injection of syngeneic Line 10 hepatocarcinoma cells. Thus, active specific immunotherapy may produce responses to tumor-associated antigens (TAA) that prevent recurrence of disease. Recently, Hoover et al. [11] reported a prospective, randomized trial in which active specific immunotherapy with autologous colorectal carcinoma cells mixed with BCG prolonged the duration of the disease-free interval and increased survival in patients with Dukes B_2 and C colorectal carcinoma.

The purposes of this report were to confirm the feasibility of active specific immunotherpy as an adjuvant therapy in colorectal carcinoma and to determine the optimal number of autologous tumor cells in the vaccine. Administration of 1×10^6 irradiated tumor cells with 10^7 BCG organisms cured 60% of guinea pigs with lung metastases established by prior i.v. injection of 5×10^5 Line 10 hepatoma cells. However, immunization with 1×10^7 irradiated tumor cells mixed with the same number of BCG organisms cured all guinea pigs of established pulmonary metastases [7]. As a consequence, Hoover et al. [10] immunized patients with the higher dose of tumor cells and observed delayed-type hypersensitivity (DTH) responses to autologous TAA that persisted for as long as 6 months. Since humoral and cell-mediated immunity directed to autologous TAA are detectable in the majority of patients with colorectal carcinoma in the perioperative period (Jessup et al. submitted for publication), active specific immunotherapy may boost preexisting tumor immunity rather than induce a de novo response as suggested in the guinea pig model. Thus, we compared a low-dose vaccine with fewer tumor cells to a high-dose vaccine similar to that used by Hoover et al. [10] to determine if a lower number of tumor cells may boost a preexisting response to autologous TAA to the same extent that a high-dose vaccine does. This is important because the number of tumor cells isolated from the primary tumor may be limited and if a low-dose vaccine is as effective as a high-dose vaccine, more patients may be candidates for active specific immunotherapy. However, our results indicate that the vaccine with the higher number of autologous tumor cells is the more effective immunogen.

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Materials and methods

Patients. The 52 patients in this study were admitted to the surgical service of the University of Texas M. D. Anderson Hospital and Tumor Institute at Houston and underwent potentially curative colon or rectal resections. This study was not a randomized trial because its intent was to determine the clinical safety and immunological efficacy of a low-dose vaccine. Therefore, the 11 patients who entered into the immunotherapy arm of this study were operated upon by three surgeons (J. M. J., D. M. O., and R. G. M.) between February, 1981 and March, 1982 whereas the 41 control patients were operated upon by the other surgeons on our staff (C. M. M., F. C. A., and M. M. R.) during this time period. No patient who was offered immunotherapy refused it, but patients were offered immunotherapy only if they could return to the Outpatient Department of the UT M. D. Anderson Hospital for the 2 weeks required to complete the vaccination process. Patients granted informed consent for this study in accordance with institutional and federal guidelines.

Pathologic staging was performed according to Astler and Coller [1]. The clinical characteristics of the 11 immunotherapy patients are presented in Table 1. There were 29 control patients with Dukes B_2 lesions: (median age 66 years, range 23–83) 1 black, 1 Hispanic and 27 whites; 11 patients were female. Of the primary lesions 3 were on the right side of the colon, 3 on the left side of the colon, 8 in the sigmoid colon, and 15 in the rectum. There were 12 control patients with Dukes C lesions: (median age 66 years range 33–82 2 blacks, 10 whites; 9 patients were female. Of these primary lesions 1 was in the right colon, 2 in the left colon, 3 in the sigmoid colon, and 6 in the rectum. The immunotherapy group was younger (median age 55 years 34–75) than the control group but included a preponderance of patients with rectal primaries (8 of 11 primary lesions) and poor prognostic factors (Table 1). All patients were well nourished. Of the 41 control patients 11 consented to cutaneous DTH testing to autologous tumor and mucosa, and/or to donation of peripheral blood mononuclear cells (PBMC) for mixed lymphocyte-tumor interaction (MLTI) assessment, while the other 30 patients did not participate in the immunologic investigations. Patients were examined at 3 month intervals in the Outpatient Department of UT M. D. Anderson Hospital. This involved a physical examination and measurement of white blood cell count, hemoglobin concentration, serum alkaline phosphatase, serum glutamic oxaloacetic transaminase (SGOT), and carcinoembryonic antigen (CEA) by the Clinical Pathology Laboratory of the UT M. D. Anderson Hospital. Once a year barium enemas or proctoscopies were performed to check for recurrences or new primaries.

Table 1. Clinical characteristics of patients with Dukes B_2 and C colorectal carcinoma who received active specific immunotherapy

Patient	Sex	Race[a]	Age	Site[b]	No. + nodes	Average Diameter (cm)[c]	Dukes
1	F	H	53	RC		6	B_2
2	M	W	62	R		5	B_2
3	F	H	37	R		5	B_2
4	M	W	63	R		4	B_2
5	F	W	56	S		4	B_2
6	F	W	75	R		3	B_2
7	F	W	59	R	3	3	C_2
8	M	W	49	R	3	4	C_2
9	M	W	61	R	3	3	C_2
10	M	B	40	R	15	8	C_2
11	M	W	34	TC	9	7	C_2
			55 (34–75)		6.2 ±2.9	4.6 ±0.5	

[a] H denotes Hispanic patient whereas B signifies a Negro and W a white patient

[b] Site of primary lesion: RC = right colon; TC = transverse; S = sigmoid colon; R = rectum

[c] Average diameter is the mean of 2 largest perpendicular diameters in cm of primary cancer in the operative specimen as measured by the surgical pathologist

Preparation of tumor and mucosa cell suspensions. Specimens removed at surgery were immediately examined by a surgical pathologist. Then samples of tumor and uninvolved mucosa were harvested and transported at 4 °C within 10 min in minimal essential medium (MEM, Grand Island Biologic Co., Grand Island, NY) containing 300 units/ml penicillin and 300 μg/ml streptomycin. Mucosa was harvested approximately 10 cm from gross tumor. Tissue dissociation was performed according to the methods of Peters et al. [13]. The samples of tumor and mucosa were minced into 3 to 5-mm pieces, with the removal of necrotic debris and adipose tissue. The pieces were transferred to trypsinizing flasks and sufficient digestion medium (MEM with 0.14% collagenase type I and 0.03% DNase type I (Sigma Chemical Co., St. Louis, Mo) with 100 units penicillin or 100 μg streptomycin/ml, respectively) was added to cover the tissue. After incubation at 37 °C for 30 to 40 min and gentle agitation to facilitate the tissue dissociation, the supernatants were decanted and replaced with fresh digestion medium. Each supernatant was kept at 4 °C and then centrifuged at 200 × g for 10 min. Again, the supernatant was decanted and the cell pellet was resuspended in 5 ml 0.3%. DNase to remove clumps, and then it was diluted with MEM. The cells were collected, washed twice with MEM, and chilled to 4 °C. The yields of cells ranged from 8 to 23×10^6 cells/g tissue; yields from tumor tissue were slightly greater than those from mucosa. Equal volumes of freezing medium (MEM, pH 7.2 plus 15% dimethyl sulfoxide (Sigma Chemical Co., St. Louis, Mo) and 10% human serum albumin (Travenol Laboratories, Inc., Glendale, Calif.) were added by drops to the cell suspensions at 4 °C. Between 1 and 50×10^6 cells/ml were frozen in Nunc vials in a programmable freezer (Neslab Instruments, Portsmouth, NH) in which the temperature dropped at a rate of −1 °C/min until thy reached −80 °C; then they were stored in liquid nitrogen. Cell samples were cryopreserved in human serum albumin to ensure that xenoantigens were not introduced.

Cells were recovered according to the method of Holden et al. [9] The vial contents were rapidly thawed in a 37 °C water bath, and then the cell suspension was transferred to a 50-ml tube where MEM was added a drop every 15 s until the contents were diluted 15-fold. After centrifugation, the cells were resuspended in MEM, washed again, and counted. Viability for the tumor and mucosa cells was determined by trypan blue dye exclusion and ranged from 55% to 90% prior to cryopreservation and from 40% to 75% after recovery.

Preparation of PBMC. Blood was collected either on the morning of surgery prior to the administration of any medication or 7 to 28 days after surgery. Mononuclear cells were separated from heparinized blood (20 units preservative-free heparin/ml blood, Fisher Scientific Co., Springfield, N.J.) by density gradient centrifugation on Lymphocyte Separation Medium (Litton Bionetics, Kensington, Md) according to the method fo Boyum [3]. After two washes, the mononuclear cells were cryopreserved in the same manner as described above for tumor and mucosa cells.

Immunization protocol. Patients who received active specific immunotherapy were sensitized by a modification of the method of Hoover et al. [10]. Cryopreserved autologous tumor cells were recovered and washed with Hanks' balanced salt solution (HBSS). In the low-dose vaccine 3×10^6 viable autologous tumor cells were subjected to 20,000 rads from a ^{137}Cs source. After radiation, the cells were pelleted by centrifugation at $500 \times g$ for 10 min to which 10^7 colony forming units of BCG (Connaught Laboratories, LTD., Willowdale, ONT.) were added in 0.10 ml of HBSS. For the high-dose vaccine, 1×10^7 autologous tumor cells were added to BCG after recovery. The mixture of BCG and irradiated tumor cells was then inoculated in 0.1 ml i.d. into the thight with a similar injection in the opposite thight 7 days later. After another 7 days, 3×10^6 irradiated autologous tumor cells were injected i.d. into an arm. Simultaneously, 3×10^6 irradiated autologous mucosal cells were also injected i.d. Patients in the high-dose vaccine group received 1×10^7 irradiated tumor cells with BCG in the initial injections and 3×10^6 irradiated tumor cells for the booster injection. Injections for skin testing were performed in a coded fashion to minimize observer bias. Induration was measured 24 and 48 h after the booster injection. A positive response was noted by induration greater than or equal to 7 mm at 24 h that persisted for 48 h. The cell suspensions received 20,000 rads because tritiated thymidine (^{3}H-Tdr) incorporation studies with tumor cell suspensions revealed that DNA synthesis still occurred with radiation doses of 15,000 rads and was completely inhibited only at 20,000 rads. Although DNA synthesis at 5–15,000 rads may represent nonproductive terminal differentiation, ethics dictate that tumor cells inoculated into patients be incapable of subsequent proliferation.

All patients who received active specific immunotherapy displayed a positive response to at least one of the following cutaneous recall antigens provided by the UT M. D. Anderson Hospital Research Pharmacy: mumps, trichophyton, purified protein derivative (PPD), blastmycin, or streptokinase-streptodornase. All patients were PPD negative prior to immunotherapy. Isoniazid (300 mg, orally, once a day) was administered only to those patients who developed signs or symptoms of an anamnestic response to BCG or systemic BCG infection.

The MLTI assay. The MLTI assay was performed essentially as described by Vanky and Stjernsward [15]. Tumor, mucosa, and autologous PBMC were recovered from cryopreservation and incubated with 50 μg/ml mitomycin C (Sigma Chemical Co., St. Louis, MO.) in RPMI 1640 at 37 °C for 1 h. Untreated autologous PBMC (1×10^5 cells) were incubated in quadruplicate in RPMI 1640 with 10% heat-inactivated agamma human serum (KC Biological Co., Lenexa, Kan.) with an equal number of mitomycin C-treated cells for 5 days at 37 °C in 5% CO_2. DNA synthesis was estimated by harvesting the plates with a Titertek automatic sample harvester (Flow Laboratories, Inc., McLean, Va.) after a 16-h postlabel with 1 μCi/well of ^{3}H-Tdr (New England Nuclear, Boston, MA; sp. act. 2 MCi/mmol). The stimulation index (SI) was calculated as: $SI_T = ((PBMC + Tm) - (Tm))/(PBMC + PBMCm)$ where (Tm) and (PBMC + Tm) were the activity of mitomycin C-treated tumor cells alone and PBMC cultured with treated tumor cells, respectively, and (PBMC + PBMCm) was PBMC incubated with mitomycin C-treated PBMC. The mean (Tm) was subtracted from the mean (PBMC + Tm) to determine the counts attributable to lymphocyte proliferation in the presence of treated tumor cells, inasmuch as a significant amount of thymidine was incorporated into the Tm population. The SI for mucosa was calculated in a similar fashion, and the significance was determined by the two-tailed Student's *t*-test comparing mean (PBMC + Tm) − (Tm) to mean (PBMC + PBMCm) using the sum of the variances for (PBMC + Tm) and (Tm) to estimate the variance of lymphocyte proliferation to tumor antigen.

Results

Biochemical and clinical response to active specific immunotherapy

A total of 11 patients with Dukes B_2 and C colorectal carcinoma were vaccinated with lethally irradiated autologous tumor cells mixed with BCG. Only 3 patients had febrile responses (temperatures less than 38.5 °C for 48 h). None developed cellulitis or pain at any of the injection sites and no tumor has recurred in any of the injection sites. Ulcers of 3 to 5-mm diameter occurred in all sites injected with BCG, but they healed within 6 to 8 weeks. There has been only one complication associated with this protocol but it was not directly associated with administration of BCG or tumor cells. The patient concerned developed what initially appeared to be an anamnestic re-

Table 2. Hematologic and biochemical responses to active specific immunotherapy

Patient group	Alkaline phosphatase[b]	SGOT[c]	WBC[d] (× 1000/ml)	HB[d] (g/dl)
Immunotherapy[a]				
Preop	89 ± 7	18 ± 3	8.4 ±0.9	12.5 ±0.9
Postop	111 ±17	22 ± 4	9.4 ±1.3	11.6 ±0.4
Postvac	149 ±48	19 ± 3	7.1 ±0.3	11.9 ±0.5
3 Months	136 ±31	28 ± 8	6.2 ±0.5	12.6 ±0.4
Controls				
Preop	109 ±29	30 ± 8	7.8 ±0.4	13.4 ±0.6
Postop	148 ±58	46 ±19	10.4 ±1.3	11.8 ±0.5
3 Months	116 ±17	75 ±21	6.4 ±1.1	13.3 ±1.3

[a] Serum and heparinized blood were obtained from patients either the morning of surgery (preop), 1 week after surgery (postop), after completion of immunotherapy 3–4 weeks after surgery (postvac), or at the time of first clinic visit (3 months)

[b] Mean ±SEM of serum alkaline phosphatase in mU/ml where normal range is 30–85 mU/ml

[c] Mean ±SEM of SGOT in mU/ml where normal range is 7–40 mU/ml

[d] Mean ±SEM of white blood cell count (WBC) or hemoglobin (HB) concentration

sponse to BCG, even though the patient had not reacted to an i.d. injection of PPD prior to immunization. Because this patient developed more erythema within 4 days of the initial i.d. injection than the other patients, 300 mg of isoniazid orally once a day was administered. After 4 weeks the patient developed a daily fever of 40 °C 2 h after ingestion of the isoniazid. Cessation of isoniazid immediately stopped the fevers. This patient did not have any clinical evidence of disseminated BCG disease. None of the other patients received isoniazid.

Liver chemistry values and hematologic indices were monitored in all patients. After operation the control patients demonstrated an elevation in alkaline phosphatase without an increase in SGOT that persisted at the first clinic visit 3 months after surgery (Table 2). Patients who received immunotherapy had a similar elevation in alkaline phosphatase without elevation of SGOT. Completion of vaccination did not cause a significant further increase in the alkaline phosphatase. There were no significant effects upon the hematologic indices, bilirubin, or CEA (data not shown). Thus, the administration of BCG and irradiated autologous tumor cells to patients resulted in a low morbidity and complication rate.

DTH responses to active specific immunotherapy

Two doses of tumor cells were evaluated in the vaccine. Seven patients received 3×10^6 irradiated tumor cells in the vaccine (low-dose vaccine), whereas 4 received 1×10^7 tumor cells (high-dose vaccine). Six of seven patients who received the low-dose vaccine developed a positive DTH response to autologous tumor cells at the conclusion of the immunization, whereas all the high-dose vaccine patients displayed an initial response (Table 3). The cutaneous DTH response to irradiated autologous tumor cells injected after surgery of both vaccine groups was significantly better than that of the controls (Table 3). In addition, although patients were not routinely tested for DTH to TAA prior to vaccination because of a concern that such treatment might alter the response to immunotherapy, two patients were challenged with irradiated autologous tumor cells prior to sensitization. These patients developed only 3 and 4-mm indurations before immunotherapy and 11 and 13-mm indurations, respectively, afterwards. A primary bronchogenic carcinoma appeared 9 months after vaccination in the one patient who was hyporesponsive after active specific immunotherapy; this suggests that the response to the TAA of her colon carcinoma may have been inhibited by the undetected lung cancer. Thus, 10 of 11 patients in the two immunotherapy groups displayed a positive initial cutaneous DTH response to autologous tumor cells, whereas only 2 of 10 control patients developed a positive response to autologous TAA ($\chi^2 = 10.5$, $p < 0.005$, Table 3).

Although both vaccines induced similar early DTH responses to TAA, the high-dose vaccine induced a longer-lasting response. When patients were skin tested 3 months after vaccination, all the patients who had received the low-dose vaccine showed no response to the cutaneous challenge, whereas 3 patients who received the high-dose vaccine maintained positive cutaneous reactions for 3 months and 2 patients maintained their DTH response for as long as 6 months (Table 3).

Correlation of cutaneous responses to autologous TAA with in vitro responses

Because cutaneous DTH responses to autologous TAA were augmented by active specific immunotherapy, we investigated whether in vitro assays of cell-mediated immunity parallel the skin test response. MLTI assays were performed after operation in the controls and after vaccination in the immunotherapy group. The proliferative response to TAA positively correlated with the DTH response to autologous tumor cells (Fig. 1).

No clear effect of active specific immunotherapy was discernible in the seven patients who were studied in the MLTI before and after immunotherapy. The SI of two patients increased, decreased in one, and one that was negative before vaccination remained negative (Table 4). Interestingly, two patients had positive proliferative responses prior to immunotherapy that persisted after vaccination. Thus, four of the seven patients had positive MLTI responses prior to vaccination which suggests these patients were presensitized to their tumors. While the proliferative response correlated with the DTH response to TAA when the control and immunotherapy groups were combined, the effect of immunotherapy upon the in vitro response to autologous TAA in the individual patient was not clear.

Specificity of the response to active specific immunotherapy.

Assessment of the specificity of the response is difficult. Utilization of allogeneic tumor cells is not optimal because

Table 3. Delayed-type hypersensitivity responses in patients receiving active specific immunotherapy

	Prevaccine[a]		Postvaccine		3 months	6–9 months
	T	M	T	M	T	T
Immunotherapy						
Low-dose ($n = 7$)	2.0 ±2.0		9.6 ±1.9[b] (6)	2.2 ±1.0	3.8 ±1.2	
High-dose ($n = 4$)			8.5 ±1.4[b] (4)	2.8 ±2.2 (1)	7.3 ±1.1[c,d] (3)	7.3 ±4.1 (2)
Controls ($n = 10$)	2.5 ±1.2	3.3 ±1.6				

[a] 3×10^6 irradiated autologous tumour (T) or mucosa (M) cells were injected i.d. and induration measured at 24 h. Mean ±SEM of average of 2 largest perpendicular diameters is presented. Values in parentheses are number of patients with induration ⩾7 mm at 24 h that persisted for 48 h

[b] $P < 0.01$ vs DTH response of control to tumor cells

[c] $P < 0.05$ vs DTH response of control to tumor cells

[d] $P < 0.05$ vs DTH response of low-dose immunotherapy group to tumor cells

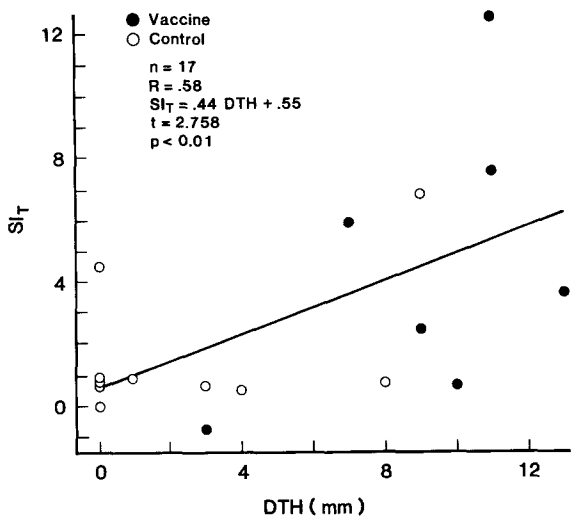

Fig. 1. MLTI assays were performed on PBMC harvested from patients 24 h after they had been injected in the arm with 3×10^6 irradiated autologous tumor cells. There is a positive correlation between the proliferative response to mitomycin C-treated autologous tumor cells (SI_T) and the skin test response

Table 4. Lymphocyte proliferative responses to autologous tumor and mucosa cells

	SI_T[a]		SI_M	
Immunotherapy	Pre-vaccine	Post-vaccine	Pre-vaccine	Post-vaccine
1	1.30	2.40	−1.30	0.30
2	0.10	3.60	0.01	0.70
3	11.66	5.90	4.50	−4.40
4	4.10	0.66	0.93	0.81
5	6.58	12.50	−2.35	0.80
7	0.01	−0.60	0.06	0.50
9	6.70	7.54	0.99	2.13

[a] 1×10^5 Mitomycin C-treated tumor cells were incubated with 1×10^5 PBMC collected from patients before (prevaccine) or after (postvaccine) immunotherapy for 5 days at 37°C in 5% CO_2. Cultures were postlabeled with ^{3}H-Tdr. Results are expressed as a Stimulation Index (SI_T or SI_M), where

$$SI_T = \frac{(Tm + PBMC) - TM}{PBMC_m + PBMC}$$

and (Tm + PBMC) is mean cpm of PBMC incubated with Tm, Tm is mean cpm of mitomycin C-treated tumor cells, and ($PBMC_m$ + PBMC) is mean cpm of PBMC incubated with mitomycin C-treated autologous PBMC. SI_M was calculated in a similar fashion. A SI ≥ 2.00 is a positive response

the response may be directed to histocompatibility antigens rather than to TAA. However, autologous mucosa cells serve as reasonable specificity controls because they contain the same microbial products as the tumor cell suspensions [10] and the same major histocompatibility antigens but may lack TAA. Active specific immunotherapy appears to induce specific immune responses, inasmuch as only one patient developed a DTH response to autologous mucosa cells after vaccination (Table 3). In fact, the response of the immunotherapy patients to autologous mucosa was similar to that of the control patients (Table 3).

The results from the MLTI assay corroborated the specificity of the response to the vaccine determined by the cutaneous DTH testing. Only one of seven immunotherapy patients had a significant proliferative response to autologous mucosa cells. In addition, two patients in the immunotherapy group displayed a positive response to autologous TAA before sensitization (Table 4). Thus, results with the MLTI assay confirmed the specificity of the response to the vaccine and suggested that immunotherapy may both induce de novo responses to autologous TAA and boost preexisting responses.

Clinical course of patients treated with active specific immunotherapy

The patients with B_2 colorectal carcinomas who received active specific immunotherapy were free of disease between 25 and 43 months later (Fig. 2A). In contrast, 7 of 29 control patients with Dukes B_2 lesions had a recurrence within 39 months of operation. In the patients with Dukes C colorectal carcinoma, 3 of 5 vaccinated patients remain free of disease more than 30 months later whereas 6 of 12 patients had a recurrence (Fig. 2B). When the groups were combined, the recurrence rate was 18% for the immunotherapy group in contrast to 32% for the control group. However, there was no significant difference in the disease-free interval between the control and the immunotherapy groups (Fig. 2C). Thus, active specific immunotherapy may decrease the recurrence rate in patients with colorectal carcinoma, but a larger randomized trial is necessary to confirm the clinical efficacy of this type of immunotherapy.

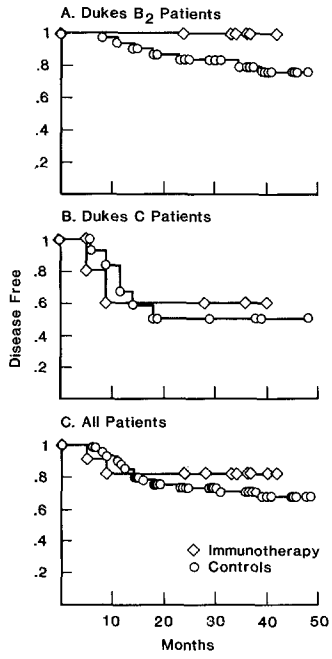

Fig. 2. Disease-free interval for patients with Dukes B_2 and C colorectal cancer

Discussion

Active specific immunotherapy using irradiated autologous tumor cells mixed with BCG provides a safe means of vaccinating patients. In this study, no complications were the direct result of the injection of either BCG or autologous tumor cells. Isoniazid-induced hepatotoxicity was observed in one patient when it was used as prophylaxis against a possible anamnestic response to BCG. Although Hanna et al. [8] have demonstrated that isoniazid does not inhibit induction of systemic tumor immunity in the guinea pig, isoniazid may be immunosuppressive [2]. Thus, isoniazid should be reserved for patients with documented, systemic BCG infection. There were no significant changes in liver function as measured by alkaline phosphatase and SGOT. Also active specific immunotherapy did not cause either a systemic inflammatory response or tumor enhancement.

Low-dose vaccine induced positive DTH responses to autologous TAA that waned within 3 months. Immunization with the high-dose vaccine also induced positive DTH responses to autologous TAA, but these responses persisted for as long as 6 months after immunization. These results are similar to those obtained in the guinea pig hepatoma model [7] in which a low-dose vaccine of BCG and 10^6 tumor cells induced a weak protection against lung metastases and a high-dose vaccine induced complete protection. Thus, the high-dose vaccine consisting of two doses of 10^7 viable BCG organisms mixed with 10^7 irradiated tumor cells followed by an injection of irradiated tumor cells alone is the preferred immunogen.

Active specific immunotherapy has mixed effects upon in vitro cell-mediated responses. We have previously observed (Jessup et al. submitted for publication) that a positive MLTI response to autologous TAA in the perioperative period correlates with a good prognosis in patients with Dukes B_2 and C colorectal carcinomas. The proliferative cell is a T4+T cell and, thus, has the phenotype of the helper T cell and the DTH effector cell. When lymphoproliferative responses to autologous tumor cells were compared with DTH responses in both control and immunized patients, there was a good correlation (Fig. 1). However, when lymphproliferative responses to autologous TAA were measured in seven immunotherapy patients, no consistent effect of vaccination was noted upon the lymphoproliferative response of individual patients. Thus, the in vitro MLTI assay does not offer any advantages over the DTH response to autologous tumor cells as a means of monitoring the response to active specific immunotherapy.

Active specific immunotherapy appears to induce tumor-specific immunity. We (unpublished observations) have confirmed the finding of Hoover et al. [10] that tumor and mucosal cell suspensions have similar degrees of microbial contamination. Further, autologous mucosa cells will share organ-specific antigens and major histocompatibility complex determinants with autologous tumor cells. Yet, patients who received active specific immunotherapy developed significantly greater DTH responses to autologous tumor cell suspensions than to mucosal preparations. These results were confirmed in the MLTI assay since only one patient had a positive response in the MLTI to autologous mitomycin-C-treated mucosal cells after vaccination. Our results substantiate this observation and suggest that TAA are indeed present on tumor cells. Thus, the vaccination process does not seem to induce a proliferative response in vitro or a DTH response in vivo to antigens present in the mucosal cell preparations. This is somewhat surprising because the preparations in both the present trial and the study by Hoover et al. [10] utilized mucosa cells from the operative specimen that contained the "transitional mucosa" described by Filipe and Branfoote [4] and Lev and Grover [12]. This mucosa is in a premalginant state and is likely to display the same antigens on mucosal cell membranes as are associated with tumor cell membranes.

The present trial of active specific immunotherapy was not intended to assess the clinical efficacy of the vaccine. Nonetheless, the results are encouraging. None of the Dukes B_2 patients have developed recurrences even though the majority of these patients had rectal primaries. The patients with Dukes C lesions who received active specific immunotherapy had a disease-free interval similar to that of our control patients. The Gastrointestinal Tumor Study Group [5] recently reported that the survival for Dukes B_2 lesions above the peritoneal reflection is 80% at 5 years while the survival of patients with Dukes C lesions is approximately 63%. Since patients died an average of 6 months after recurrence (data not shown), the survival of our control groups is similar to that of the Gastrointestinal Tumor Study Group. While active specific immunotherapy did not significantly decrease recurrence rate or disease-free interval, it did not enhance tumor growth. Thus, a larger, prospectively randomized trial is essential to establish the efficacy of this type of adjuvant therapy. Such a trial is now being performed by the Eastern Cooperative Oncology Group.

In conclusion, active specific immunotherapy is a relatively nontoxic method of vaccination that does not lead to tumor enhancement, but that does induce immunity to autologous TAA in patients with Dukes B_2 and C lesions. Monitoring the response to immunotherapy should be performed by cutaneous DTH testing with irradiated autologous tumor cells. While low-dose vaccine induced a transient state of immunity, a high-dose vaccine should be utilized consisting of 10^7 irradiated autologous tumor cells because it induced a more persistent state of immunity. Further prospective randomized trials are necessary to establish the efficacy of active specific immunotherapy.

References

1. Astler VB, Coller FA (1954) The prognostic significance of direct extension of carcinoma of the colon and rectum. Ann Surg 139: 846–852
2. Bhide SV, Maru GB, Sawai MM, Ranadine KJ (1978) Isoniazid tumorigenicity in mice under different experimental conditions. Int J Cancer 21: 318–326
3. Boyum A (1968) Isolation of mononuclear cells and granulocytes from human blood. Scand J Clin Lab Invest 21: 77–86
4. Filipe MI, Branfoot AC (1974) Abnormal patterns of mucus secretion in apparently normal mucosa of large intestine with carcinoma. Cancer 34: 282–290
5. Gastrointestinal Tumor Study Group (1984) Adjuvant therapy of colon cancer-results of a prospectively randomized trial. New Engl J Med 320: 737–743
6. Hanna Jr MG, Peters LC (1978) Specific immunotherapy of established visceral micrometastases by BCG-tumor cell vaccine alone or as an adjuvant to surgery. Cancer 42: 2613–2625

7. Hanna Jr MG, Brandhorst JC, Peters LC (1979) Active specific immunotherapy of residual micrometastases. An evaluation of sources, doses, and ratios of BCG with tumor cells. Cancer Immunol Immunother 7: 165–173
8. Hanna Jr MG, Pollack VA, Peters LC, Hoover HC (1982) Active specific immunotherapy of established micrometastases with BCG plus tumor cell vaccines. Cancer 49: 659–664
9. Holden HT, Oldham RK, Ortaldo, JR, Herberman RB (1976) Cryopreservation of the functional reactivity of normal and immune leukocytes and of tumor cells. In: Bloom BR, David JR (eds) In vitro methods in cell-mediated and tumor immunity. Academic Press, New York pp 723–729
10. Hoover Jr HC, Surdyke M, Dangel R, Peters LC, Hanna Jr, MG (1984) Delayed cutaneous hypersensitivity to autologous tumor cells in colorectal cancer patients immunized with an autologous tumor cell: Bacillus Calmette-Guerin vaccine. Cancer Res 44: 1671–1676
11. Hoover Jr HC, Surdyke MG, Dengel RB, Peters LC, Hanna Jr MG (1985) Prospectively randomized trial of adjuvant active specific immunotherapy for human colorectal cancer. Cancer 55: 1236–1243
12. Lev R, Grover R (1981) Precursors of human colon carcinoma: a serial section study of colectomy specimens. Cancer 47: 2007–2015
13. Peters LC, Brandhorst JC, Hanna Jr MG (1979) Preparation of immunotherapeutic autologous tumor cell vaccines from solid tumors. Cancer Res 39: 1353–1360
14. Silverberg E (1983) Cancer statistics. CA 33: 9–25
15. Vanky F, Stjernsward J (1976) Lymphocyte stimulation test for detection of tumor-specific reactivity in humans. In: Bloom BR, David JR (eds) In vitro methods in cell-mediated and tumor immunity Academic Press, New York pp 597–606

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