In Vitro Responses of Lymphocytes from Cancer-Bearing Patients to Autochthonous Tumor Tissues[1] (35284)

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(Introduced by E. E. Baker)

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There is an increasing clinical interest in the immune response of tumor-bearing patients towards their autochthonous tumor-specific antigens (1–3). Based upon animal studies, there is a growing conviction that the immune response to tumors is a major part of the host defence against the growth of neoplastic cells. However, the term "immune response" involves two types of immune reactivity—humoral and cellular—and current evidence suggests that both responses do not function similarly in tumor immunity. In general, experimental induction of tumor-specific antibodies has been associated with enhanced tumor growth (4); cellular immunity has more often been associated with tumor rejection (3, 4). Although there are a number of studies which demonstrate that man develops humoral immunity toward autochthonous tumors (5), there are few studies on human cellular immune response toward tumor in such patients. Studies estimating cellular immune responsiveness have been limited by the technical difficulties in measuring a response against tumor antigens. In animal tumor systems, cellular hypersensitivity experiments have been carried out using syngeneic transplantation (5), skin sensitivity (5), or lymphocyte-target cells assays in tissue culture (6, 7). In man, syngeneic experiments are unlikely; skin testing has had limited investigation (13) and has suffered from the question of non-specific inflammation and the need to use soluble preparations of tumor. Tissue culture experiments have generally been too demanding for extensive investigation, although recently a few investigators have begun to utilize these methods (8, 9, 14–16). A number of studies have recently demonstrated that peripheral blood lymphocyte transformation and proliferation in tissue culture, induced by antigens, is primarily a manifestation of cellular immunity (10, 11). We have, therefore, attempted to use this experimental system to demonstrate that a delayed hypersensitivity reaction occurs in man against tumor-specific antigens. We have used autochthonous tumor tissue homogenates to induce proliferation in tissue cultures of the patients own blood lymphocytes. Unfortunately, in this study, we could not demonstrate any statistical evidence of lymphocyte response, although 1 patient in 10 appeared to produce some evidence of blast transformation on exposure to tumor homogenates.

Materials and Methods. Ten patients undergoing surgery for tumor excision or laparotomy were the subjects. These patients, and the character of their tumors are listed in Table I. Tumor tissue, approximately 1 cm^3, and a similar-sized piece of normal tissue from an adjacent area were obtained during surgery and were quickly frozen to —70° (usually within 10 min). These frozen specimens were stored for periods between 2 days and 3 weeks before use in the lymphocyte cultures. On the day of the lymphocyte culture, the tissues were thawed, weighed, homogenized with a Potter-Elvehjem glass tissue grinder, and suspended in incubation media at a concentration of 3 mg/ml. Lymphocyte cultures were established as previously described (11) with medium 199 containing 10% fetal calf serum. One-ml cultures containing 1×10^6 lymphocytes were generally employed. Two to four replicate cultures per experimental variable were es-

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TABLE I. Index of Response of Lymphocytes to Phytohemagglutinin, and to Normal and Tumor Tissue Antigens.

Initials	Age	Sex	Pathological diagnosis	Storage period for tumor tissue (days)	Nos.[a]	Response of DNA synthesis 2/1	3/1	4/1	Response of blast transformation 2/1	3/1	4/1
E.T.	54	F	Malignant carcinoid of stomach	4		—	—	—	6.43	1.41	1.31
C.W.	94	F	Adenocarcinoma of breast	2		2.07	0.98	0.86	17.70	1.10	0.86
M.W.	78	F	Adenocarcinoma of sigmoid	6		1.36	1.00	0.86	10.00	0.86	0.89
				20		14.91	0.81	0.60	12.48	1.92	0.98
D.D.	83	F	Basal cell carcinoma	2		1.36	1.07	0.67	18.83	1.50	1.77
L.G.	83	F	Adenocarcinoma of rectum	3		1.63	3.01	1.33	42.27	2.46	1.45
J.H.	72	M	Adenocarcinoma of prostate	3		6.75	—	1.16	12.11	—	1.06
J.C.	81	M	Renal cell carcinoma[b]	3		—	1.05	0.67	15.69	0.42	0.11
A.C.	82	F	Adenocarcinoma of pancreas[c]	6		3.03	—	1.45	34.61	—	3.61
A.D.	68	F	Duct cell carcinoma of breast	4		55.48	0.62	0.55	46.31	0.95	0.63
H.D.	84	M	Adenocarcinoma of rectum	19		11.34	0.83	0.81	17.59	0.65	1.33
	Mean					10.78	1.17	0.90	23.75	1.25	1.27
	SD						0.76	0.31		0.64	0.89

[a] 1, unstimulated lymphocytes; 2, PHA-stimulated lymphocytes; 3, normal tissue-stimulated lymphocytes; 4, tumor tissue-stimulated lymphocytes.

[b] Evidence of diffuse metasis.

[c] Tumor removed and patient studied 1 week after X-ray therapy to the tumor.

tablished and the cultures were maintained in water-saturated 5% CO_2–95% air at 36° for 7 days. Lymphocyte proliferation and blast transformation were determined after 7 days. Cytologic examination and classification were carried out using routine smears stained with Wright's stain (12). At least 100 lymphoid cells were counted on each slide and the results were recorded as percentage blastoid forms. DNA synthesis in these cultures was estimated by determining the quantity of ^{3}H-thymidine (^{3}HT) incorporated into chemically extracted DNA after exposure to 1 μCi of ^{3}HT/ ml of culture (NEN) (sp act 4 Ci/mmole). DNA was isolated prior to counting by precipitation with 0.3 *M* perchloric acid, washing, and final extraction into the acid by heating at 100° for 10 min. The acid-extracted DNA was added to dioxane–naphthaline–PPO fluor mixture and counted in the Beckman scintillation counter, dpm 200. The mean of the replicates for each of the experiments was calculated and the responses were expressed as a stimulation ratio ("index of response") by dividing the mean for each group by the mean of the untreated controls. Experimental variables included lymphocyte cultures without any additional additives, cultures with phytohemagglutinin M (PHA) (Difco); cultures containing homogenized normal tissue; and cultures containing homogenized tumor tissue.

Results. Our results are summarized in Table I. In general, our donors were elderly patients, all of whom carried malignant carcinoma. However, only patient AC had any gross evidence of residual tumor at the time the lymphocyte reactivity was studied. Six of

the 10 patients were tested for the presence of intact delayed hypersensitivity by skin testing with a battery of common antigens (Candida, mumps, and Trichophyton). Four patient responded to at least one of these. Patients LG and AC did not. Except for patient AC, none of the patients studied had severe constitutional symptoms and all recovered from their surgery without ill effects.

Lymphocytes obtained from all the patients responded well to phytohemagglutinin stimulation with large numbers of cells undergoing blast transformation. Relatively small quantities of tritiated thymidine were incorporated into the DNA of PHA-stimulated cells because the tritium pulse was added after 6 days of culture, when the maximum rate of DNA synthesis had passed (12).

As a group, the lymphocyte responses of these patients to both autochthonous tumor and normal tissue were not significantly different. Using the simple t test, a comparison of lymphocyte response to tumor or normal tissue as measured by DNA synthesis gave a $t = 0.96$ and the probability that these responses were different was $p => 0.4$; when measured by "blast" transformation, the $t = 0.06$, $p => 0.9$. Of the 10 patients studied, only patient AC gave a response of blast transformation which was significantly different than that observed in the normal tissue control. One of the patients, LG, appeared to demonstrate a response to normal tissue.

Discussion. In the 10 patients studied, we were unable to demonstrate a general lymphocyte response to tumor antigens, even though these cells could respond to PHA. Only the cells of one patient appeared to respond to substances present in homogenized tumor tissue. This effect is in marked contrast to the reports of Hellstrom *et al.* (8, 9), who have demonstrated responses of blood lymphocytes to tumor cells when assayed by a colony inhibition method. Our data is more consistent with the report of Savel (15) who found that approximately 12% of his patients had blood lymphocytes which responded to saline extracts of their autologous tumors. The small responses in the lymphocyte stimulation system may be the result of poor cellular hypersensitivity to autochthonous tumors. However, there are many possible experimental artifacts which may produce negative results despite functioning cellular immune reactions against the tumor. Tumor-specific antigen may have been lost during freezing and thawing, or homogenization of the samples. Reactive lymphocytes may have been removed from the circulation by metastatic antigen-bearing cells remaining after surgery, or these cells may have been removed with the tumor at the time of surgery. It is possible that 0.3 mg of tumor tissue did not contain enough antigen for stimulation of 1 million lymphocytes, or, alternatively, did contain too large a quantity of antigens which might have suppressed the lymphocyte response (11). However, the recent demonstration that lymphocytes from patients with melanoma (16) or leukemia (14) are more likely to respond to tumor cells, or extracts of these tumors, tends to support the idea that the tumor which we studied did not contain sufficient quantities of foreign antigen.

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