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Migration of Peripheral Leukocytes in the Presence of Carcinoembryonic Antigen. Studies in Patients with Chronic Inflammatory Diseases of the Intestine and Carcinoma of the Colon and Pancreas (32568)

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(Introduced by Victor Herbert)

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Carcinoembryonic antigen (CEA) was originally described by Gold and his group as a tumor-specific cell surface antigen, found only in the sera of patients with malignant tumors arising from the endodermally derived epithelium of the digestive system (1, 2). Subsequently, reports of circulating CEA in patients with bronchogenic carcinoma, breast carcinoma, prostatic carcinoma, alcoholic liver disease, alcoholic pancreatitis, chronic inflammatory disease of the intestines, and uremia have questioned this specificity (3–5).

Cell-mediated hypersensitivity reactions, both *in vivo* and *in vitro*, to antigens prepared from fetal tissue or tumor cells have been reported from a number of laboratories (6–9) in patients with Crohn's disease, ulcerative colitis, or carcinoma of the gastrointestinal tract. These studies have been performed with supernatants or with cell extracts containing surface membranes or other particulate material. Because of the type of preparations used, the nature of the antigen responsible for the reactions is not clear. An understanding of the cell-mediated immune response to CEA is of importance both from a diagnostic and, possibly, therapeutic point of view.

The present study employs an *in vitro* correlate of cell-mediated immunity to investigate the response to purified CEA in patients with conditions associated with circulating CEA.

Materials and Methods. Selection of subjects. Four patients with adenocarcinoma of the colon, four patients with carcinoma of the pancreas, four patients with active ulcerative colitis, ten patients with Crohn's disease, and five normal individuals were studied. None of the patients in the carcinoma group were receiving cytotoxic drugs; none were considered to be terminally ill. No patient had significant hepatic or renal disease, and there was no evidence of malignancy in the group with inflammatory disease. None of the patients were receiving corticosteroids or immunosuppressive medications.

Preparation of the antigens. CEA was purified from the large hepatic metastasis of an adenocarcinoma of the colon according to recently described lithium diiodosalicylate (LIS) techniques (10, 11). Antigen was prepared from normal adult colonic mucosa according to the method of Wolberg (6). Specificity of the CEA was ascertained by precipitation in agar gel double diffusion with reference CEA and mono-specific anti-CEA antibody.[1]

Leukocyte migration test. The leukocyte migration test was performed according to the method of Soborg and Bendixen (12). The diagnoses were not known to the persons performing the tests. Inhibition of migration of 20% or more was considered significant.

Protein concentration of the antigens used. Toxicity studies were carried out over a wide range of protein concentrations. These studies demonstrated that the antigens were nontoxic to human peripheral leukocytes as assayed by direct migration in protein concentrations up to 500 μg/ml. The antigens were used in protein concentrations of 50 and 250 μg/ml in RPMI 1640 cell culture medium containing 10% heat-inactivated

[1] We are indebted to Dr. H. Kupchik and Dr. N. Zamcheck for providing us with reference materials.

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TABLE I. LEUKOCYTE MIGRATION TESTS AND ASSAY FOR CIRCULATING CEA.

Patient	Diagnosis	% Migration inhibition[a] CEA	% Migration inhibition[a] Normal Colon	Circulating CEA (ng/ml)
RL	ileocolitis	15	10	3.3
HK	ileocolitis	0	0	4.0
JH	ileocolitis	5	11	2.4
KS	ileitis	0	0	6.0
SK	ileitis	3	12	0
NO	ileitis	17	0	0.6
EG	ileitis	0	1	0.8
SR	ileocolitis	0	0	4.1
JR	ileocolitis	17	0	1.8
BL	ileitis	9	3	N.D.
EM	ulcerative colitis	0	12	4.5
GQ	ulcerative colitis	1	7	1.2
FF	ulcerative colitis	0	0	0.9
RZ	ulcerative colitis	0	0	1.7
JB	carcinoma of colon	0	6	16.0
EC	carcinoma of colon (post op)	5	20	1.4
PS	carcinoma of colon	0	15	>20
MK	carcinoma of colon (post op)	5	0	2.0
BB	pancreatic carcinoma	0	0	3.3
WB	pancreatic carcinoma	31	16	>20
JM	pancreatic carcinoma	9	19	12.6
AM	normal-healthy control	16	20	2.0
SS	normal-healthy control	0	12	0.5
AL	normal-healthy control	3	0	1.7
ES	normal-healthy control	0	0	0.1
EK	normal-healthy control	0	0	0.9
ES	normal-healthy	0	0	N.D.

[a] CEA and Normal Colon antigens used in protein concentrations of 250 μg/ml.

fetal calf serum, fresh penicillin, streptomycin, and L-glutamine.

Circulating CEA. Radioimmunoassays for circulating CEA were carried out according to the method of Lo Gerfo *et al.* (4). Levels greater than 2.5 ng/ml were considered positive.

Results. The results of the leukocyte migration tests using each antigen in protein concentration of 50 and 250 μg/ml were similar. Significant inhibition of leukocyte migration by CEA was not found in patients with Crohn's disease, ulcerative colitis, or malignancies of the gastrointestinal tract, with the exception of one case (WB), a patient with pancreatic carcinoma (Table I). This patient had a circulating CEA level of over 20 ng/ml.

A total of ten patients had circulating CEA levels above 2.5 ng/ml at the time their leukocytes were tested. Two patients in the carcinoma group (EC, MK) were postresection for carcinoma of the colon and had circulating CEA levels below 2.5 ng/ml at the time of this study.

Discussion. Cell-mediated immunity has been implicated in the pathophysiology of a wide variety of neoplastic and inflammatory disease processes. In ulcerative colitis and Crohn's disease, cell-mediated immune responses have been studied both *in vivo* and *in vitro* using a variety of test systems, yet no clear-cut picture has emerged to indicate the role of cell-mediated immunity in these disorders (13, 14). Weeke and Bendixen have reported data suggesting organ-specific cellular reactivity in patients with ulcerative colitis which is absent in those with Crohn's disease (8). They employed the leukocyte migration technique and used a crude antigen preparation derived from pooled extracts of fetal colonic and jejunoileal mucosa. Straus *et al.*, employing the same technique, but using a crude antigen preparation derived from meconium, found a higher incidence of migration inhibition in patients with Crohn's disease than in those with ulcerative colitis (9). The meconium preparation was shown to be rich in CEA, and it may be reasonable to suppose that CEA was present ia Bendixen's antigen preparation. Our failure to demonstrate cell-mediated immunity directed toward CEA with this *in vitro* system suggests that the phenomena described by Bendixen and Straus cannot be attributed to CEA. Further, it would appear that the histologic alterations

of the gut seen in ulcerative colitis and Crohn's disease are not the result of delayed-type hypersensitivity directed toward CEA.

Observations indicating a role for cell-mediated tumor rejection immune processes in human cancer are abundant (15, 16). Evidences have been presented that patients with carcinoma of the colon also possess cell-mediated immunity against autochthonous and allogeneic colonic carcinoma (17, 18). Lymphocytes from patients with colonic carcinoma are cytotoxic for fetal gut epithelium but not adult colonic mucosa, suggesting that lymphocytes were reacting against fetal antigens (19). The identification of a tumor-specific or tumor-associated immunogen responsible for the rejection process is of prime importance. In a previous study Lejtenyi *et al.* (20) found no evidence for cell-mediated immune reaction to CEA in patients with colonic carcinoma using the lymphocyte stimulation technique. The present study, employing a different antigen purification technique and another test system, also failed to demonstrate consistent *in vitro* cell-mediated immunity to CEA. These studies suggest that, while CEA may be a useful tool for diagnosis, it does not seem to be an antigen toward which cell-mediated host-response phenomena are directed.

Experimental evidence indicates that chemically induced rat sarcomas and hepatomas express at least two classes of tumor antigens. The first is unique for each tumor, while the second is a fetal antigen common to tumors of the same type. Because of the specificity of the rejection antigens in these tumor systems, it has been possible to establish that the embryonic antigens are not responsible for host-mediated rejection processes (21). A similar role may be played by CEA in human disease.

Summary. The leukocyte migration technique was employed to study *in vitro* cell-mediated immune responses to purified CEA in patients with Crohn's disease and active ulcerative colitis and in those with colonic and pancreatic carcinoma.

No significant inhibition of leukocyte migration was demonstrated by CEA, with the exception of one patient with pancreatic carcinoma. Thus, with the leukocyte migration technique, no consistent *in vitro* cell-mediated immunity to CEA was demonstrated supporting the hypothesis that CEA is not the antigen toward which cell-mediated host response phenomena are directed.

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