choose between differing light sources (*13*). Cardinali *et al.* measured the effectiveness of the sources used in our experiments in inhibiting pineal hydroxyindole-*O*-methyltransferase activity in the rat (*14*), and observed spectral sensitivity similar to that reported here. Whether the same receptor cells are involved in these functions and in vision has not been determined; the separation of photic information mediating these various responses could occur further along the visual pathways from the retina (*15*).

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Immunity to Colon Cancer Assessed by Antigen-Induced Inhibition of Mixed Mononuclear Cell Migration

Abstract. *A purified preparation of mixed human peripheral blood lymphocytes and monocytes was used in an inhibition-of-migration assay for cell-mediated immunity to cancer of the colon. This preparation was reproducibly antigen-responsive and migrated with greater reliability than did a more complex cell mixture. Of 27 patients with this disease, cells from 24 showed inhibited migration in response to colon carcinoma antigen. Uninhibited migration patterns were found in each of the 52 cancer-free controls, including eight patients with nonmalignant disease initially diagnosed as cancer of the colon, and in nine patients with surgically cured adenocarcinoma of the colon.*

The lymphocyte-mediated cytotoxicity (LC) test and related techniques have been widely used for the in vitro demonstration of immunity against tumors in animals and man (*1*). Assays of this type are also useful for studying serum "blocking" activity, an effect that is probably mediated by tumor antigen–antibody complexes and that may be the in vitro counterpart of in vivo "enhancement" of tumor growth (*2*). Several factors limit the clinical applicability of the LC test, however. Target tumor cells are required, incubation of cultures requires 48 to 72 hours, and cytotoxic effect must be assessed by time-consuming morphologic or isotope-release techniques. Tests of this type are not useful for assessing immunity to preparations of tumor-specific antigens. Finally, the LC test is of limited diagnostic and prognostic usefulness in that it is frequently negative in the presence of growing cancer, is often positive in tumor-free relatives and contacts of cancer patients, and, when positive in a cancer patient, tends to remain so after surgical cure (*3*).

Another in vitro correlate of cellular immunity, inhibition of leukocyte migration, was originally described by Soborg (*4*) and Bendixen (*5*) in bacterial infection and autoimmune disease, and attempts have also been made to demonstrate human tumor immunity by this means (*6*). Although the test is simple and rapid, results obtained with soluble antigens in delayed hypersensitivity are characterized by a lack of reproducibility, poor correlation with skin test reactivity, and the need to accurately distinguish the antigen-responsive mononuclear cell migration pattern from the antigen-unresponsive granulocyte margin (*7*). In studies of human tumor immunity with this assay, negative responses to tumor antigen were reported in a significant proportion of patients (*6*). We suspected that the incomplete correlation between clinical status and in vitro tumor immunity reflected as inhibition of leukocyte migration was not necessarily the result of absence of tumor immunity, but might be related to the complex and variable leukocyte preparations used. In this report, we describe the use of purified populations of antigen-responsive lymphocytes and migrating monocytes in a migration inhibition test, and we correlate the results of this assay with clinical status of colon carcinoma.

Tumor antigens were prepared as membrane-rich dilutions of homogenates of colon adenocarcinomas obtained at surgery. Homologous colon adenocarcinoma antigen was generally used, although in a few cases tumor antigen from the patient being studied gave identical results. One gram of tumor tissue was homogenized for 15 minutes in nine volumes of phosphate-buffered saline at 60,000 rev/min in a VirTis blade-type homogenizer. The homogenate was frozen, thawed, and rehomogenized for 15 minutes, and particulate matter was removed by filtration through several layers of sterile gauze. The filtrate was exhaustively dialyzed against Seligmann's buffered salt solution (a modified Hanks solution free of calcium and magnesium) and stored at -20°C in RPMI 1640 tissue culture medium at 1 : 100 dilutions (1 g of original tumor per 100 ml of medium). For use as antigen, the concentrated portions of tumor extract were further diluted to 1 : 3000 for final inclusion in migration chambers. Whole blood (40 ml) from patients with colon adenocarcinoma and controls was added to 1.4 ml of 5 percent ethylenediaminetetraacetic acid (EDTA), diluted with 100 ml of Seligmann's buffered salt solution containing 500 mg of EDTA, and divided into two parts. Under each portion was injected 20 ml of a mixture of 24 parts of 9 percent Ficoll (Pharmacia, Uppsala, Sweden) and 10 parts of 34 percent Hypaque (Winthrop Laboratories, New York), and samples were sedimented at 400*g* for 30 minutes (*8*). Platelets contaminating the monocyte-lymphocyte layer were removed by sedimentation through triple sucrose layers (6, 12, and 16 percent) at 200*g* for 15

minutes; platelets were then in the 12 percent layer and mononuclear cells in the pellet. Lymphocytes and monocytes were washed three times in Seligmann's buffered salt solution and suspended with or without antigen in RPMI 1640 containing 10 percent horse serum. Lymphocyte-monocyte mixtures were packed into capillary tubes (inner diameter, 0.9 to 1.1 mm; Kimax, Toledo, Ohio) by centrifugation at 180*g* for 5 minutes. The tubes were broken at the fluid-cell interface and secured in Sykes-Moore migration chambers with stopcock grease, the chamber was filled with RPMI 1640 containing 10 percent horse serum with or without antigen, and migration was allowed to proceed for 18 hours. Areas of migration were photographed and projected onto bond paper, and their areas were measured by planimetry. The migration index (MI) was expressed as area of migration in the presence of antigen divided by the area of migration without antigen.

Results in 40 patients with adenocarcinoma of the colon and 52 cancer-free controls are shown in Fig. 1. Twenty-seven tumor patients with Dukes' class A, B, or C colon adenocarcinoma were studied 3 days before to 3 days after resective surgery and showed a mean MI of 0.64 ± 0.14. Only three patients showed an MI above 0.80. One of these three patients was a 72-year-old woman who underwent sigmoid resection for obstructing diverticulitis; a small (0.8 by 1.0 cm) adenocarcinoma was found in the surgical specimen. A second patient in this group was a 52-year-old man with widely metastatic terminal disease. This patient failed to react to candida, mumps, and tuberculin antigens given intradermally, results suggesting that the absence of antigen-induced inhibition of migration in this patient may have reflected the effect of widespread malignancy on the capacity to mount a cellular immune response.

The group of 52 controls was composed of healthy volunteers, patients with noncancerous gastrointestinal diseases, and patients with the initial diagnosis of cancer of the colon who were subsequently shown to have nonmalignant disease. The controls failed to demonstrate tumor antigen–induced inhibition of migration both as a group (MI, 0.98 ± 0.09) and as individuals; no control subject had an MI of less than 0.80. The importance of age differences between patients with cancer of the colon (mean age, 64 ± 13 years) and the initial 25 controls (mean age, 43 ± 21 years) became apparent, and an attempt was made to select the final 27 control subjects so that their mean age (60 ± 16 years) would approximate that of the 27 cancer patients. The MI for this older control subgroup was 0.96 ± 0.08, and no individual showed an MI of less than 0.80. Also studied was a group of nine patients with surgically resected colon adenocarcinomas documented by histologic examination who had been clinically free of disease for 6 months to 15 years (mean, 3.8 years). These subjects showed a mean MI of 1.06 ± 0.07, with no values below the arbitrary level of 0.80. In addition, four patients with metastatic adenocarcinoma who were undergoing chemotherapy with 5-fluorouracil also failed to exhibit antigen-induced inhibition of migration (MI's of 0.95, 0.93, 0.91, and 0.96). Finally, eight control patients with initial clinical diagnoses of colon cancer who were ultimately shown to have benign disease also did not demonstrate tumor antigen–induced inhibition of migration (MI, 0.99 ± 0.08). Five of these patients were found to have sigmoid diverticulitis with bleeding and obstruction; one had ulcerative colitis localized to a segment of transverse colon; and another had a large calcified internal hemorrhoid. One patient with a history of resected colon adenocarcinoma had jaundice and an abnormal hepatic radioiosotopic scan at the time of this study. A needle biopsy specimen of the liver was free of malignancy and revealed Laennec's cirrhosis with associated alcoholic hepatitis. The jaundice disappeared during 2 weeks of abstinence from alcohol in the hospital. The mean MI of the 27 patients with active colon cancer differed from the mean MI of each other group ($P < .001$).

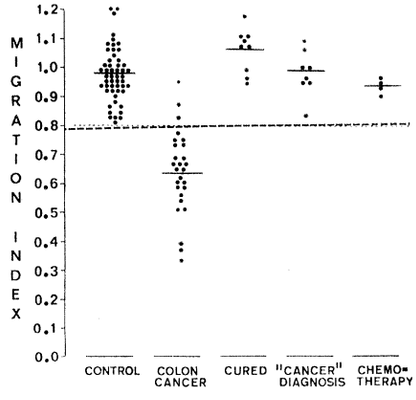

Fig. 1. Tumor antigen–induced inhibition of mixed mononuclear cell migration in patients with colon cancer and controls. The control group consisted of 52 healthy volunteers and patients with nonmalignant diseases. The colon cancer group consisted of 27 patients with colon adenocarcinoma studied 3 days before to 3 days after resective surgery. The cured group consisted of nine patients with histologically proven colonic carcinoma who had been clinically cancer-free for a mean follow-up period of 3.8 years. The "cancer" diagnosis group was composed of eight patients in the control group who had the clinical diagnosis of colon carcinoma at the time of study but were subsequently shown to have benign disease. The chemotherapy group consisted of four patients who had been receiving 5-fluorouracil for treatment of colon carcinoma for at least 1 month. The migration index equals the area of migration in the presence of antigen divided by the area of migration in the absence of antigen. The mean migration index of the colon cancer group is significantly different from each of the other four groups ($P < .001$ by unpaired *t*-test).

These results indicate that inhibition of mixed mononuclear cell migration may be a valid indicator of the presence of actively growing colon cancer. The data do not reveal whether the reaction observed has specificity for colon cancer antigen contained in the tumor extract or for a common tumor antigen. Migration inhibition studies with colon cancer extracts in patients with other gastrointestinal malignancies and with antigenic preparations from gastric and pancreatic tumors in patients with colon cancer are needed to resolve this question.

We were impressed with the variability imparted to inhibition of crude leukocyte migration by the presence of granulocytes, platelets, and contaminating erythrocytes. In our experience with crude tumor antigen, these cell types appear not to be pertinent to either migration per se or to its inhibition. This might partially explain differences between our results and those obtained for inhibition of leukocyte migration. Using lymphocytes from tuberculin-positive patients and purified protein derivative (PPD) as antigen, Read and Zabriskie (*9*) concluded that the polymorphonuclear leukocyte is required for antigen-induced inhibition of mononuclear cell migration. Thus, the nature and form of the antigen may be an important variable in leukocyte migration inhibition. That the polymorphonuclear leukocyte's contribution to in vitro immune reactions varies with the nature of the antigenic stimulation is also suggested by the report that activity of mixed leukocyte cultures is enhanced by removal of this cell type (*10*).

The reason this mononuclear cell migration inhibition test is negative in surgically cured cancer patients is not clear. Unlike lymphocyte-mediated cytotoxicity, the lymphocyte function resulting in inhibition of migration of monocytes may require recent and vigorous in vivo lymphocyte stimulation by tumor antigen in order to maintain tumor antigen responsiveness.

The four patients with metastatic cancer undergoing chemotherapy did not show a reduction in tumor mass, and we suspect that lack of migration inhibition resulted from the effect of chemotherapy or of disseminated disease on the patients' capacity to mount a cellular immune response rather than from diminished in vivo stimulation by tumor antigen.

Removal of granulocytes, erythrocytes, and platelets from the antigen-responsive and migrating cell population used in this assay is a relatively efficient process requiring about 2 hours for cell preparation. Although its usefulness for the demonstration of cellular immunity to other solid tumors remains to be established, inhibition of mixed mononuclear cell migration correlates well with clinical status of colon adenocarcinoma and may prove to be of diagnostic and prognostic value in this condition.

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Behavioral Development after Forelimb Deafferentation on Day of Birth in Monkeys with and without Blinding

Abstract. *Four infant monkeys underwent somatosensory deafferentation of both forelimbs within hours after birth. Ambulation, climbing, and reaching toward objects developed spontaneously in each case. Thumb-forefinger prehension could be trained by operant shaping methods. Two infants deafferented at birth and blinded by eyelid closure were retarded in motor development by only 1 to 2 weeks. Results indicate that topographic sensory feedback and autogenetic spinal reflexes are not necessary after birth for the development of most types of movement performed by the forelimb musculature in monkeys.*

Previous research has shown that a wide range of purposive movement is possible after the elimination of spinal reflexes and somatosensory feedback accomplished by serial section of the dorsal roots of spinal nerves (*1*). Adolescent monkeys are capable of using deafferented limbs effectively for grasp, ambulation, and climbing, both while able to see and while blindfolded; they can also pick up raisins from a shallow well between thumb and forefinger. These movements are not affected by further dorsal rhizotomy; they continue to remain possible after the complete deafferentation of the spinal cord [see (*2*)].

These results indicate that the primate central nervous system is capable of generating movements of almost all types autonomously in the absence of guidance from sensory cues from the organism's own body. However, the subjects in all of these studies were adolescents with considerable motor experience prior to deafferentation. The question remained, therefore, as to whether somatic sensation is necessary in ontogeny for the development of normal patterns of coordination. The present report describes the effects of deafferentation of both forelimbs on the first day of life with and without the occlusion of vision by sewing the eyelids closed.

Four neonates (one baboon and three rhesus monkeys) were separated from their mothers within hours of birth and anesthetized. Bilateral dorsal rhizotomy (C_2 or C_3 to T_4) was then carried out intradurally under magnification (× 16; checking of the root section was at × 25 to × 40 by two observers). Intensive supportive therapy and nursing care were provided in the early postoperative period in order to

Table 1. Age (in weeks) of appearance and comparative retardation of different types of motor activity. The values are the mean ages of the first definite appearance of the behaviors.

Type of motor activity	(1) Deafferented only*	(2) Normal†	(3) Blinded deafferented	(4) Blinded only	Retardation (1) vs. (3)
Visually guided reaching	1.1	0.4			
Crouching, arms crossed	1.5	‡	2.5	‡	1.0
Sitting-crouching, arms uncrossed	1.8	0.6	4.0	0.4	2.2
Standing on all fours	2.4	.6	3.5	.7	1.1
First step	3.1	.3	4.5	.6	1.4
Sequential steps	3.3	.7	4.5	.7	1.2
Crude ambulation	3.6	2.0	5.5	2.0	1.9
Mean retardation	(1) vs. (2) = 1.8		(3) vs. (4) = 3.5		1.5

* Time of emergence of each of the behaviors was very consistent across animals in the group. † Normal data derived from Hines (*8*). ‡ This stage is exhibited only in deafferented animals.