Decreased T Lymphocyte Migration in Patients with Malignancy Mediated by a Suppressor Cell Population

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Abstract. The migration and concentration of lymphocytes at sites of antigenic challenge are an integral part of the expression of delayed cutaneous hypersensitivity, as well as of tumor and graft rejection. In this study, we have analyzed the migration of T lymphocytes from patients with malignancy. We used casein and concanavalin A (Con A)-stimulated mononuclear cell supernatants to stimulate T cell locomotion. Peripheral blood T lymphocytes from 30 patients with established malignancy, 10 patients with indolent malignancy or benign tumor, and 42 normal adult controls were tested. Data are expressed as a migration index (MI), which represents the difference in micrometers between the distance migrated in response to a stimulus and the distance migrated in response to media alone. We observed a marked depression in casein-stimulated T lymphocyte migration in patients with established malignancy (mean MI±1 SD = 17.0±9 μm) as compared with normal adult controls (mean MI±1 SD = 35.3±10 μm). Similar results were observed with migration in response to Con A supernatants. T cells from patients with established malignancy had a mean MI of 5.8±4 μm to Con A supernatants as compared with 24.5±5 for controls. This depressed migration was apparent both in the distance that cells migrated and in the number of cells that migrated into the membrane. Of 10 patients with indolent malignancy or benign tumor, T cell migration in 8 was not significantly decreased as compared with controls. When we mixed equal concentrations of normal control T lymphocytes with T lymphocytes from patients with cancer and added the mixture directly to the upper compartment of the chemotaxis chamber, the response of the normal T cells to casein was inhibited by an average of 48%. We observed inhibition of this migration of normal cells when we added as little as 10% of patient cells to normal cells. When we mixed normal control T lymphocytes from different donors and added them directly to the upper compartment of the chemotaxis chamber, T lymphocyte migration in response to casein was not significantly altered. If T cells from patients with cancer were cultured overnight, the suppressive effect on lymphocyte locomotion was lost. Our results indicate that there is a population of T lymphocytes in patients with cancer that suppress normal T lymphocyte migration. This suppressor activity may partially explain the subversion of immunosurveillance in established neoplastic states, as well as the defective inflammatory reaction to intradermal injection of antigen observed in many patients with malignancy.

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Introduction

Depressed cell-mediated immune responses have been well documented in patients with cancer, including patients with lymphoid as well as nonlymphoid tumors. Depressed lymphocyte proliferation in response to phytohemagglutinin (1–4), as well as depressed cutaneous hypersensitivity reactions (1, 2, 5–8) and prolonged skin allograft rejection (9, 10) have been observed in individuals with tumors. These observations indicate an impairment of the T lymphocyte effector arm of the immune response. The importance of the mobilization of mononuclear cells to a tumor site is indicated by studies that show that the prognosis is more favorable for patients with certain types of tumor when the tumor is accompanied by an extensive mononuclear (MN)[1] cell infiltration. In fact, the claim has been made that among patients with these tumors, those who show the most intense MN cell infiltration survive the longest (11).

1. *Abbreviations used in this paper:* Con A, concanavalin A; MEM, minimum essential medium; MI, migration index; MN, mononuclear; SRBC, sheep erythrocytes.

Many groups have studied abnormalities of monocyte chemotaxis in patients with cancer (12–17). Recently, techniques have been developed to analyze lymphocyte migration in vitro (18–23). In this study, we show that T lymphocyte migration is depressed in patients with established malignancy, and that it can be attributed to the presence of a suppressor cell population.

Methods

Patients and controls. Heparinized peripheral venous blood samples were obtained from 30 patients with pathologic diagnoses of malignant neoplastic disease; these included 6 with adenocarcinoma of the breast, 5 with lung cancer (2 oat cell and 3 adenocarcinoma), 3 with adenocarcinoma of the colon, 3 with transitional cell bladder carcinoma, 2 with squamous cell cervical cancer, 2 with adenocarcinoma of the prostate, 2 with lymphoma (1 diffuse histiocytic and 1 nodular sclerosing Hodgkin's disease), 1 with pleomorpholiposarcoma, 1 with multiple myeloma, 1 with malignant melanoma, 1 with carcinoma of the ovary, 1 with endometrial cancer, 1 with a mucoepidermoid parotid gland tumor, and 1 patient with widespread malignancy whose primary was unknown; ages of these patients ranged from 18 to 83 with a mean ±1 SD of 55.6±18. 10 patients with benign tumors or indolent nonmetastatic malignancy were also studied and included 2 with carcinoma in situ of the cervix, 1 with basal cell skin carcinoma, 1 with Bowen's disease of the vulva, 1 with a dermoid cyst, 1 with a leiomyoma, 1 with a prolactinoma, 1 with a cholesteatoma, 1 with an endometrial polyp, and 1 with a carcinoid of the bronchus. Follow-up studies over a 9–16 mo period showed that none of the patients in this group had developed tumor progression or evidence of metastasis. The mean age ±1 SD of patients in this group was 32.1±10 with a range of 19–50 yr. The patients were seen at The University of New Mexico Medical Center, Albuquerque Veterans Administration Medical Center, Presbyterian Hospital Center, or St. Joseph's Hospital, all in Albuquerque, New Mexico. Two patients had received chemotherapy before the start of the study but had ended their treatment 7 mo earlier. Two patients had received radiotherapy (one 5 mo earlier and one 4 h pre-venipuncture). Seven patients had had surgical resection of tumor mass between 1 mo and 3 yr (three within 2 mo) before the start of the study. 19 patients without previous therapy were studied. No patients were receiving immunosuppressive nor immunoenhancing agents at the time of study. Seven patients had metastases to several anatomic locations. The control population consisted of 42 healthy adults of both sexes. The mean age ±1 SD of the control group was 32.2±15 with a range of 19–64 yr.

Isolation of T lymphocytes. Human peripheral blood MN cells were separated from heparinized venous blood by Ficoll-Hypaque density gradient centrifugation. The MN band was removed, washed twice in Eagle's minimum essential medium (MEM), and resuspended to 1×10^7 cells/ml in 10% fetal calf serum and MEM containing 0.1% gentamycin. Sheep erythrocytes (SRBC) were washed three times in phosphate-buffered saline, pH 7.4 and resuspended to a 5% concentration in MEM containing 10% fetal calf serum. Equal volumes of the MN cell suspension and the SRBC suspension were mixed and incubated at 37°C for 5 min. This preparation was centrifuged at 200 *g* for 5 min and further incubated overnight at 4°C. Cell pellets were resuspended, and rosetting T lymphocytes were separated from nonrosetting cells by Ficoll-Hypaque density gradient centrifugation. The cell pellet that contained T lymphocytes and SRBCs was suspended in 9 ml distilled water, cooled to 4°C, and vortexed several times for 20–30 s before 1 ml of 10× Hank's balanced salt solution was added to restore isotonicity. The final preparation of E-rosette positive cells contained <4% monocytes, as determined by peroxidase stain (24), and was >95% E-rosetting lymphocytes. Patient cell preparations with 5% or more peroxidase-staining MN cells were not used in this study. In some cases MN cells were passed through a glass wool column before incubation with SRBC (21). Isolated T lymphocytes were washed and resuspended to 2×10^6 cells/ml in the media appropriate for each assay.

T lymphocyte locomotion assay. T lymphocyte locomotion assays were performed in blind-well Boyden chambers (Nuclepore Corp., Pleasanton, CA) using a modification of the leading front technique developed for neutrophils by Zigmond and Hirsch (25). The assay performed here used an 8-μm-pore-size cellulose nitrate membrane (Sartorius Filters, Inc., Hayward, CA) to separate the upper and lower compartments. Casein or supernatant from Con A-stimulated mononuclear cells was added to the lower compartment of the chemotaxis chamber to stimulate lymphocyte migration. T lymphocytes (4×10^5 cells or 2×10^5 cells) were added to the upper compartment and allowed to migrate into the membrane for 3 h at 37°C, after which the membrane was fixed in formaldehyde, stained with hematoxylin, and cleared with isopropyl alcohol and xylene, as previously described (20). Cell migration was measured by the leading front method, which used the microscope micrometer to measure the distance that the leading three cells had traveled into the membrane. Morphologically, these cells were lymphocytes, as determined by light microscopy. Data were expressed as mean migration in micrometers ±1 SD or as a migration index (MI), which represents the difference between the lymphocyte response to a locomotor stimulus and that observed with media alone. In some cases, the number of cells responding to the stimulus was determined by counting those cells per 400× field that migrated beyond 20 μm into the membrane. In each case, results represent the mean number of cells per 400× field (five fields were counted) on each of two duplicate membranes.

Preparation of agents used to stimulate lymphocyte migration. Stock casein (Fisher Scientific Co., Allied Corp., Pittsburgh, PA) preparations were prepared by adding casein (2 mg/ml) to MEM and agitating periodically for 2 h at 37°C. Casein was then used at a final concentration of 0.4 mg/ml in the lower compartment of the chemotaxis chamber to stimulate locomotion.

Lymphocyte chemotactic factor from Con A-stimulated MN cells was prepared as previously described (22). Briefly, 5×10^6 peripheral blood MN cells in MEM that contained 1% human AB serum were cultured for 24 h with 0.5 μg/ml of Con A (Sigma Chemical Co., St. Louis, MO). Supernatants that contained T lymphocyte chemotactic factor were removed and mixed with 25 mM α-methyl-D-mannoside to block the binding of any residual Con A to lymphocytes in the chemotaxis assay system. Control media for the factor consisted of culture media that contained 0.5 μg/ml of Con A plus 25 mM α-methyl-D-mannoside. The addition of Con A plus α-methyl-D-mannoside did not alter random lymphocyte migration.

Lymphocyte culturing procedure. In some cases, T lymphocytes were resuspended in MEM supplemented with 1%, 56°C, heat-inactivated human AB serum, 2 mM *l*-glutamine, and 0.1% gentamycin. The suspensions were incubated at 37°C for 24 h without mitogen or antigen. After culture, cells were washed and then adjusted to 2×10^6 cells/ml in MEM. Viability, as determined by trypan blue dye exclusion, was >80%.

Results

Defective T lymphocyte migration in patients with established malignancy. We prepared and tested T lymphocytes from 30

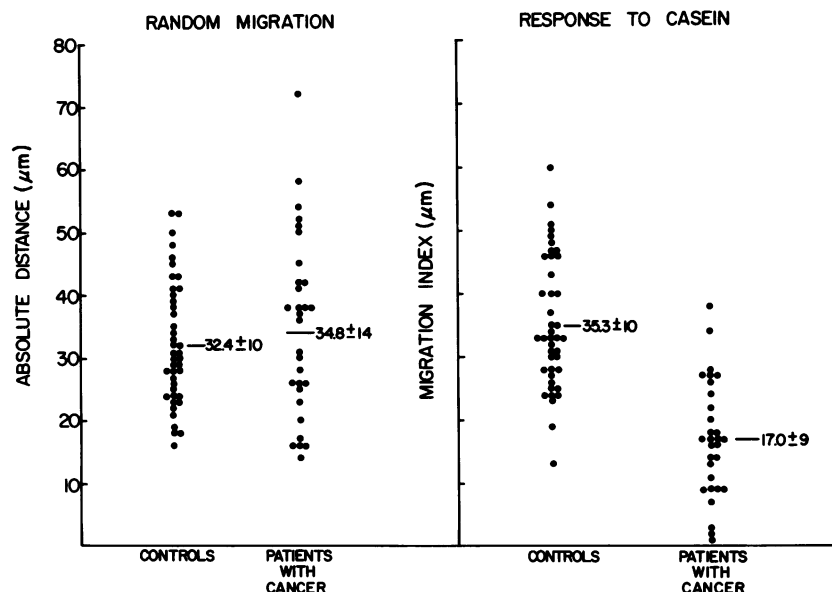

Figure 1. Comparison of the migration of T lymphocytes from normal controls and from patients with established malignancy in response to random migration is expressed as the distance in micrometers traveled, and casein-stimulated migration is expressed as a migration index. The mean migration for each group is indicated by the bar.

patients with diagnoses of established malignant neoplastic disease for locomotor activity in response to casein. In each case, we tested one or two normal controls simultaneously. As shown in Fig. 1, random T lymphocyte migration, expressed as the absolute distance traveled by T lymphocytes with media alone in the lower compartment, was equivalent in both normal controls (mean migration ±1 SD = 32.4±10 μm) and patients with cancer (mean migration ±1 SD = 34.8±14 μm). However, T lymphocytes from patients with established malignancy showed significantly reduced migration ($P < 0.001$ by t test) in response to casein (mean MI±1 SD = 17.0±9 μm) as compared with T lymphocytes from normal controls (mean MI±1 SD = 35.3±10 μm). This significant difference was also apparent when the absolute migration of T cells from patients or controls in response to casein was compared ($P < 0.001$). Since the mean age of patients with malignancy was 56±18 with a range from 18 to 83 yr whereas controls averaged 32±15 with a range of 19 to 64 yr, we analyzed our data to determine if a correlation between age and T cell migration existed in either group. We found no correlation between age and T cell responsiveness to casein in any group. We obtained the data shown in Fig. 1 with T cells prepared directly from MN cells by E-rosette formation with SRBC. We obtained similar results with patient MN cells depleted of adherent cells by passage through glass wool before preparation of E-rosettes.

Because previous studies have shown that casein enhances T lymphocyte random migration and that it is therefore chemokinetic (21), we conducted additional studies (Fig. 2) to determine if the migration of T lymphocytes in response to Con A-stimulated MN cell supernatants containing lymphocyte chemoattractant activity (22) was abnormal. We tested T lymphocytes from six patients with established malignancy for locomotor activity in response to Con A-stimulated MN cell supernatant and we compared their activity to that of normal controls. As shown in Fig. 2, the response of T cells from patients with established malignancy to Con A supernatants was suppressed (MI±1 SD = 5.8±4 μm) as compared with normal adult controls (mean MI±1 SD = 24.5±5 μm). Random locomotion was similar in both patients and controls (mean ±1 SD = 28±10 and 26±8 μm, respectively).

Since the leading front technique measures only the migration of the leading three cells, we performed experiments to determine if suppressed T lymphocyte migration in patients with cancer is also reflected in the number of cells that respond. Fig. 3 shows the response of T lymphocytes from three patients with malignancy and suppressed lymphocyte migration to various concentrations of casein; both the distance migrated by

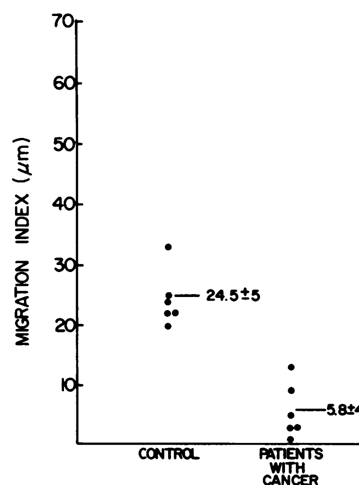

Figure 2. Comparison of the migration of T lymphocytes from normal controls and from patients with established malignancy in response to Con A-stimulated MN cells supernatants. Data are expressed as an MI, and the mean MI for the whole population is indicated by the bar.

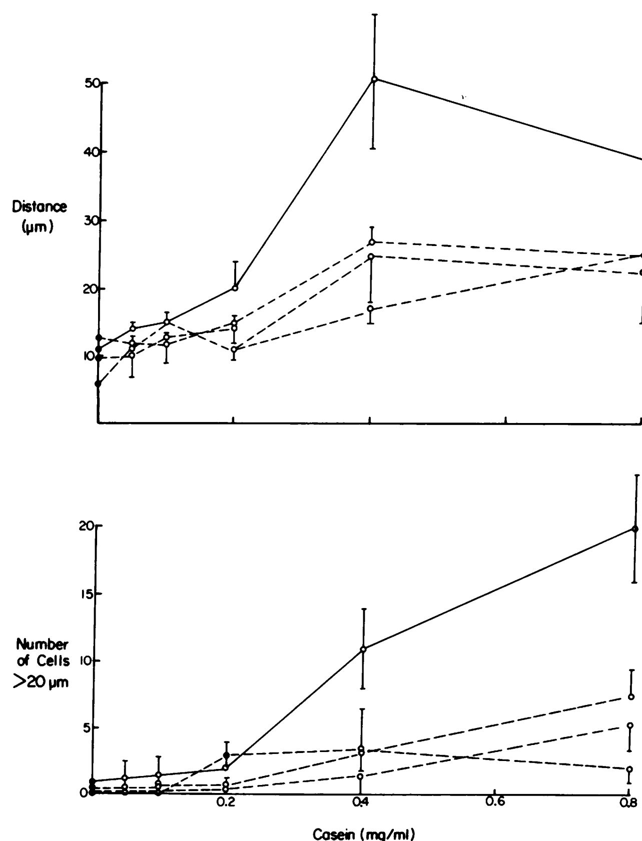

Figure 3. Comparative migration of T cells from three patients with established tumor and normal control T cells as measured by the leading front assay or by counting the number of cells that migrate beyond 20 μm into the membrane. The mean ±1 SD for two simultaneously run normal controls (solid line) is compared with the response of T cells (mean ±1 SD) from each of three different patients with established tumor (dashed lines).

the leading front technique and the number of cells that migrated beyond 20 μm are shown. Migration as measured by both methods was reduced in the patients with cancer as compared with normal controls. This decreased migration was apparent at all concentrations of casein tested. When we compared the number of cells that had migrated beyond 20 μm in response to 0.8 mg/ml of casein to the total number of cells on the membrane, we found that 7–12% of normal cells had migrated to this level as compared with only 0.8–4% of patient cells. When we used Con A supernatant as a stimulus, our results were similar and showed both a decreased distance migrated and a decreased number of responding cells. When we reduced the migration time from 3 h to 2 h, we still found decreased migration of patient T cells with respect to normal T cells, although the distance that both normal and patient cells migrated was proportionally reduced.

T lymphocyte migration in patients with benign tumor or indolent malignancy. All of the patients studied in Figs. 1 and 2 had malignant neoplastic disease. We performed additional studies with T lymphocytes from 10 patients with benign tumor or indolent malignancy to determine if depressed T lymphocyte migration was associated with these conditions. Fig. 4 compares lymphocyte migration in response to casein in each of these 10 patients to migration of T cells from the original 30 patients with established malignancies. The 10 patients with benign neoplasm or indolent malignancy showed an MI±1 SD of 40.3±24, which is comparable to the MI of normal controls (mean MI±1 SD = 35.3±10) and much greater than the MI of patients with established malignancy (mean ±1 SD = 17.0±9). Statistical analysis by *t* test showed that T cell migration in response to casein as measured by MI or absolute distance was significantly suppressed ($P < 0.001$) in patients with established tumor as compared with both normal controls and patients with benign tumor. Furthermore, T cell migration in response to casein did not differ significantly ($P = 0.213$) among patients with benign tumor and normal controls, nor did random lymphocyte migration differ significantly ($P = 0.7$) among patients with established malignancy (mean ±1 SD = 34.8±13 μm) and patients with benign tumor or indolent malignancy (mean ±1 SD = 37±9 μm).

Suppression of normal T lymphocyte migration by T cells from patients with cancer. Fig. 5 shows the results of two representative experiments in which equal concentrations of normal and patient T lymphocytes were added directly to the upper compartment of the chemotaxis chamber. Absolute distance, plotted on the left, revealed no significant difference in random migration when either 4×10^5 or 2×10^5 control cells were used in the migration assay. Similarly, we saw no significant difference in random migration of patient cells at these two cell concentrations. When 2×10^5 patient cells and 2×10^5 control cells were mixed, random migration was equivalent to control or to patient values. As shown on the right of Fig. 5, halving the cell concentration did not affect the response to casein with either control or patient T lymphocyte suspensions. When we added 2×10^5 patient cells to 2×10^5 control cells, we saw a marked reduction in the MI of control cells in response to casein, but random migration was unaffected. The suppression of normal T cell migration by patient T cells was demonstrable both by the leading front assay and by counting the cells that migrated beyond 20 μm into the membrane (Fig. 6). Furthermore, the addition of 10–50% normal cells from one normal donor to normal cells from another donor had a negligible effect on the leading front assay, and enhanced the number of cells that migrated beyond 20 μm. The addition of as few as 10% T cells from a patient with established tumor markedly suppressed normal T cell migration as measured by both methods of analysis. The data shown in Fig. 6 are representative results from two patients with a T cell migration of <50% of the mean of normal controls as measured by the leading front assay.

The data in Fig. 7 show results of mixing experiments obtained with cells from 11 patients with established malignancy, expressed as a percentage of the simultaneous normal control lymphocyte response to casein. Six of the patients were tested

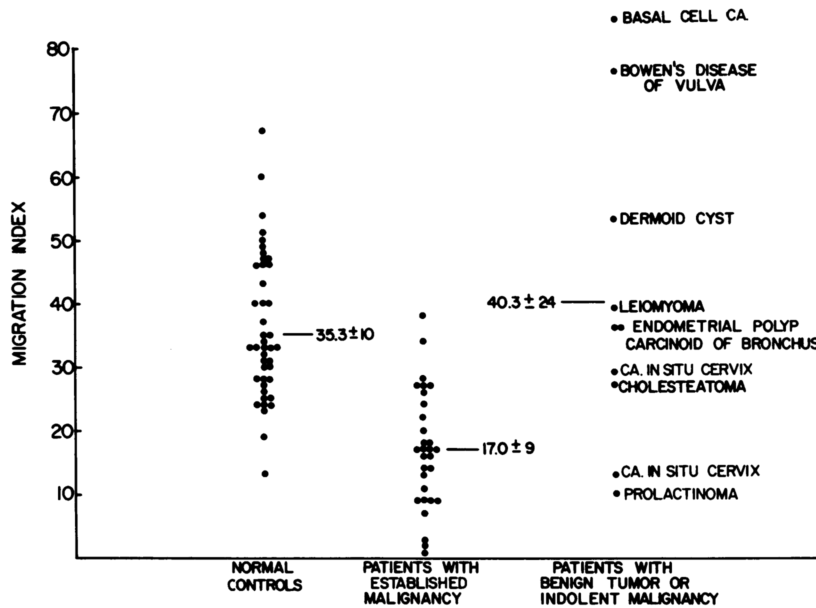

Figure 4. T lymphocyte migration in response to casein comparing lymphocytes from patients with established malignancy to those from patients with indolent malignancy or benign tumors and simultaneously tested normal controls. The mean MI for each group is indicated by the bar. CA refers to carcinoma.

twice with different normal T cells. As shown, when we mixed 2×10^5 T lymphocytes from two normal adult donors and added them directly to the upper compartment of the chemotaxis chamber, T cell responses to casein were not altered. On the contrary, when we mixed equal concentrations (2×10^5 cells) of normal control T lymphocytes with T lymphocytes from patients with cancer and added them directly to the upper compartment of the chemotaxis chamber, the response of the normal

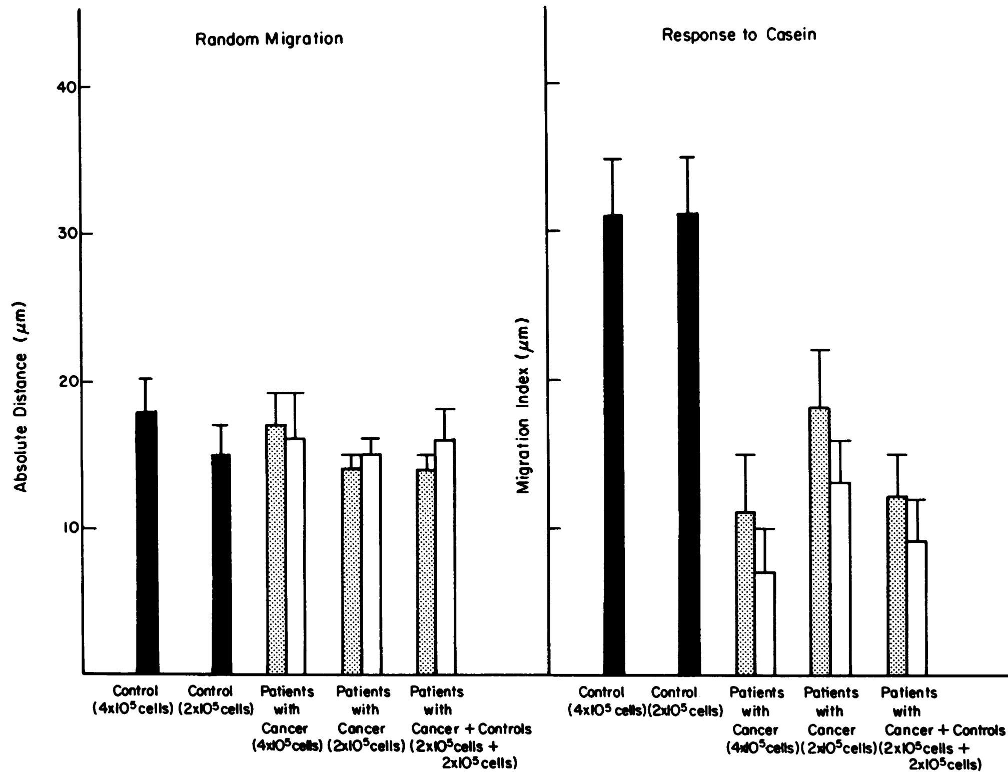

Figure 5. Suppression of normal donor T lymphocyte migration by the addition of T cells. Results show both random and casein-stimulated migration. Stippled bars and solid bars represent two different experiments. In experiment 1 (stippled bar), the patient had a mucoepidermoid parotid gland tumor, while in the second experiment (solid bar) the patient had bladder cancer.

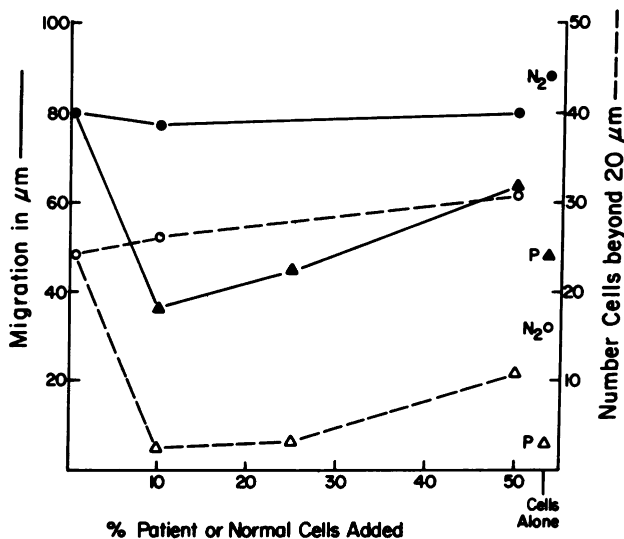

Figure 6. Suppression of casein-stimulated normal T cell migration by the addition of T cells from a patient with established tumor. Results show the effect of the addition to normal T cells (3×10^5 cells/chamber) of various percentages of T cells from either a second normal donor (circles) or a patient with established tumor and suppressed T cell migration (triangles). T cell migration measured by the leading front technique (solid line) or by counting the number of cells migrating beyond 20 μm (dashed line) is shown. The response of 3×10^5 T cells alone from the second normal donor (N_2) or from the patient with established tumor (P) is indicated.

T cells to casein was inhibited by an average of 47.5%. We noted no association between the degree of suppressive activity and previous chemotherapy, radiation treatment, or the degree of metastatic disease. In experiments with three different subjects where cells were cultured for 24 h at 37°C before being added to normal T cells in the migration assay, the suppressive effect by cancer patient cells was lost. These results indicated that there was a population of T lymphocytes in patients with established malignancy that suppressed normal T lymphocyte migration and that the suppressor activity was lost after 24 h of 37°C incubation. We could not attribute the observed suppression to a direct cytotoxic effect since mixtures of patients' and normal T cells after incubation for 3 h at 37°C did not show decreased viability as measured by trypan blue dye exclusion. We attempted to identify a soluble suppressor factor by culturing T lymphocytes from three patients with suppressed T cell migration (<50% of normal response) for 3 h in the same media used in chemotaxis assays. When we suspended normal T cells in this supernatant, we observed no suppression of lymphocyte migration in response to casein.

Discussion

This study demonstrates that T lymphocyte migration is suppressed in patients with established neoplastic disease. We observed impaired locomotor activity of these cells with the chemokinetic stimulus casein (21) as well as with the chemotactic Con A supernatant from MN cells (22). The data accumulated so far suggest that suppressed T lymphocyte locomotion in response to casein is associated primarily with established malignancy, since we did not see the same degree of suppression in patients with benign tumor or indolent malignancy. In the patients studied there was no correlation between the age of the patient or tumor burden and degree of impairment of locomotor activity. In fact, in some instances cells from younger patients with early established malignancy showed greater impairment in migration than those cells from older patients with more advanced lesions. Our studies demonstrate that decreased T lymphocyte migration in these patients may be the result of an

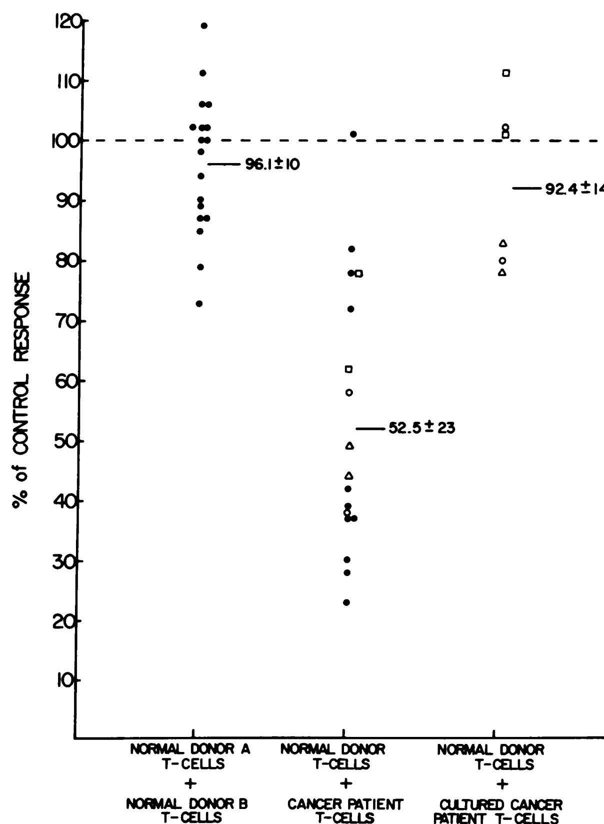

Figure 7. Loss of T-suppressor activity after overnight culture of T lymphocytes from patients with malignancy. The effects of mixing T lymphocytes from two different normal donors, T lymphocytes from patients with cancer with those from normal donors, and 24-h cultured T lymphocytes from patients with cancer with normal T cells, are shown. Open symbols indicate T cells from the same patient with cancer tested before and after culture. Duplicate open symbols indicate cells from the same patient with cancer tested in conjunction with different normal donor cells. Results are presented as a percentage of the simultaneously tested normal control T lymphocyte response. The mean response of each group is indicated by the bar.

active suppressor T cell population. These experiments represent the first in vitro demonstration that lymphocyte migration may be regulated by a suppressor cell population that is coincident with malignant disease.

The impaired ability of T lymphocytes to migrate well in the cancer milieu may help explain the depressed cutaneous hypersensitivity reactions to chemicals (5, 6), the depressed delayed skin test reactions to antigen (1, 2, 5, 7, 8), and the delayed skin allograft rejection (9, 10). Interestingly, in this study, patients with indolent malignancy or benign tumor demonstrated near-normal T lymphocyte migration in response to casein. In at least one other study (1), patients with benign dysplasia have shown full immunocompetence. Thus, assessment of in vitro T lymphocyte migration may be a way to evaluate impaired T cell function associated with cancer.

Several human and animal studies have demonstrated suppressor cell populations that reduce mitogen-stimulated lymphocyte proliferation, mixed lymphocyte reactivity, immunoglobulin synthesis, and tumoricidal activity in neoplastic disease (26–31). Our study demonstrates for the first time that a suppressor cell might regulate T lymphocyte migration in patients with cancer. Normal T-cell migration was suppressed when as few as 10% patient T cells were added to normal cells. Although our data imply that suppression is mediated by a T cell, we cannot be sure that monocytes are not mediators, since T cell preparations from patients contained up to 4% monocytes as determined by peroxidase staining. If these monocytes are responsible for inhibition, they must be effective at very low concentrations since we found that as few as 10% of patient cells mediate inhibition of normal cell migration. Although active suppression occurred, we could not identify a soluble factor in patient T cell supernatants that could account for the inhibition of normal cell migration. This suppressor cell may be similar to the short-acting suppressor cells previously described in other assay systems (31–35), where suppression was also lost after a 37°C incubation. There are several possible explanations for the loss of suppressor activity we observed. First, suppressed migration may be due to the presence of a short-lived suppressor cell, and this loss of suppression after incubation may represent the loss of these suppressor cells (33). It is possible that in our system, suppression is mediated by a short-acting arachidonic acid product. It has been shown that prostaglandin E_2 in concentrations as low as 10^{-8} M suppresses the lymphocyte locomotor response to casein (23). Alternatively, suppressed migration may be due to tumor-derived factors such as tumor antigen-antibody complexes bound to the cell surface of T lymphocytes (36). Such complexes could be shed after overnight incubation, releasing the T cell population from the suppressed locomotor responses shown before culture. It is also possible that surface-bound tumor antigen-antibody complexes not only suppress T lymphocyte locomotion but that they induce a population of suppressor cells that can regulate the migration of lymphocytes whose surfaces are not bound to tumor antigen-antibody complexes. Parrott and co-workers have described a heterogeneity of locomotion to casein in human peripheral blood T lymphocyte subpopulations (19). T lymphocytes with receptors for the Fc portion of IgG ($T\gamma$) did not move in response to casein, whereas T lymphocytes with receptors for the Fc portion of IgM ($T\mu$) and those without receptors for the Fc portion of IgM or IgG ($T\phi$) moved very well toward casein. It is interesting that, as described by Pichler et al. (37), overnight incubation of $T\gamma$ at 37°C in the presence of immune complexes results in a population shift from $T\gamma$ through $T\phi$ to $T\mu$. Such a shift after culture for 24 h could also explain the loss of suppression in the lymphocyte migration assay after overnight culture of cancer patient T cells.

Gupta and Tan have speculated that with a large population of $T\gamma$ in the peripheral blood, as is found in patients with Hodgkin's disease (38, 39), the T lymphocyte response to casein may be diminished. These authors have proposed that the maldistribution of nonresponsive subpopulations of T cells is responsible for the depressed locomotor behavior of the peripheral blood T lymphocytes. If such were the case in the patients with cancer studied here, a mixture of normal peripheral blood T cells with T cells from patients with cancer should still show normal migration, since a large enough population of $T\mu$ cells would be present and their migration measurable. As we have shown in our system, normal migration was not observed when normal and cancer patient lymphocytes were mixed, implying that decreased migration is the result of an active suppression and not simply because of the absence of responding cells.

In summary, our data suggest that suppressed T lymphocyte migration is a generalized phenomenon in patients with established neoplastic disease. This suppressed migration may be attributable in part to the action of a suppressor cell population. Moreover, the observed suppressor activity may partially explain the decreased cellular immunity observed in malignant states.

Acknowledgments

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