Phytohaemagglutinin-induced lymphocyte transformation in patients before and after resection of large intestinal cancer

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SUMMARY Lymphocyte responses to stimulation with phytohaemagglutinin (PHA) have been compared in 20 patients with large intestinal cancer, 10 patients whose large intestinal cancers have been removed, and in 30 age- and sex-matched control individuals. Responses were markedly reduced in the tumour-bearing patients and were unaffected by substituting normal for autologous plasma during incubation. Responses, however, were normal after tumour resection suggesting that the defect in lymphocyte responsiveness is a reversible one and related to the presence of tumour tissue.

There is now considerable evidence that immune reactivity is altered in patients with cancer, thus poor responses have been found to the intradermal injection of tuberculin (Hughes and MacKay, 1965) and mumps antigen (Logan, 1956) and to skin testing with dinitrochlorobenzene (Schier, Roth, Ostroff, and Schrift, 1956), whilst by contrast lymphocytic reactivity to cancer cells has been similarly described for a wide variety of tumours (Hellström, Hellström, Pierce, and Yang, 1968). Few studies have been made of individual types of cancer and intestinal tumours would seem to be of particular interest. A circulating antigen, found also in extracts of foetal colonic mucosa (CEA) has been especially well described in large intestinal cancer (Gold and Freedman, 1965a and b, 1967). The levels of antigen appear to be related to the amount of tumour present (Lo Gerfo, Lo Gerfo, Herter, Barker, and Hansen, 1972), and usually fall after its removal (Laurence, Stevens, Bettelheim, Darcy, Leese, Turberville, Alexander, Johns, and Neville, 1972). Furthermore, cytotoxicity of autologous lymphocytes for colonic tumour tissue has also been demonstrated (Nairn, Nind, Guli, Davies, Rolland, McGiven, and Hughes, 1971). We have therefore sought to explore cellular immune re-activity in patients with large intestinal cancer in more detail, and have compared the responses to stimulation with PHA of lymphocytes from patients before and after removal of the tumour with those of age- and sex-matched controls.

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Method

Heparinized venous blood samples were obtained from patients with large intestinal cancer, from patients whose large intestinal tumours had been resected, and from control patients with non-neoplastic illnesses or from normal volunteers, but matched for sex and within decennial age groups. Red cells were removed by the addition of dextran 70 in a ratio of 1:3 and allowing the blood to stand for 30 minutes in an incubator at 37°C before removing the supernatant plasma and buffy coat layer. This was centrifuged at 220 *g* for five minutes, the supernatant removed and retained for use in the incubation media, and the cell button resuspended in TC 199 medium and respun as before. The supernatant was removed and discarded and the cells were resuspended in 1 ml of medium. A 50 μ l aliquot was then removed and the lymphocyte content calculated and by reference to this the concentration of the remainder adjusted to approximately 4000 lymphocytes per cmm. In cultures 0.4 ml of the lymphocyte suspension was mixed with the same volume of plasma (obtained by centrifuging the original supernatant fluid at 2200 *g* for 10 minutes), 0.1 ml of a 1 in 5 dilution of PHA (in TC199 medium), and 0.6 ml of warmed TC 199 medium, save only one tube which acted as a background control and to which 0.7 ml of TC 199 medium was added, but no phytohaemagglutinin. In each experiment cells and plasma were taken from a patient and a matched

control, and set up so that cells were incubated in autologous and homologous plasma for 72 hours at 37°C. After this time 0.5 μ Ci of tritiated thymidine was added to each tube (specific activity 2 Ci/mM) and incubated for 60 minutes at 37°C before harvesting. During this hour 20 μ l of cell suspension was removed from each tube and examined microscopically in an equal volume of Trypan Blue to assess cell viability. Any cultures where 20% or more of cells had taken up the dye were discarded.

At the end of incubation tubes were centrifuged at 2300 *g* at 0°C for five minutes, the supernatant was removed and discarded, leaving the cell pellet undisturbed and 3 ml of ice-cold 0.85M perchloric acid added and mixed. The precipitate thus produced was washed twice with 3 ml aliquots of perchloric acid, incubated at 70°C for 30 minutes, and 0.5 ml 2M potassium dihydrogen phosphate added to neutralize the perchloric acid before recentrifugation. Of the supernatant, 1.5 ml was then added to 15 ml of Xylene-Triton-PPO scintillation fluid in a counting vial and the radioactivity was measured in a liquid scintillation counter.

All results are expressed as the mean of duplicate or triplicate cultures in disintegrations per minute and converted to the logarithmic scale to compensate for their skewed distribution.

Blood samples were obtained from 20 patients with tumours of the colon or rectum, all being proven subsequently at operation. Blood samples were also obtained from 10 patients with cancer of the colon or rectum who had had successful resections from two weeks to 108 months previously (median six months). None of them showed clinical evidence of recurrence of the tumours. Six patients were tested both before and after operation. The control group were matched for age and sex, and consisted of three healthy volunteers and 27 hospital inpatients suffering from non-malignant diseases such as venous thrombosis, ischaemic heart disease, cerebrovascular disease, peptic ulcer, and diverticulosis. All people both in the cancer and control groups were in reasonably good general health. None had received immunosuppressive therapy or blood transfusions and none had evidence of active infection. The mean age of the cancer patients (57.5) was slightly greater than the mean age of the controls (52.5) but not significantly so.

Results

Figure 1 compares the results obtained for tumour-bearing patients with those of the normal controls, where patients' cells and normal cells were incubated in autologous plasma. The ranges shown represent the mean values $\pm$ twice the standard error. Thy-

Fig 1 PHA-induced lymphocyte transformation in patients with large intestinal cancer (mean $\pm$ 2 SEM) ($P < 0.001$).

PCPP, patients' cells incubated in cancer plasma; NCNP, normal cells in non-cancer plasma.

midine uptake was considerably greater in normal cells than in patients' cells, and using a paired *t* test for statistical comparison the difference was highly significant ($P < 0.001$).

Five tumours were found to be unresectable at operation and thymidine uptake was on average slightly lower in lymphocytes obtained from these patients than in the remainder of the cancer group, but the values obtained were scattered throughout the cancer range (fig 1) and the overall mean differences were not statistically significant.

Crossover experiments in which control and patients' cells were incubated in both control and patients' plasma did not reveal any consistent differences that would suggest the presence of a plasma-inhibiting factor (see table), thus there was if anything, a slight increase in thymidine uptake by normal cells in the presence of patients' plasma.

Figure 2 compares the results obtained in cancer

	Normal Cell in Non-cancer Plasma	Normal Cells in Cancer Plasma	Patients' Cells in Non-cancer Plasma	Patients' Cells in Cancer Plasma
Mean log disintegration per minute	4.1045	4.1737	3.6891	3.5946
SEM	±0.0993	±0.0760	±0.0996	±0.0944

Table Comparison of the effect of cancer plasma and non-cancer plasma on PHA-induced lymphocyte transformation

Fig 2 PHA-induced lymphocyte transformation in patients after tumour resection (mean $\pm$ 2 SEM). PCPP, patients' cells in cancer plasma; NCNP, normal cells in non-cancer plasma.

patients after tumour resection with those of their matched controls. Results were all within the normal range except in one patient. She had had a resection only two weeks previously so that this low value may reflect the persistence in her circulation of abnormal lymphocytes that were present before operation. However, even including this value there was no significant difference in the mean thymidine uptake of lymphocytes from the controls and patients after resection.

Thymidine uptake was measured both pre- and postoperatively in six patients. There was a marked increase in uptake postoperatively in four of them. In two patients, however, it was if anything, slightly lower and may suggest the presence of recurrent tumour, although at the time there was no clinical evidence for this.

Discussion

These results clearly show that, by comparison with those obtained from non-cancer controls, lymphocytes from patients with cancer of the large intestine respond poorly to stimulation with phytohaemagglutinin. The difficulty of matching a patient to a control with the same 'degree of illness' is obvious, but we have attempted to do this in each case by selecting only those whose general health appeared good, without excessive weight loss or anaemia, and who had neither received immunosuppressive therapy nor blood transfusions. Despite this matching we cannot be absolutely certain that the depressed lymphocyte responses which we have found are simply due to the presence of tumour tissue, but it seems the only likely explanation. The ability to respond to PHA can be regarded as a measure of cell-mediated immunity and our findings therefore appear to suggest that this is defective in patients with large intestinal cancer. Furthermore, as the poor response was unaffected by substituting normal for cancer plasma the defect is probably in the lymphocytes themselves.

Similar findings have been reported in unselected groups of patients with a wide variety of tumour types (Garrioch, Good, and Gatti, 1970; Whittaker, Rees, and Clark, 1971; Hagan, Frøland, and Weberg 1971), although previous work with intestinal cancer has yielded somewhat conflicting results. Lejtenyi, Freedman, and Gold (1971) claimed that lymphocyte responses to PHA were normal in colonic cancer patients, though examination of their data suggests that tritiated thymidine uptake was, in fact, reduced on average by half in the small group of patients examined. These findings contrast with those obtained in a mixed group of cancer patients, including some with colonic cancer in whom the proportions of transformed cells differed little, if at all, from normal values (Al-Sarraf, Sardesai, and

Vaitkevicius, 1971). The significance of these results is hard to assess because of the mixed varieties of cancer studied and because in the same investigation cell transformation was inhibited markedly by allogeneic cancer or non-cancer plasma, an effect which we and others have been unable to detect (Golob, Israsena, Quatrale, and Becker, 1969).

Our results using lymphocytes from patients whose bowel tumours had been removed showing almost normal responses to stimulation with PHA suggest that the defect in lymphocyte function is a reversible one and dependent on the presence of tumour tissue. It is of interest that Hughes and MacKay (1965) have reported a return of strong tuberculin sensitivity after apparently curative removal of bronchial carcinoma, but no such data have been reported for intestinal cancer.

Diminished lymphocyte reactivity to PHA in the cancer-bearing patients contrasts with the cell-mediated immunoreactivity which can be shown by other techniques. Thus growth of colonic cancer target cells *in vitro* is inhibited by previous exposure to lymphocytes from tumour-bearing patients (Hellström *et al*, 1968) and lymphocytotoxicity for colonic cancer cells can be demonstrated (Nairn *et al*, 1971). Delayed hypersensitivity reactions can also be produced by intradermal injections of soluble fractions obtained from the membranes of tumour cells (Hollinshead, Glew, Bunnag, Gold, and Herberman, 1970).

There are at least three possible explanations for our findings. First the ratio of circulating T-dependent to B-dependent lymphocytes may be reduced in tumour-bearing patients, so that our low results might reflect a quantitative rather than a qualitative lymphocyte abnormality. Secondly, such patients may produce a factor or factors which in some way block the effect of PHA, perhaps by interfering with 'PHA receptor sites' on the surface of their lymphocytes. Thirdly, the intensity and duration of the stimulus to the lymphocytes by tumour-specific antigens may be such that their ability to respond to further exogenous stimulation is impaired.

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