may also be a necrotizing leuco-encephalopathy with anterior horn cells of the cord affected.

Prof. Uys: This syndrome of neuropathy in association with a malignant tumour is often seen clinically and confirmed pathologically, but it has not always been possible to demonstrate the specific neuropathological changes.

Prof. Ferguson: I am surprised at the clinical fluctuation of this neuropathy, with regression almost to normal, when one sees the marked pathological changes with demyelination as well as axonal degeneration in this patient. Particularly surprising was the rapid reversal.

Dr Philcox: Structural changes do not seem to correlate well with function, and certainly myelin degeneration is able to recover fully and regenerate fairly rapidly.

REFERENCES

Inhibition of mitogen-induced lymphocyte proliferation by autologous serum in oesophageal carcinoma

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Summary

The reactivity of lymphocytes from patients with oesophageal carcinoma in response to mitogenic stimulation with phytohaemagglutinin was assessed in four culture systems in an attempt to demonstrate an inhibitory effect of cancer serum on lymphocyte transformation. The metaphase index (number of cells at metaphase/1000 live cells) was used to determine lymphocyte reactivity of patients and controls in the presence of: (a) autologous plasma; (b) pooled AB serum; (c) pooled plasma from patients with oesophageal cancer, and (d) fetal calf serum. The division of lymphocytes from cancer patients was significantly depressed in the presence of autologous plasma (P<0.001) when compared with division of lymphocytes of control subjects. No significant difference was evident in pooled AB serum, homologous cancer plasma or fetal calf serum. The depressed lymphocyte reactivity in cancer patients appears to be related to a serum inhibitory factor.


Many patients with cancer manifest nonspecific suppression of cellular immunity which can be improved by successful tumour ablation. Peripheral blood lymphocytes in a variable but significant percentage of patients with cancer respond poorly to mitogenic stimulation in vitro. Depressed lymphocyte reactivity may result from a nonspecific defect in lymphocyte activation or the presence of a serum inhibitory factor. The existence of an inhibitory effect on normal lymphocyte transformation by sera from cancer patients is controversial, however, since serum suppression of lymphocyte reactivity has been found by some investigators but not by others.

As part of another study lymphocytes from patients with oesophageal cancer were cultured to produce dividing cells for chromosome analysis, but it was found that cells failed to divide in autologous plasma following mitogen stimulation. This observation gave impetus to an investigation of in vitro lymphocyte reactivity in response to phytohaemagglutinin stimulation in patients with oesophageal carcinoma. The reactivity of lymphocytes from cancer patients in autologous plasma, homologous cancer plasma, pooled AB serum and fetal calf serum was assessed and compared with that of age-correlated normal subjects.

Materials and methods

Culture systems

The initial cultures of lymphocytes from control subjects and cancer patients were made in Eagle's minimal
essential medium (Grand Island Biological Co., Grand Island, NY, USA) supplemented with 10% serum or plasma. For each culture 4.5 ml of medium was added to a McCartney bottle. The medium was then made up to 5 ml with one of the following: (a) autologous plasma; (b) pooled plasma from cancer patients; (c) pooled AB serum; or (d) fetal calf serum.

Lymphocytes from both cancer patients and control subjects were cultured in all four systems, as follows: (a) medium + autologous plasma (56 patients, 25 controls); (b) medium + pooled homologous cancer serum (17 patients, 25 controls); (c) medium + pooled AB serum (17 patients, 11 controls); and (d) medium + fetal calf serum (57 patients, 25 controls).

Lymphocyte culture technique

Two-millilitre samples of peripheral blood were collected in heparinized syringes (Pularin, 5,000 IU/ml) and allowed to sediment standing vertically for 1½-2 hours at 4°C. The plasma was expelled by carefully depressing the plunger of the syringe. The plasma was then centrifuged at 1,200 rpm for 5 minutes to remove any red blood cells. The leucocytes (buffy coat) and red blood cells were mixed by inverting the syringe gently several times. In the few instances where separation of the red blood cells and plasma did not readily occur, the whole blood sample was centrifuged.

Six drops of blood (red blood cells and buffy coat) were added to each culture system. Phytohaemagglutinin-P (PHA) (Difco, Detroit, Mich., USA) was the routinely used mitogen, and a concentration of 0.25 mg/ml gave optimum results, with the maximum number of cells synthesizing DNA 60-72 hours after exposure to PHA. Cultures were incubated at 37°C for 70 hours; 0.01% colchicine 0.25 ml/dl was then added and incubation continued for a further 2 hours. The cultures were then harvested according to the technique of Hungerford.\(^1\)

The fixed-cell suspensions were dropped onto chilled glass slides, flame-dried and stained with 10% Giemsa in Sorenson’s phosphate buffer, pH 6.8, for 7 minutes.

Determination of lymphocyte reactivity

The slides were examined at a total magnification of 400 × and the number of cells at metaphase (indicated by the presence of spread chromosomes) per 1,000 live cells were counted. The areas counted were randomly selected on each of two slides counted for each sample. The metaphase index so obtained measures only the number of lymphocytes that have divided, and differs from the standard mitotic index since metaphases were hypotonically broken and a metaphase-arresting agent (colchicine) used. As a measure of lymphocyte reactivity the metaphase index also differs from and cannot be equated with other standard techniques using labelled thymidine, since lymphocyte transformation is not estimated. In several instances, numerous transformed cells were present without any evidence of cell division. The metaphase indices of lymphocytes grown in the four culture systems were compared using Student’s t test.

Results

Lymphocyte reactivity of cancer patients

The metaphase indices for patient lymphocytes in each of the culture systems are shown in Fig. 1. Significantly depressed division after PHA stimulation is evident in autologous plasma when compared with pooled homologous cancer plasma ($P < 0.001$), pooled AB serum ($P < 0.001$) and fetal calf serum ($P < 0.001$). When cultured in autologous plasma, over 70% of patient lymphocytes showed complete absence of division, compared with 12% in normal subjects. There was no significant difference between lymphocyte reactivity in pooled cancer plasma, pooled AB serum and fetal calf serum. In many instances numerous lymphoblasts were observed without evidence of cell division when patient lymphocytes were cultured in autologous plasma. This suggests that cell multiplication may merely be retarded and not totally inhibited, or alternatively that although the cells are capable of undergoing blastoid transformation they are incapable of completing cell division.

Lymphocyte reactivity of control subjects (Fig. 2)

Significantly depressed division of control lymphocytes was evident in pooled homologous cancer plasma when compared with fetal calf serum ($P < 0.05$). No significant difference in lymphocyte reactivity was detected when comparing autologous plasma, pooled AB serum and homologous plasma from cancer patients.
Comparison of reactivity of patients and controls
(Fig. 3)

Mitogenic stimulation of patient lymphocytes was significantly depressed in autologous serum in comparison with control subjects ($P < 0.005$). No significant difference was evident in lymphocyte reactivity of patients and control subjects when challenged with PHA in homologous cancer plasma, pooled AB serum and fetal calf serum. Lymphocytes from both control subjects and cancer patients showed the highest rates of division in the presence of fetal calf serum.

![Graph showing comparison of lymphocyte reactivity of cancer patients and controls as measured by the metaphase index (mean ± SE).](image)

Fig. 3. Comparison of lymphocyte reactivity of cancer patients and controls as measured by the metaphase index (mean ± SE).

Discussion

Autologous plasma depressed lymphocyte reactivity in patients with oesophageal carcinoma as measured by mitogenic stimulation with PHA. Similar immunosuppressive activity has been demonstrated in autologous sera following operation, trauma or burns. Since lymphocyte reactivity of patients with oesophageal cancer is abnormal in autologous plasma, the differentiation between an underlying cellular defect and a suppressive effect of the plasma is necessary. A cellular defect is unlikely because lymphocyte reactivity of cancer patients was significantly improved in pooled AB serum and fetal calf serum, and in those media did not differ from that of control subjects. However, there is marked variation in normal lymphocyte response to mitogens in standardized media; it is therefore important to establish the relationship of the reactivity of normal lymphocytes in various homologous culture systems to their reactivity in autologous plasma. Although the response to PHA is generally maximal after 72 hours, the lymphocytes of patients with tumours may divide later than those of control subjects.

The stimulatory effect of PHA on lymphocytes is dose-dependent and the interaction of PHA with serum constituents may partly account for the variable responses of lymphocyte populations to specific PHA concentrations. Mangi et al. have shown a tenfold variation in the response of lymphocytes from a single individual in different samples of non-pooled homologous plasma, compared with a twofold variation in pooled plasma. To standardize results in the present investigation, therefore, pooled oesophageal cancer patients' plasma, pooled AB serum and fetal calf serum were used for constant reference. The lymphocytes of cancer patients showed markedly depressed reactivity in autologous plasma, although in many instances numerous lymphoblasts were present. This would suggest that lymphocyte division may have been retarded and not totally inhibited, or alternatively that cells may recognize and respond to the mitogen but are incapable of completing division. Binding of PHA does not in itself activate the transducer of the mitogenic signal, and blocking of binding sites is an unlikely explanation for depressed lymphocyte reactivity, since this could be improved by changing the culture system. Mannick et al. demonstrated an improvement of lymphocyte responsiveness to PHA stimulation after repeated washing in vitro. This would suggest that the non-specific defect in lymphocyte activation in patients with cancer may be caused by suppressive factors loosely associated with the cell membrane.

PHA-induced blastogenesis is impaired in patients with oesophageal cancer, and our investigation would suggest the presence of a serum inhibitory factor. An immunosuppressive peptide fraction which has been shown to cross species barriers and inhibit T-cell mediated immune responses without affecting B-cell responses had been isolated from the sera of cancer patients. Lymphocyte reactivity in the presence of autologous cancer serum is inversely related to the extent of the disease, and serum blocking activity is commingled in divided tumour. Other factors such as a decrease in T-cell numbers, protein energy malnutrition and infection with oncogenic viruses may also contribute to the impaired lymphocyte reactivity in cancer patients. The depression of lymphocyte reactivity by autologous serum represents one factor contributing to immunosuppression in patients with oesophageal carcinoma.

This study was supported by the National Cancer Association of South Africa.

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