Lymphocyte Stimulation by Phytohemagglutinin and Tumor Cells of Malignant Effusions

E. Robinson,1 S. Sher, and T. Mekori2

Department of Oncology, Rambam University Hospital, and the Aba Khoushy School of Medicine in Haifa, The Technion, Haifa, Israel

SUMMARY

Lymphocyte cultures from 15 cancer patients and 15 normal controls were stimulated by phytohemagglutinin (PHA) and by tumor cells obtained from malignant effusions. Seventy-three % of the cancer patients did not react to PHA, compared to only 7% nonresponding controls. Lymphocytes from cancer patients were not stimulated by their own tumor cells in mixed cultures as opposed to lymphocytes from 60% normal controls that reacted to the allogeneic tumor cells. Control lymphocyte cultures from patients with localized solid neoplasm were stimulated by allogeneic tumor cells.

When PHA was added to the patients' mixed lymphocyte-tumor cultures, an increase in the thymidine uptake occurred in 20% of the cultures, which is similar to that obtained in the patients' lymphocyte cultures stimulated by PHA alone (27%). The addition of PHA to the mixed cultures of the normal controls raised the percentage of reacting cases from 60 to 73%.

These results support the view that lymphocytes of cancer patients in an advanced stage of disease mount a poor reaction toward their specific tumor antigens and to nonspecific stimulants such as PHA. The possible explanation for these findings is discussed.

INTRODUCTION

The host-tumor relationship is now extensively investigated in many centers. Much evidence has been accumulated regarding the host defense mechanism against neoplastic disease which is of immunological nature. The lymphocytes play an important role in this cell-mediated immune response (7, 12). Sensitized lymphocytes to a target cell kill these cells when cultured together. Nonsensitized lymphocytes are cytotoxic to the target cells only when cultured together with PHA. This phenomenon was interpreted as an immunological reaction or attributed to the so-called allogeneic inhibition (5, 6, 8).

The purpose of the present investigation was to test the effect of cells obtained from malignant effusions on cultured lymphocytes obtained from tumor-bearing patients or normal donors with and without the addition of PHA.

MATERIALS AND METHODS

Thirteen patients with ascites and 2 patients with pleural effusion were studied.

Preparation of Lymphocytes. Peripheral blood, 50 ml, was collected from controls and cancer patients, and the lymphocytes were separated following a technique described by Ben Ezra and Hochman (1).

Tumor Cells. The cells were obtained sterilely from abdominal or pleural fluid punctures. All patients had cytologically proven malignant cells in the effusions. The fluid was centrifuged at 700 rpm for 7 min. The supernatant was discarded and the tumor cells were transferred to centrifuge tubes and washed 3 times with Medium M 199, which contained 15% fetal calf serum and was supplemented with 100 units penicillin and 100 µg streptomycin per ml. The viability of the cells was checked by 0.2% trypan blue staining; 90% of the cells used were viable. Mitomycin C, 0.05 mg/ml, was used for 30 min to inactivate the tumor cells. After this, the cells were washed twice.

The MLTC was made by mixing the inactivated tumor cells with lymphocytes obtained from the tumor cell donor and normal donors.

The MLTC with lymphocytes and tumor cells was carried out in different lymphocyte : tumor ratios at ranges of 1:2, 1:1, 2:1, 5:1, 10:1, and 20:1. The ratios were obtained by keeping the number of lymphocytes constant at 1 × 10^6 cells/culture tube, adding tumor cells in decreasing concentrations but keeping a constant volume of 2 ml in each culture. As a control for the specific lymphocyte stimulation by tumor cells from effusions, lymphocytes from other cancer patients were stimulated by autologous tumor cell obtained from operative specimen. For nonspecific stimulation of lymphocytes, cultures with 0.05 ml PHA (Wellcome Research Laboratories, Beckenham, England) were set up. All the combinations were set up in triplicate and incubated at 37° with 5% CO₂ atmosphere for 5 days in Medium 199. Lymphocyte transformation was tested by the incorporation of 2 µCi tritiated thymidine indicating DNA synthesis during 3 hr of incubation.
RESULTS

The results for stimulation of normal lymphocytes and patients' lymphocytes to PHA and tumor cells are represented in Table 1.

A high spontaneous transformation in cultures of normal lymphocytes was observed in 4 of the 15 controls (27%), whereas lymphocytes from the cancer patients did not show such a spontaneous transformation.

The lymphocytes from only 4 out of 15 patients (27%) reacted to PHA stimulation (Tables I and 2) (the same percentage observed as high spontaneous transformation in the cultures of normal lymphocytes). The addition of PHA to the cultures of the normal controls increased the number of responders to 14 cases (93%). (We considered as a negative reaction results < 1000 dpm.)

The MLTC from effusions were set up in different lymphocyte:tumor ratios, as described above. There was no substantial reaction in lymphocyte:tumor dilutions of 1:2 and 1:1; whereas in the 2:1, 5:1, 10:1, and dilutions, a nonchangeable positive reaction was obtained. Therefore, the results are given for the lymphocyte:tumor cell ratios with 2:1 as a representative dilution.

The results of the MLTC show that none of the 15

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<tr>
<th>Table 1</th>
<th>The results of lymphocyte transformation to PHA and to tumor cells</th>
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<td><strong>Case</strong></td>
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* Spontaneous transformation.

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patients studied reacted to the autochthonous tumor cells (100%). The addition of PHA to the mixed cultures stimulated the lymphocytes in 3 out of 15 patients (20%). When lymphocytes from healthy donors were cultured with autologeneic tumor cells, 9 out of 15 showed increase in thymidine uptake (60%). The addition of PHA increased the percentage of reacting cases to 11 (73%).

Of the 9 MLTC (with tumor cell from localized solid tumors) we found in 4 a stimulation of lymphocytes by the tumor cells. The lymphocytes of those patients reacted to PHA like the lymphocytes of normal donors.

DISCUSSION

Lymphocyte stimulation in culture has been a useful technique in the study of cellular immunity to human-tumor associated antigens.

Fossati et al. (3) developed a technique to study the cellular immunological response of breast cancer patients against tumor antigens. They reported that the lymphocytes of the peripheral blood of 3 out of 5 patients had a specific killing effect on autochthonous tumor cells. The lymphocytes of 9 out of 13 other patients killed at least 1 of the target tumor cell lines. Additional evidence for existence of cellular reactivity was observed by Hellström, who studied 13 breast cancers and other tumors and found a specific killing effect of the patients’ peripheral lymphocytes on autologous and allogeneic tumor cells growing in vitro (4). Lymphocytes from cancer patients have been shown to undergo blast transformation and to incorporate increased amounts of tritiated thymidine when cultured with autologous tumor cells (14). In the 15 patients that we have examined, we were surprised to find no increase of DNA synthesis in the MLTC. Therefore, lymphocytes from patients with localized solid tumors were stimulated by autologous tumor cell. In 4 of the 9 cases studied, stimulation was found. The lymphocytes of those patients reacted to PHA as lymphocytes from normal donors. A possible explanation to the finding with tumor cells from effusions is that all the examined patients suffered from metastatic carcinoma. It is known that in patients with advanced disease, and after radiotherapy and chemotherapy, the lymphocytes react less to PHA (10) and produce a smaller reaction when tested by the lymphocyte transfer test (11).

Anyway this is the reason why no specific stimulation was obtained in the patients’ lymphocytes to the autochthonous tumor cells. Another explanation may be the decrease in the antigenicity of tumor cells in effusions not found in cases of malignant solid tumors (12). It may also be that in effusions there exists a factor inhibiting lymphocyte stimulation, a factor that was not eliminated by the 3 washings of the tumor cells. Previously, we have found that, in the ascitic fluid of mice with tumor, there exists a factor that prolonged the survival of allograft (2).

It has been reported (6) that PHA facilitates the interaction between lymphocytes and target cells. We therefore added PHA to the MLTC. The results show (Table 1) that the addition of PHA to the MLTC increases the thymidine uptake in some of the normal cases but decreases the uptake in other controls.

The addition of PHA to the MLTC of the patients resulted in a positive reaction in 3 cases only (20%). This percentage is almost identical to that obtained in the patients’ lymphocytes cultured with PHA (27%). It is therefore correct to believe that lymphocytes of cancer patients in an advanced stage of disease fail to react to specific and nonspecific stimuli.

REFERENCES

Lymphocyte Reactivity to PHA and Tumor Cells

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Cells of Malignant Effusions

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