Microcytotoxicity Assays of Tumor Immunity in Patients with Bronchogenic Carcinoma Correlated with Clinical Status

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SUMMARY

Peripheral blood lymphocytes from patients with bronchogenic carcinoma were tested in microcytotoxicity assays against cultured bronchogenic cancer cells, other types of tumor cells, and skin fibroblasts. Lymphocytes from patients who were postresection with no clinical evidence of residual or recurrent tumor were more frequently toxic against bronchogenic carcinoma than were lymphocytes from normal donors or from patients with clinically evident disease. Lymphocytes from patients with minimal or no tumor were more frequently toxic against bronchogenic cancer than against skin fibroblasts. Serum samples from a few patients rendered lymphocytes toxic for bronchogenic cancer cells, but this serum activity could not be correlated with the patient's clinical status.

INTRODUCTION

Microcytotoxicity assays have been used extensively to search for cell-mediated immune reactions against human tumors. Many investigators (1–3, 7, 8, 11–13, 21, 34) have demonstrated tumor-specific reactions with these assays. Peripheral blood lymphoid cells kill or inhibit the growth of autochthonous tumors or allogeneic tumors of the same histological type but do not inhibit the growth of normal fibroblasts or tumors of a different tissue of origin. Recently, however, other investigators have found a high incidence of toxic reactions with lymphocytes from normal individuals and have observed nonspecific toxicity with lymphocytes from cancer patients (18, 20, 22, 30, 32, 33).

Whether or not these assays might be used clinically to aid in prognosis or to monitor therapy remains undetermined. Studies of human bladder carcinoma, however, suggest that there is a correlation between the results of microcytotoxicity assays and the clinical stage of disease. Lymphocytes from patients with superficially invasive bladder carcinomas appear to be more frequently reactive than lymphocytes from patients with advanced disease (2, 23, 24).

In this study, lymphocytes from patients with bronchogenic carcinoma were tested against cultured bronchogenic carcinoma cells and against control target cells, i.e., other types of tumors and normal fibroblasts. The effects of patient serum samples on the lymphocyte-mediated reactions were also studied and attempts were made to correlate the results with the patient's clinical status.

MATERIALS AND METHODS

Patients. Only patients with an established tissue diagnosis of bronchogenic carcinoma were included in this study. This group of 46 patients ranged in age from 39 to 84 years with a mean of 60 years. Eighty % were males and 20% were females. The patient's histological tumor types included epidermoid carcinoma (36%), adenocarcinoma (39%), and small- and large-cell undifferentiated carcinoma (25%). At the time tested all patients were classified according to their clinical status: Status A, postresection, no evidence of disease; Status B1, preoperative; Status B2, postresection with residual or recurrent disease; or Status B3, nonoperative. The patient distribution according to histological tumor type and clinical status when studied is presented in Table I. None of the patients received chemotherapy prior to testing. No Status A patient and only 1 Status B1, I Status B2, and 4 Status B3 patients were irradiated prior to testing.

Normal Volunteers. This group included 39 medical students and laboratory technicians with no previous history of blood transfusion or serious medical illness. The volunteers ranged in age from 16 to 59 years with a mean of 30 years. Seventy % were males and 30% were females.

Lymphocytes and Sera. Lymphocytes were separated from fresh heparinized venous blood by a modified Ficoll-Hypaque technique. Leukocyte-rich plasma was first separated from RBC by sedimentation with plasmagel (Roger Bellon, Paris, France) 8 ml/40 ml blood, at 37° for 30 to 45 min and then centrifuged at 250 x g for 15 min at 37°. The cell pellets were suspended in 30 ml of Dulbecco's phosphate-buffered saline (Grand Island Biological Co. Grand Island, N.Y.), divided into 2 aliquots of 15 ml each, layered onto 15-ml Ficoll-Hypaque cushions with a specific gravity of 1.078 in 50-ml tubes and centrifuged at 300 x g for 40 min. The cell pellets were washed twice in Waymouth's medium (MB-752/1, Grand Island Biological). Smears of
lymphocytes, 5 to 20 monocytes and/or atypical large
the final preparations revealed 75 to 95% typical small
lymphocytes, and 0 to 5% polymorphonuclear leukocytes.
Viability counts ranged from 98 to 100% as determined by
trypan blue exclusion, and the final concentrations were
adjusted to 7.5 x 10^6 cells/ml Waymouth's medium.

Fresh serum samples were obtained from clotted blood on
the same day that the lymphocytes were drawn. Serum
samples were heat inactivated at 56° for 30 min.

**Target Cells.** Fresh sterile pieces of either primary or
metastatic bronchogenic carcinoma were obtained at operation,
wnched and debrided of necrotic tissue, and finely
minced with scissors. One portion of the minced tumor was
tripsinized, and the single-cell suspensions were then cul-
tured at concentrations of 2 to 4 x 10^6 cells/ml in
Waymouth's medium containing penicillin and strep-
tomycin and supplemented with sodium pyruvate, L-gluta-
mime, nonessential amino acids, and 30% heat-inactivated
fetal calf serum in 30-ml plastic tissue culture flasks. A few
drops of medium were mixed with another portion of the
tumor mince to permit layering of fine pieces into culture
flasks. After 24 to 48 hr the pieces became adherent to the
flasks and were then covered with additional medium.
Tumor cells were also obtained from cytology-positive
pleural and pericardial effusions. The fluid was mixed 1:3
with Waymouth's medium. The diluted sera were decanted prior to
incubation for 45 mm with heat-inactivated normal volunteer
serum. The target cells in other groups of wells were
preincubated for 45 mm with Waymouth's medium. The diluted sera were decanted prior to
preparation of the target cells. A majority of the cells became attached to the bottoms of the
wells, and on the following day the medium containing
nonattached cells was decanted.

The cultures were used in the assay as soon as possible
after the 1st subculture with 1 exception, Tumor HT-035, a
melanoma kindly supplied by Dr. G. Heppner. Cultures
were not maintained for periods longer than 6 weeks; for
subsequent use they were frozen in dimethyl sulfoxide and
stored in liquid nitrogen. All target cells were cultured for
*Mycoplasma* and, if positive, were not used in the assays.
The histological types and sources of bronchogenic carcino-
mas used as target cells are presented in Table 2.

**Microcytotoxicity Assays.** The Hellström microcytotox-
icity assay (13) was used in these studies.

Cultures of rapidly growing target cells that had not yet
reached confluence were trypsinized and added to the wells
of Falcon No. 3040 Microtest Plates in amounts of 150 to
300 cells/well depending upon plating efficiency. Overnight
a majority of the cells became attached to the bottoms of the
wells, and on the following day the medium containing
nonattached cells was decanted.

Target cells in certain wells were preincubated for 45 min
with Waymouth's medium with or without 20% fetal calf
serum. The target cells in other groups of wells were
incubated for 45 min with heat-inactivated normal volunteer
and cancer patient serum samples diluted 1:6 in Way-
mouth's medium. The diluted sera were decanted prior to
addition of the lymphocytes. Groups of wells treated
throughout the test with medium only (i.e., no lymphocytes
added) were included. In all studies a minimum of 8
replicate wells were included for each different group. After a 2-hr incubation, heat-inactivated normal AB+ human serum was added to a final concentration of 10%. Following an additional 40-hr incubation, the plates were washed and stained with crystal violet and the surviving (i.e., attached) target cells were counted with stereoscopic magnification. Each well was identified by a code which was broken only after completion of the counts. For each replicate group of wells the TCC* and standard error were calculated. The statistical significance of differences between the TCC’s of 2 different groups was determined by Student’s t test.

For the purposes of this report an “experiment” is defined as the study of the effects of a group of lymphocyte samples, which generally included 2 normal and 1 to 3 patient samples collected and tested on the same day, against 1 to 4 different target cultures. A “test” is defined as the study of these lymphocyte samples against each separate individual target. Most of the tests were comprised of 2 separate normal lymphocyte-target cell “combinations”, and 1 to 3 patient lymphocyte-target cell combinations, i.e., a total of 3 to 5 lymphocyte-target cell combinations. Thus each experiment was comprised of 1 to 4 tests with 3 to 5 lymphocyte-target cell combinations per test. The results of all technically successful experiments are included in this report. Only 4 experiments were excluded.

In this study the lymphocyte sample from the normal volunteer that gave the highest TCC was arbitrarily designated the base-line or control lymphocyte sample. The effects of other lymphocyte samples were compared to this base-line sample, and toxicity was defined as a greater than 20% reduction in TCC after exposure to a lymphocyte sample compared to the base-line sample significant at least at a level of p ≤ 0.05 as determined by Student’s t test.

The toxicity of a lymphocyte sample was calculated from the TCC obtained after exposure to the lymphocyte sample compared to the base-line lymphocyte sample following a 45-min preincubation with Waymouth’s medium. The effects of normal volunteer and patient serum samples on the reactivity of any given lymphocyte sample were assessed by comparing the TCC obtained following preincubation with the serum sample with that obtained following preincubation with medium and other serum samples and subsequent exposure to the lymphocyte sample. The results of a typical experiment are presented in Table 3.

A total of 74 lymphocyte and serum samples from the 46 lung cancer patients were tested with 94 lymphocyte and serum samples from the 39 normal volunteers in a total of 54 experiments. The cultures of bronchogenic cancers used as targets included 2 epidermoid carcinomas, 7 adenocarcinomas, and 6 undifferentiated carcinomas (Table 2).

RESULTS

Effects of Lymphocytes. Toxicity was observed in 39 of 76 (51%) of patient lymphocyte-bronchogenic cancer combinations. A lower incidence of toxicity was observed with patient lymphocytes when tested against other types of tumors; i.e., reactivity was observed in 16 of 44 (36%) of patient-lymphocyte-target cell combinations in which the target cells were tumors other than lung cancer, but this difference is not statistically significant. A definitely lower frequency of toxicity was observed with patient lymphocytes against skin fibroblasts than against bronchogenic cancer. Reactions were observed in only 15 of 63 (24%) of patient lymphocyte-fibroblast target cell combinations. This difference is significant at p < 0.001 by the standardized Z test.

In contrast, lymphocytes from normal volunteers were not more frequently toxic on bronchogenic cancer than on other cells. Toxicity occurred in 6 of 35 (17%) of normal lymphocyte-bronchogenic cancer target combinations, in 3 of 20 (15%) normal lymphocyte-"other tumor" combinations, and in 5 of 26 (19%) normal lymphocyte-fibroblast combinations. Since the normal volunteer lymphocyte sample that permitted the higher TCC was arbitrarily designated as the base-line sample, only the other member of a pair of normal lymphocyte samples could be analyzed for "toxicity" in any 1 test. Nevertheless, these values for the incidence of toxicity with normal lymphocytes are similar to those previously reported from this laboratory (26) in which the lymphocyte sample that permitted the highest TCC, whether from normal or patient, was designated the base-line sample. With this definition of a "base line," toxicity was observed in 30 of 191 (16%) of normal lymphocyte-tumor and in 19 of 94 (20%) of normal lymphocyte-fibroblast combinations with lymphocytes separated by Ficoll-Hypaque.

Lymphocytes from patients with clinical Status A, i.e., those patients who were postresection with no evidence of tumor, were more frequently reactive than lymphocytes from patients with clinical Status B, i.e., those who were either preoperative, postoperative, or nonoperable with clinically evident disease. Toxicity was observed in 75% (30 of 40) of patient lymphocyte-bronchogenic cancer combinations in which lymphocytes were obtained from status A patients. With status B patients, toxicity was observed in only 25% (9 of 36) of lymphocyte-bronchogenic cancer combinations. The probability that this difference is due only to chance is less than 0.001 by the standardized Z test. As shown in Table 4, this increased level of reactivity with Status A patient lymphocytes was not completely specific for lung cancer cells, since there was also a higher level of reactivity against other types of tumors, i.e., 53% versus 23% and against fibroblasts, i.e., 34% versus 13%, with lymphocytes from Status A patients versus those from Status B patients.

The patient’s histological type of tumor had little effect on the pattern of lymphocyte reactivity with 1 exception. Status B patients with adenocarcinoma were less frequently toxic against bronchogenic carcinoma than were Status B patients with either epidermoid or undifferentiated carcinomas. No toxicity was observed in 16 lymphocyte-bronchogenic cancer combinations with lymphocytes from patients with adenocarcinomas (Table 4).

In the studies of 4 patients from whom at least 3 different
lymphocyte samples were tested over a period of 6 or more months, fairly consistent patterns of reactivity were observed against bronchogenic carcinoma cells (Table 5). For example, Patient K. T. was nonreactive preoperatively but was toxic in each of 3 experiments postresection when tested against 3 different bronchogenic carcinomas. The results obtained with Patient D. W. are of interest. On 3 different occasions during the 1st 2 postoperative months, when this patient had no evidence of disease, lymphocytes were toxic against the patient’s own bronchogenic carcinoma and against autochthonous skin fibroblasts. Six months later with no evidence of disease, reactivity against the fibroblasts was lost but toxicity against the tumor persisted. At 16 months after resection the patient had demonstrable metastatic disease, although the patient’s lymphocytes remained toxic for tumor and nontoxic for fibroblasts.

**Effects of Patient Serum on Lymphocyte Reactivity.**

Patient serum samples tested alone in the absence of lymphocytes did not affect the survival of target cells when compared with normal volunteer sera or with Waymouth’s medium. However, a few patient serum samples altered the reactivity of lymphocytes on target cells as previously reported (Ref. 27; Table 6).

Fourteen of 76 patient serum samples selectively increased the survival of a target culture incubated with lymphocytes. Preincubation with these sera significantly increased (greater than 20%; \( p \leq 0.05 \) or less) the TCC of 1 of the targets after exposure to a certain lymphocyte sample when compared to the TCC obtained with the same lymphocyte sample following preincubation with medium or with other patient or normal volunteer serum samples. The effect was usually observed against only 1 of several target cells tested in any given experiment and was observed with only 1 or 2 rather than with all lymphocyte samples tested. The effect was observed with normal as well as with patient lymphocytes and was observed against 7 bronchogenic cancer targets but it was also observed against 3 other types of tumor targets and against 4 fibroblast cultures. Similar results were obtained with 4 of 94 normal volunteer serum samples.

Of greater interest were reductions in TCC’s observed on exposure to lymphocytes after preincubation with certain patient serum samples compared to preincubation with medium or other serum samples. This effect was observed only with patient sera and only against bronchogenic cancer targets (Table 6). Seven of 74 patient serum samples

### Table 3

**Results of Experiment 4/4/74: microcytotoxicity assays of normal and patient lymphocyte reactivity against cultured bronchogenic cancer cells and autochthonous skin fibroblasts following preincubation of target cells with Waymouth’s medium or with dilutions of normal and patient sera**

<table>
<thead>
<tr>
<th>Test*</th>
<th>Target cells</th>
<th>Lymp-</th>
<th>TCC/well after preincubation with medium or serum from:</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>hone-</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>hode</td>
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</tr>
<tr>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
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<td>HT115*</td>
<td>M*</td>
</tr>
<tr>
<td></td>
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</tr>
<tr>
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<td></td>
<td>N2*</td>
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<td>M</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>N1*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>N2*</td>
</tr>
<tr>
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<td></td>
<td></td>
<td>P1*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P2*</td>
</tr>
</tbody>
</table>

* A "test" is defined as that portion of an experiment in which the serum and lymphocyte samples were assays against 1 specific target culture.

* Lymphocyte-target cell combination refers to the assay of 1 lymphocyte sample against 1 target.

* Serum samples were heat inactivated at 56° for 30 min and diluted 1:6 in Waymouth’s medium for use in the assays.

* Groups of wells under this heading were preincubated with medium (Waymouth’s plus 20% fetal calf serum) prior to addition of lymphocytes.

* M, medium; N1, and N2, 2 normal volunteers of serum and lymphocyte samples; P1, and P2, 2 patient donors of serum and lymphocyte samples.

* HT115 target cells were cultured from a brain metastasis of bronchogenic adenocarcinoma. HS115 target cells are autochthonous skin fibroblasts.

* HS Medium only was added to this group of wells, i.e., no lymphocytes were added.

* Mean ± S.E. (8 replicate wells/group).

* Of the 2 normals tested, N2 is the volunteer whose lymphocytes yielded the higher TCC. This italicised TCC value is arbitrarily designated the base-line value against which the TCC’s of the other groups are compared. After preincubation with medium, lymphocytes Samples 1 and P2 are selectively toxic on HT115 versus HS115 whereas lymphocytes from normal Volunteer N1 are toxic on both tumor and fibroblasts compared to base-line Sample N2.
selectively and significantly (greater than 20% reduction; \( p \leq 0.05 \) or less) decreased the survival of bronchogenic cancer target cells when preincubated with the target cells prior to addition of lymphocytes compared to preincubation with medium or with other patient or normal serum samples. The effect was usually observed with all lymphocyte samples tested in any given experiment. The effect was observed with none of 94 normal serum samples. It was not possible, however, to correlate either the lymphocyte-dependent, growth-promoting or the lymphocyte-dependent toxic effects of serum samples with the patient’s clinical status.

**DISCUSSION**

In many laboratories it has been demonstrated that lymphocytes from cancer patients are specifically toxic in microcytotoxicity assays against autochthonous tumors or against allogeneic tumors of the same tissue of origin \((1-3, 7, 8, 11-13, 21, 34)\). Serum samples from patients may modulate the lymphocyte reactivity; tumor-type specific blocking, unblocking, and lymphocyte-arming serum effects have been demonstrated that appear to correlate well with the patient’s clinical status \((4, 10, 14-17, 19)\).

A number of investigators have encountered difficulties in the clinical use of microcytotoxicity assays. Major problems include frequent toxicity of “normal” lymphocytes and nonspecific toxicity of patient lymphocytes \((18, 20, 22, 30, 32, 33)\). Results obtained with microcytotoxicity assays in the authors’ laboratory over a 4-year period have been reported recently \((26, 27)\). With improvements in technique and with use of Ficoll-Hypaque for lymphocyte separation, it has been possible to reduce but not to eliminate toxicity of normal lymphocytes and to demonstrate that lymphocytes from cancer patients are more frequently toxic on tumor cells but no more frequently toxic on fibroblasts than are lymphocytes from normal donors. It has not been possible, however, clearly to demonstrate specificity against tumors of the same tissue of origin.

In these experiments only patients with bronchogenic carcinoma were studied. An attempt was made to correlate the toxic reactions of patient lymphocytes against bronchogenic cancer cells with the patient’s clinical status, regardless of whether or not the toxic reactions were specific for lung cancer targets. The results suggest that lymphocytes from Status A patients with bronchogenic carcinoma, *i.e.*, those who are cured or who have minimal disease not clinically detectable, are more frequently toxic against bronchogenic cancer than are lymphocytes from Status B patients who have clinical evidence of disease, *i.e.*, those with presumably larger tumor burdens. The increased frequency of toxicity was not specific or at least not completely specific for bronchogenic cancer targets since lymphocytes from Status A patients were also more frequently toxic on other types of tumors and on fibroblasts than were lymphocytes from Status B patients. Nevertheless, toxicity was observed in a significantly higher proportion of lymphocyte-target cell combinations with lymphocytes from Status A patients tested against bronchogenic cancer than against fibroblast targets and in a slightly higher proportion (not significant) with Status A lymphocytes tested against bronchogenic carcinoma than against other types of tumors. These observations suggest the possibility that both selective and nonselective reactions are responsible for the toxicity observed with Status A patient lymphocytes.

There are many possible explanations for both the selective and nonselective toxic reactions. The data from this study are insufficient to draw conclusions concerning specific mechanisms, but in view of the current interest in “nonspecific” lymphocyte toxicity, a brief discussion is appropriate. By definition all Status A patients were

### Table 4

Reactivity of bronchogenic cancer patient lymphocytes in microcytotoxicity assays correlated with patient’s clinical status and histological tumor type

<table>
<thead>
<tr>
<th>Patient status*</th>
<th>Histological type of patient’s bronchogenic cancer</th>
<th>Target cells</th>
<th>% of lymphocyte-target cell combinations in which lymphocytes were toxic*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Adenocarcinoma</td>
<td>Bronchogenic cancer</td>
<td>60 (6/10)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Other tumors*</td>
<td>33 (1/3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fibroblasts</td>
<td>18 (2/11)</td>
<td></td>
</tr>
<tr>
<td>Epidermoid</td>
<td>Bronchogenic cancer</td>
<td>65 (11/17)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Other tumors*</td>
<td>58 (7/12)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fibroblasts</td>
<td>38 (5/13)</td>
<td></td>
</tr>
<tr>
<td>Undifferentiated</td>
<td>Bronchogenic cancer</td>
<td>100 (13/13)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Other tumors*</td>
<td>66 (2/3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fibroblasts</td>
<td>50 (4/8)</td>
<td></td>
</tr>
<tr>
<td>All types combined</td>
<td>Bronchogenic cancer</td>
<td>75 (30/40)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Other tumors*</td>
<td>55 (10/18)</td>
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</tr>
<tr>
<td></td>
<td>Fibroblasts</td>
<td>34 (11/32)</td>
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</tr>
<tr>
<td>B Adenocarcinoma</td>
<td>Bronchogenic cancer</td>
<td>0 (0/16)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Other tumors*</td>
<td>23 (3/13)</td>
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</tr>
<tr>
<td></td>
<td>Fibroblasts</td>
<td>8 (1/13)</td>
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</tr>
<tr>
<td>Epidermoid</td>
<td>Bronchogenic cancer</td>
<td>50 (4/8)</td>
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<td></td>
<td>Other tumors*</td>
<td>33 (2/6)</td>
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</tr>
<tr>
<td></td>
<td>Fibroblasts</td>
<td>14 (1/7)</td>
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<tr>
<td>Undifferentiated</td>
<td>Bronchogenic cancer</td>
<td>42 (5/12)</td>
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<td></td>
<td>Other tumors*</td>
<td>14 (1/7)</td>
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<td>Fibroblasts</td>
<td>15 (2/13)</td>
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<tr>
<td>All types combined</td>
<td>Bronchogenic cancer</td>
<td>25 (9/36)</td>
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<td>Other tumors*</td>
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<tr>
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<td>Fibroblasts</td>
<td>13 (4/33)</td>
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</tr>
</tbody>
</table>

* Status A patients were postresection with no clinical evidence of disease. Status B patients had clinically demonstrable tumors and were either postoperative, nonoperable, or postoperative with residual or recurrent disease.

* Toxicity is defined as a greater than 20% reduction (significant at least at \( p < 0.05 \)) in TCC after exposure to a lymphocyte sample compared to the base-line sample. The base-line sample in any test is the lymphocyte sample from a normal volunteer that yields the highest TCC.

* "Other tumors" includes miscellaneous nonbronchogenic cancers, *i.e.*, melanomas, carcinomas of colon, bladder, cervix, etc.
Table 5
Sequential microcytotoxicity assays of bronchogenic cancer patient lymphocyte reactivity against cultured target cells

<table>
<thead>
<tr>
<th>Patient</th>
<th>Histological type of tumor</th>
<th>Date of experiment</th>
<th>Patient clinical status* when tested</th>
<th>Bronchogenic cancer target cells</th>
<th>% toxicity vs. tumor target</th>
<th>% toxicity vs. “Control” target cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>K. T.</td>
<td>Adenocarcinoma</td>
<td>12/18/73</td>
<td>B1</td>
<td>HT072†</td>
<td>5</td>
<td>HS072                 — 8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1/25/74</td>
<td>A</td>
<td>HT072</td>
<td>30*</td>
<td>HS072                 0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4/18/74</td>
<td>A</td>
<td>HT115</td>
<td>41†</td>
<td>HS115                 27</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8/1/74</td>
<td>A</td>
<td>HT128</td>
<td>38†</td>
<td>HS132                 10</td>
</tr>
<tr>
<td>R. J.</td>
<td>Epidermoid carcinoma</td>
<td>5/2/73</td>
<td>A</td>
<td>HT056</td>
<td>43†</td>
<td>HT035                 40†</td>
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<tr>
<td></td>
<td></td>
<td>5/10/73</td>
<td>A</td>
<td>HT046</td>
<td>34†</td>
<td>HT035                 39†</td>
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<td></td>
<td></td>
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<td>A</td>
<td>HT115</td>
<td>51†</td>
<td>HS115                 39†</td>
</tr>
<tr>
<td>C. F.</td>
<td>Undifferentiated carcinoma</td>
<td>6/8/73</td>
<td>A</td>
<td>HT046</td>
<td>22†</td>
<td>HS062                 6</td>
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<tr>
<td></td>
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<td>HT072</td>
<td>23†</td>
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<tr>
<td></td>
<td></td>
<td>12/4/74</td>
<td>A</td>
<td>HT144</td>
<td>40†</td>
<td>HS144                 5</td>
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<tr>
<td>D. W.</td>
<td>Undifferentiated carcinoma</td>
<td>7/31/73</td>
<td>A</td>
<td>HT072</td>
<td>53†</td>
<td>HS072                 48†</td>
</tr>
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<td></td>
<td></td>
<td>9/12/73</td>
<td>A</td>
<td>HT072</td>
<td>24†</td>
<td>HS072                 36†</td>
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<tr>
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<td></td>
<td>9/18/73</td>
<td>A</td>
<td>HT072</td>
<td>31†</td>
<td>HT035                 48†</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1/25/74</td>
<td>A</td>
<td>HT072</td>
<td>40†</td>
<td>HS072                 — 15</td>
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<tr>
<td></td>
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<td>11/14/74</td>
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<td>HT144</td>
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</tbody>
</table>

* Status A patients were postresection with no clinical evidence of disease. Status B patients had clinically demonstrable tumors and were either preoperative (B1), nonoperable (B2), or postoperative with residual or recurrent disease (B3).

Toxicity is defined as a greater than 20% reduction (significant at least at p < 0.05) in TCC after exposure to a lymphocyte sample compared to the base-line sample. The base-line sample in any test is the lymphocyte sample from a normal volunteer that yields the highest TCC.

Control target cells included fibroblasts and nonbronchogenic carcinomas.

HT, tumors; HS, fibroblasts. Tumors and fibroblasts designated by the same number are obtained from the same patient.

†p < 0.05, Student's t test.
‡p < 0.01, Student's t test.
§p < 0.001, Student's t test.

postoperative, and it is possible that operation per se rendered lymphoid cells toxic by perhaps "activating" macrophages or inducing "autoimmunity" against normal tissues. Lymphocytes from the one Status A patient who was repeatedly tested against her own tumor and fibroblasts were consistently toxic against both types of autochthonous cells for two months after operation. Since most of the operated patients received blood transfusions, sensitization against histocompatibility antigens could account for some of the toxicity against allogeneic targets (25).

Selective toxicity could be explained by either immunological or nonimmunological mechanisms. It is possible that fibroblasts are simply less sensitive to some nonspecific toxic effects than are tumor cells. A recent report, however, suggests that tumor cells and fibroblasts do not differ in their sensitivity to nonspecific toxic reactions when studied as target cells in matched pairs (31). Therefore, it is tempting in the present study to attribute the increased frequency of toxicity observed with patient lymphocytes against tumor cells versus fibroblasts to reactions against some types of tumor antigens. There is little evidence in this study to suggest that the reactions were directed against "tissue-type"-specific tumor antigens (17), since toxic reactions with lymphocytes from Status A patients were only slightly more frequently toxic against bronchogenic cancer than against other types of cancers. However, melanomas were the other type of tumor most often used as targets in this study. Several investigators have noted cross-reactions with lymphocytes from other types of cancer patients against melanoma targets and/or with melanoma lymphocytes against other types of tumors (9, 18, 21). Furthermore, the melanoma target most frequently used was the only "long-term" culture (i.e., in continuous culture for more than 2 months) in this study. Nonspecific toxicity has been encountered more frequently with long-term than with short-term target cultures (18).
Tumor Immunity in Patients with Bronchogenic Carcinoma

Table 6
Selective increase or decrease in target cell survival following preincubation with certain human sera compared to preincubation with medium or with other human sera and subsequent addition of lymphocytes in microcytotoxicity assays

Target cells were preincubated for 45 min with medium or with 1:6 dilutions of heat-inactivated human serum samples. The sera were then decanted and lymphocytes were added in amounts of 150,000/well. The plates were incubated for an additional 40 hr, then they were rinsed and stained, and the surviving cells were counted.

<table>
<thead>
<tr>
<th>No. of serum samples that</th>
<th>No. of serum samples that</th>
</tr>
</thead>
<tbody>
<tr>
<td>effected an increase in survival</td>
<td>produced a decrease in survival</td>
</tr>
<tr>
<td>of a target cell in the presence of lymphocytes*</td>
<td>of a target cell in the presence of lymphocytes*</td>
</tr>
<tr>
<td>Bronchogenic cancer patient sera (74 samples tested)</td>
<td>Bronchogenic cancer patient sera (74 samples tested)</td>
</tr>
<tr>
<td>Normal volunteer sera (94 samples tested)</td>
<td>Normal volunteer sera (94 samples tested)</td>
</tr>
<tr>
<td>Bronchogenic cancer</td>
<td>Normal volunteer sera</td>
</tr>
<tr>
<td>(73 serum samples tested)</td>
<td>(94 serum samples tested)</td>
</tr>
<tr>
<td>Fibroblasts</td>
<td>4</td>
</tr>
</tbody>
</table>
| * | None of the reactive serum samples alone, in the absence of lymphocytes, altered the target cell survival when compared to medium only or with other serum samples.

In a recent report from another laboratory (35) leukocytes from 73% of patients with bronchogenic carcinoma were toxic against lung cancer cells in microcytotoxicity assays. Approximately one-third of the patients studied were postoperative and the remaining two-thirds were receiving irradiation therapy. Since it was not stated in this report what fraction of the postoperative patients were clinically free of disease and since very few patients in the present study were irradiated, it is difficult to compare the results of the 2 studies.

From previous reports (10, 16) it was anticipated that most patients with growing tumors, i.e., Status B patients, would have lymphocytes toxic for bronchogenic cancer cells but that serum from these patients would block this lymphocyte reactivity. Characteristically, however, serum blocking factors in human tumor systems have been tumor tissue-type specific (16). Since the lymphocyte toxicity against tumors observed in this study was not clearly tissue-type specific, it is not surprising that blocking activity was found in only a few samples. The low incidence of lymphocyte toxicity observed in this group of patients deserves comment. It correlates well with the clinical observation that, unlike neuroblastoma, melanoma, mammary carcinoma, or certain other cancers in which prolonged remissions are not uncommon, bronchogenic carcinoma follows a relentless, highly predictable course with little or no clinical evidence of host resistance. The results also are in accord with a recent report (6) that the circulating levels of thymus-dependent lymphocytes in bronchogenic cancer patients with clinically evident tumors are significantly lower then in normal persons even when the disease is localized or regional. Only postoperative patients who are apparently cured of bronchogenic carcinoma have normal levels of thymus-dependent lymphocytes.

The observation that serum samples from a few patients rendered lymphocytes toxic for bronchogenic cancer cells is of interest. Lymphocyte-dependent antibodies against histocompatibility antigens cannot be excluded (5, 36), but the selectivity for bronchogenic carcinoma suggests that the effect might be attributed to serum "arming" factor(s) that enable lymphocytes to specifically kill tumor cells in vitro and that have been demonstrated in both experimental (28, 29) and human (15) tumor systems. This activity, however, was found in too few patients to permit correlations with clinical course or status.

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Microcytotoxicity Assays of Tumor Immunity in Patients with Bronchogenic Carcinoma Correlated with Clinical Status

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