Studies of Lymphocyte Stimulation to Tumor-Associated Antigen

I. Uptake of Lymphocyte Protein and Nucleic Acid Precursors in Response to Autologous and Allogeneic Tumor-associated Antigen Solubilized from Human Tumors

J. A. Roth¹ and D. L. Morton²

¹ Division of Oncology, Department of Surgery, 54-140 CHS, UCLA School of Medicine, University of California, Los Angeles, California 90024, USA
² Surgical Services, Veterans Administration Hospital, Sepulveda, California 91343, USA

Summary. Lymphocyte stimulation to 3 M KCl extracts of fresh human tumors was studied by measuring the incorporation of ³H-labeled protein and nucleic acid precursors. Lymphocytes from cancer patients and normal donors were incubated with autologous and allogeneic extracts. Duplicate lymphocyte cultures were labeled with ³H-leucine (³H-Leu), ³H-uridine (³H-Udr), or ³H-thymidine (³H-Tdr). All patients were sensitized to keyhole limpet hemocyanin (KLH) prior to testing. Of the 29 cancer patients tested, many demonstrated significant uptake of ³H-Udr (90%) and ³H-Leu (62%), but not ³H-Tdr (7%) in response to soluble tumor extracts. However, most patients demonstrated uptake of all three precursors in response to KLH. Lymphocytes from cancer patients did not undergo morphologic blast cell transformation in the presence of tumor extracts. Significant incorporation of ³H-Leu and ³H-Udr was seen after 24–48 h of incubation, while significant ³H-Tdr incorporation was not detected until day 5. Stimulation by KLH was significantly greater for all isotope precursors than stimulation in response to tumor extracts. Responses of lymphocytes from normal donors to tumor extracts were noted, although they occurred less frequently than in lymphocytes from cancer patients. Lymphocytes from cancer patients incorporated ³H-Leu and ³H-Udr, but only rarely incorporated ³H-Tdr in response to 3 M KCl extracts of fresh tumors.

Introduction

Stimulation of lymphocyte blastogenesis by autologous and allogeneic tumor cells and tumor extracts is a well-established observation (Dean et al., 1975; Gainor et al., 1975; Hsu and Cooperband, 1971; Jehn et al., 1970; Levin et al., 1975; Mavligit et al., 1973a and b, 1974; Savel, 1969; Silva et al., 1976; Stjernsward et al., 1973; Vanky et al., 1974, 1975). However, there is wide variation in the response of cancer patients' lymphocytes to tumor antigen. Some investigators have been unable to demonstrate lymphocyte stimulation to tumor antigen or have only noted responses in a small fraction of samples tested (Dean et al., 1975; Hsu and Cooperband, 1971; Savel, 1969; Vanky et al., 1975). Others found lymphocyte stimulation in 36%–100% of the patients tested (Gainor et al., 1975; Jehn et al., 1970; Mavligit et al., 1973a, 1974; Silva et al., 1976; Stjernsward et al., 1973). The cause for this variability is unknown. Our study was undertaken to delineate those factors influencing lymphocyte responsiveness to autologous and allogeneic tumor-associated antigens (TAA).

Previous studies of lymphocyte stimulation concentrated on measuring lymphocyte proliferation, as detected by ³H-Tdr incorporation. However, ³H-Tdr incorporation represents a relatively late event following initial lymphocyte-TAA contact. We have shown that stimulation of protein synthesis (SPS) as measured by ³H-Leu incorporation, occurs early in the course of lymphocyte-TAA interaction (Roth et al., 1975a and b, 1976). Failure of lymphocytes to synthesize RNA and/or protein is a possible cause of variability in lymphocyte DNA synthesis in response to TAA. To investigate this hypothesis, we simultaneously measured synthesis of RNA, protein, and DNA, using tritium and ¹⁴C-labeled protein and nucleic acid precursors. Our purpose was to measure lymphocyte RNA, protein, and DNA synthesis in response to autologous and allogeneic tumor cell extracts prepared from fresh surgical specimens. The kinetics of these responses were determined, and the responses to TAA were compared with responses to a nontumor antigen (keyhole limpet hemocyanin, KLH).
Materials and Methods

Patient Population

A total of 29 patients were studied. Lymphocytes from ten patients (nine males, one female) were tested with autologous tumor extracts (three sarcomas, four melanomas, three lung carcinomas) and extracts of autologous nontumor tissue. The mean age for this group was 43 years (range 26–76 years). Three patients' tumors were Stage I (primary cancer confined to site of origin), four were Stage II (regional spread), and three were Stage III (disseminated tumor). However, only one Stage-III patient had tumor clinically evident at the time of testing.

Lymphocytes from a second group of 19 melanoma patients (13 males, 6 females) were tested with a single allogeneic melanoma extract. The mean age for this group was 45 years (range 18–72 years). Ten patients were Stage I, seven were Stage II, and two were Stage III, one of whom had tumor clinically evident at the time of testing. None of the melanoma patients was receiving chemotherapy or immunotherapy, and all were tested at least 1 month after surgical procedures.

Lymphocytes from 16 normal volunteers (11 males, 5 females) were also tested with various tumor extracts. In several instances, lymphocytes from a single donor were tested with different tumor antigens.

Antigen Preparation

Tumor antigen and antigen from normal tissue were prepared by the 3 M KCl extraction technique as previously described (Roth et al., 1975a and b, 1976). Control extracts consisted of uninvolved ipsilateral lung from lung cancer patients, normal skin from melanoma patients, and normal muscle from sarcoma patients. KLH was prepared as previously described, and each test participant was sensitized with an intradermal injection of 200 µg 2 weeks before initiation of the study (Roth et al., 1976).

Lymphocyte Stimulation Test

The technique for lymphocyte separation and the assays used to measure 3H-Leu and 3H-Tdr incorporation in response to TAA have been reported elsewhere (Roth et al., 1975a and b, 1976). Incorporation of uridine was measured with the aid of a technique similar to that for leucine incorporation. Lymphocyte cultures were prepared exactly as in the leucine incorporation assay. However, 1 h before termination, 2 µCi 3H-Udr (New England Nuclear, Boston, Mass.) was added to each well.

A typical experiment included lymphocytes suspended in 0.1 ml medium only (eight microtest plate wells); lymphocytes suspended in media containing 25 and 50 µg tumor antigen/ml (four wells for each concentration); lymphocytes suspended in KLH (four wells); and four wells containing only 0.2 ml medium. This set of cultures was prepared in duplicate on six or seven microtest plates for each subject tested. Cultures were terminated at 18, 24, and 48 h and at 5, 6, and 7 days. In some cases additional cultures were terminated at 3 and 4 days. For cultures terminated between 18 and 72 h, one set per microtest plate was labeled with 3H-Leu and one set labeled with 3H-Udr prior to termination. For cultures terminated between 4 and 7 days, one set of cultures was labeled with 3H-Udr and one set with 3H-Tdr. In preliminary experiments, some cultures were double-labeled with 3H-Udr and either 3H-Leu or 3H-Tdr. The results of these experiments did not differ significantly from those with single cultures. Lymphocytes from normal subjects and cancer patients were tested simultaneously. Lymphocytes known to be responsive to the antigens tested were included in each experiment as positive controls.

Statistical Analysis

All data were processed as previously described (Roth et al., 1976). Significant stimulation for all isotopes was defined as a statistically significant increase (P < 0.05) in the counts per minute (CPM) of antigen-stimulated cultures against unstimulated cultures. The paired Student's t-test with a correction for unequal variances was used. In addition, for 3H-Tdr labeled cultures the stimulated cultures were required to have a value at least twice that of unstimulated cultures to be considered significantly stimulated. Comparisons between TAA and KLH were made with the unpaired Student's t-test. Responses between cancer patients and normal subjects were compared by χ² analysis.

Results

A majority of cancer patients' lymphocytes demonstrated significant 3H-Udr and 3H-Leu incorporation in the presence of allogeneic and autologous TAA. Significant 3H-Tdr incorporation (twofold statistically significant increase of antigen-stimulated CPM over the antigen-absent control) was noted in only 2 of the 29 tests (Fig. 1). However, most patients demonstrated significant incorporation of all three labels in response to KLH. In 5 of the 19 allogeneic and 2 of the 10 autologous tests, 3H-Tdr incorporation was significantly higher for antigen-stimulated cultures, although the values did not exceed those measured by antigen-absent controls by a factor of 2. In these experiments, increases of TAA-stimulated cultures ranged from 23%–76%.

Addition of tumor antigen extracts to phytohemagglutinin(PHA)-stimulated lymphocyte cultures did not inhibit 3H-Tdr uptake by lymphocytes from three normal donors (Table 1). No significant difference in CPM

<table>
<thead>
<tr>
<th>Tumor Antigen</th>
<th>Allogeneic Autologous</th>
<th>CANCER PATIENTS</th>
<th>3H-Uridine</th>
<th>12/19 15/19 62%</th>
<th>8/10 5/10 8%</th>
<th>14/21 67%</th>
<th>3H-Leucine</th>
<th>17/19 9/10 90%</th>
<th>14/21 67%</th>
<th>3H-Thymidine</th>
<th>2/19 0/10 7%</th>
<th>0/10 0/10 0%</th>
</tr>
</thead>
<tbody>
<tr>
<td>3H-Uridine</td>
<td>Allogeneic Autologous</td>
<td>CANCER PATIENTS</td>
<td>3H-Uridine</td>
<td>12/19 15/19 62%</td>
<td>8/10 5/10 8%</td>
<td>14/21 67%</td>
<td>3H-Leucine</td>
<td>17/19 9/10 90%</td>
<td>14/21 67%</td>
<td>3H-Thymidine</td>
<td>2/19 0/10 7%</td>
<td>0/10 0/10 0%</td>
</tr>
<tr>
<td>3H-Leucine</td>
<td>Allogeneic Autologous</td>
<td>NORMAL SUBJECTS</td>
<td>3H-Uridine</td>
<td>14/21 67%</td>
<td>8/10 36%</td>
<td>12/21 58%</td>
<td>3H-Leucine</td>
<td>14/21 67%</td>
<td>8/10 36%</td>
<td>3H-Thymidine</td>
<td>12/21 58%</td>
<td>12/21 58%</td>
</tr>
</tbody>
</table>

Fig. 1. Stimulation of lymphocyte tritiated precursor uptake in response to melanoma antigen and KLH in cancer patients and normal donors. The mean percentage stimulation for KLH was significantly (P < 0.0025) greater than stimulation for TAA for all isotopes tested.