In Vitro Immunization against Human Tumor Cells with Tumor Cell Fractions

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SUMMARY

Tumor cell fractions isolated from tumor lines SH-3 (breast carcinoma) and RPMI-7932 (malignant melanoma) by differential centrifugations were capable of transforming lymphocytes into cytotoxic effector cells. Lymphocytes cultured alone in human AB plasma did not become cytotoxic to tumor cells. However, when cultured with tumor cell fractions sedimented at 1,000 \times g (R1), 20,000 \times g (R2), and 100,000 \times g (R3), these lymphocytes became markedly cytoxic to specific tumor targets in a 3.5-hr \textsuperscript{51}Cr release assay. R2 fractions were significantly more immunogenic than were R3 fractions (P < 0.05). Although lymphocytes sensitized with SH-3 tumor cell fractions were cytotoxic to SH-3 tumor cells, they were also cytotoxic to cells from RPMI-7932 and RPMI-8322 (malignant melanoma) tumor lines and vice versa. Cells from tumor lines HT-29 (colon carcinoma) and COLO-110 (ovary carcinoma) were significantly less susceptible to lysis by effector cells generated against SH-3. These immune cells, although capable of killing cells from tumor lines, were not able to lyse cells from autochthonous normal lymphoid lines or normal lymphocytes that have been transformed by phytohemagglutinin. Tumor cell fractions were most immunogenic at low (5- to 20-\mu l/0.75 ml) concentrations; an increase of 4- to 10-fold in their concentrations was usually followed by a decrease in immunization.

INTRODUCTION

Although immunological knowledge and techniques can be applied to the problem of cancer in man by a variety of means, one of the most promising and perhaps potentially effective immunological approaches would be to induce active immunity against cancer. Immunization with chemically or enzymatically modified tumor cells may induce tumor-specific immunity. Immunization with a cell fraction, capable of inducing specific immunity against tumor in vitro, perhaps would be the choice immunogen because of its inability to replicate and produce any immunosuppressive factor. Mouse cell-free transplantation antigens and several forms of tumor cell-free extracts have been reported to stimulate lymphocytes in vitro (2, 7, 8). Recently, subcellular fractions isolated from a lymphoid line have been shown to induce cell-mediated immunity in vitro (1).

This study demonstrates that cell fractions isolated from well-characterized human tumor lines, SH-3 (breast carcinoma) and RPMI-7932 (malignant melanoma), are able to stimulate the generation of effector lymphocytes that are markedly cytotoxic to tumor cells.

MATERIALS AND METHODS

Source of Tumor Cells. Human tumor cells were obtained from tumor lines (Table 1) SH-3 (breast carcinoma); RPMI-7932, RPMI-8322, COLO-53, and COLO-38 (malignant melanomas); COLO-110 (ovary carcinoma); and HT-29 (colon carcinoma). COLO 8 is a normal lymphoid line autochthonous to RPMI-8322, and COLO 59 is a normal lymphoid line autochthonous to COLO 53. SH-3 was provided by Dr. G. S. Seman, M. D. Anderson Hospital, Texas Medical Center; HT-29 was provided by Dr. J. Fogh, Sloan-Kettering Institute for Cancer Research, N. Y., and all other tumor and lymphoid lines were provided by Dr. G. E. Moore, Denver General Hospital, Denver, Colo. All tumor lines are Mycoplasma and virus free, as determined by electron microscopy and culture methods.

Isolation of Tumor Cell Fractions. Tumor cells were maintained in MEM containing 10% heat-inactivated calf serum. The cells were harvested by gently scraping the monolayers and then passing the cells through 22-gauge needles. The cells were then washed 3 times with plasma-free medium and suspended in either plasma-free medium or Tris buffer containing 0.25 M sucrose (pH 7.4) at a concentration of 12 \times 10^6 cells/ml. The cells were disrupted by freezing (-80°) and thawing 10 times. Various fractions were obtained from the cell-free extract by differential centrifugations and were suspended in equal volumes of medium or Tris buffer (1, 3). Residue and supernatants obtained after centrifugation of cell-free extracts at 1,000 \times g for 2 min were denoted as SH-3-R1 and SH-3-S1 or RPMI-7932-R1 and RPMI-7932-S1, respectively. Residue and supernatants obtained after centrifugation of S1 at 20,000 \times g for 20 min were designated SH-3-R2 and SH-3-S2 or RPMI-7932-R2 and RPMI-7932-S2, respectively. SH-3-R3, SH-3-S3 or RPMI-7932-R3, and RPMI-7932-S3 were the residues and supernatants obtained after 100,000 \times g centrifugation of S2 for 90 min. Fractions R1 contained a few of the cells that were not completely disrupted or lysed and some nuclei; RPMI-7932-R2 appeared to contain a few intact nuclei and broken nuclear material, granular cytoplasmic material, and plasma membrane; and R3 seemed to have plasma membranes.

Preparation of Killer Cells. Peripheral blood lymphocytes...
were isolated and purified over Ficoll-Hypaque according to the method described previously (14, 16). The purified lymphocytes were sensitized with various tumor cell fractions in MEM containing 20% heat-inactivated human AB plasma. Responding lymphocytes (1 x 10^6) and immunizing tumor cell fractions at different concentrations were mixed in 15-ml polystyrene tubes (Falcon Plastics Co., Los Angeles, Calif.). The total volume was brought up to 0.75 ml, and the tubes were incubated for 5 days at 37° in 6 to 7% CO_2 humid atmosphere. At the end of the incubation period the contents of tubes were mixed and centrifuged at 800 x g for 2 min. The supernatants were carefully removed and transferred to corresponding new microtubes. The percentage of 51Cr released into the supernatant as a result of the lysis of tumor targets and the percentage of 51Cr remaining in the residue were counted. The percentage of specific 51Cr release was determined by the comparison of the 3 values for the test release to the 3 for the minimum release with the use of a Student’s t test with 4 d.f. Significantly higher values of the test release translate to a percentage of specific 51Cr release significantly greater than zero. Analysis of variance was used to compare specific versus nonspecific sensitization. Statistical significance was determined by the comparison of the difference.

### Table 1

**Characteristics of tissue-cultured human tumor lines**

<table>
<thead>
<tr>
<th>Cell line (source)</th>
<th>Diagnosis of patient donor</th>
<th>Morphology</th>
<th>Mos. in culture</th>
<th>Growth in nude mice</th>
<th>Chromosome mode</th>
<th>Stability</th>
<th>Originator</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPMI-7932 (pleural effusion)</td>
<td>Malignant melanoma</td>
<td>Epithelial (triangular dendritic)</td>
<td>54</td>
<td>Yes</td>
<td>44 (42-58)*</td>
<td>+</td>
<td>G. E. Moore (6, 11)</td>
</tr>
<tr>
<td>SH-3* (pleural effusion)</td>
<td>Breast carcinoma</td>
<td>Epithelial</td>
<td>29</td>
<td>Yes</td>
<td>71 (68-75)</td>
<td>+</td>
<td>G. Seman, S. G. Hunter, and L. Dmochowski (13)</td>
</tr>
<tr>
<td>RPMI-8322* (solid metastases)</td>
<td>Malignant melanoma</td>
<td>Triangular dendritic</td>
<td>51</td>
<td></td>
<td>57 (51-58)</td>
<td>+</td>
<td>G. E. Moore (11)</td>
</tr>
<tr>
<td>COLO 8* (peripheral blood)</td>
<td>Normal</td>
<td>Lymphoid</td>
<td>52</td>
<td></td>
<td>46,XY</td>
<td>+</td>
<td>G. E. Moore (11)</td>
</tr>
<tr>
<td>COLO 109 (pleural effusion)</td>
<td>Ovary carcinoma</td>
<td>Epithelial</td>
<td>24</td>
<td></td>
<td>74 (70-78)</td>
<td>+</td>
<td>G. E. Moore</td>
</tr>
<tr>
<td>HT-29 (solid primary)</td>
<td>Colon carcinoma</td>
<td>Epithelial</td>
<td>52</td>
<td></td>
<td>Hypohypertroid</td>
<td>J. Fogh and G. Trempe (4)</td>
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<tr>
<td>COLO 53* (solid metastasis)</td>
<td>Malignant melanoma</td>
<td>Epithelial</td>
<td>32</td>
<td></td>
<td>70 (60-80)</td>
<td>+</td>
<td>G. E. Moore (11)</td>
</tr>
<tr>
<td>COLO 59* (peripheral blood)</td>
<td>Normal</td>
<td>Lymphoid</td>
<td>32</td>
<td></td>
<td>46,XX</td>
<td>+</td>
<td>G. E. Moore (11)</td>
</tr>
<tr>
<td>COLO 38 (primary lesion)</td>
<td>Malignant</td>
<td>Epithelial</td>
<td>38</td>
<td></td>
<td>74 (68-77)</td>
<td>+</td>
<td>G. E. Moore (11)</td>
</tr>
</tbody>
</table>

* Refers to observation of the marker chromosomes over a period of months.
* Numbers in parentheses, range.
* May be cross-contaminated with HeLa (cervix) tumor line as recently reported by Nelson-Rees (9).
* RPMI-8322 and COLO 8 are tumor and normal lymphoid lines that were established from the same patient.
* COLO 53 and COLO 59 are tumor and normal lymphoid lines that were established from the same patient.

Av. test release = av. minimum release x 100
Av. maximum release = av. minimum release

Lymphocytes were sensitized in 1 tube and their cytotoxic activity was determined in triplicates. All S.D. are of the triplicates. Statistical significance of the positivity of the percentage of specific 51Cr release was determined by the comparison of the 3 values for the test release to the 3 for the minimum release with the use of a Student’s t test with 4 d.f. Significantly higher values of the test release translate to a percentage of specific 51Cr release significantly greater than zero. Analysis of variance was used to compare specific versus nonspecific sensitization. Statistical significance was determined by the comparison of the difference.
Table 2

In vitro immunization of lymphocytes with human tumor cell fractions and its reactivity

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Effector-stimulating cell fraction</th>
<th>Source of cell fractions and tumor targets</th>
<th>Target alone</th>
<th>Target + unsensitized lymphocytes</th>
<th>% 51Cr release&lt;sup&gt;a&lt;/sup&gt;</th>
<th>% specific 51Cr release&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A-SH-3-cells</td>
<td>SH-3 (breast)</td>
<td>20 ± 1.0</td>
<td>18 ± 2.0</td>
<td>17&lt;sup&gt;d&lt;/sup&gt;</td>
<td>31&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>A-SH-3-S1</td>
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<td></td>
<td></td>
<td>23&lt;sup&gt;d&lt;/sup&gt;</td>
<td>7&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>A-SH-3-R1</td>
<td></td>
<td></td>
<td></td>
<td>27&lt;sup&gt;d&lt;/sup&gt;</td>
<td>7&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>A-SH-3-R2</td>
<td></td>
<td></td>
<td></td>
<td>31&lt;sup&gt;d&lt;/sup&gt;</td>
<td>22&lt;sup&gt;d&lt;/sup&gt;</td>
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<td></td>
<td></td>
<td>14&lt;sup&gt;d&lt;/sup&gt;</td>
<td>39&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>A-SH-3-R3</td>
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<td></td>
<td></td>
<td>21&lt;sup&gt;d&lt;/sup&gt;</td>
<td>33&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>2</td>
<td>B-SH-3-cells</td>
<td>SH-3</td>
<td>20 ± 4.0</td>
<td>20 ± 1.0</td>
<td>13&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>B-SH-3-S1</td>
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<td>19&lt;sup&gt;d&lt;/sup&gt;</td>
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<td></td>
<td></td>
<td>44&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>26&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
<td>3</td>
<td>C-SH-3-cells</td>
<td>SH-3</td>
<td>18 ± 2.0</td>
<td>18 ± 1.0</td>
<td>26&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>C-SH-3-S1</td>
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<td>34&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>C-SH-3-R2</td>
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<td>98&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>4</td>
<td>D-SH-3-cells</td>
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<td>18 ± 2.0</td>
<td>34&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>28&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>D-SH-3-R2</td>
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<td>51&lt;sup&gt;d&lt;/sup&gt;</td>
<td>29&lt;sup&gt;d&lt;/sup&gt;</td>
<td>22&lt;sup&gt;d&lt;/sup&gt;</td>
<td>20&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>D-SH-3-R3</td>
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<td>D-RPMI-7932-cells</td>
<td>RPMI-7932 (melanoma)</td>
<td>16 ± 1.0</td>
<td>21 ± 2.0</td>
<td>23&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
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<td>D-RPMI-7932-S1</td>
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<td>11&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>D-RPMI-7932-R1</td>
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<td></td>
<td>10&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
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<td>D-RPMI-7932-R2</td>
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<td></td>
<td></td>
<td>11&lt;sup&gt;d&lt;/sup&gt;</td>
<td>16&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>32&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>5</td>
<td>E-SH-3-cells</td>
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<td>20 ± 2.0</td>
<td>16 ± 1.0</td>
<td>40&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>E-SH-3-S1</td>
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<td>E-SH-3-R1</td>
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<td>30&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>50&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>6</td>
<td>F-SH-3-cells</td>
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<td>22 ± 2.0</td>
<td>25&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>F-SH-3-R2</td>
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<td>17&lt;sup&gt;d&lt;/sup&gt;</td>
<td>12</td>
</tr>
<tr>
<td>7</td>
<td>G-SH-3-cells</td>
<td>SH-3</td>
<td>18 ± 2.0</td>
<td>18 ± 2.0</td>
<td>24&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>G-SH-3-S1</td>
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<td>G-SH-3-R3</td>
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<td>11&lt;sup&gt;d&lt;/sup&gt;</td>
<td>9</td>
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<tr>
<td>8</td>
<td>H-RPMI-7932-cells</td>
<td>RPMI-7932</td>
<td>21 ± 10.0</td>
<td>22 ± 2.0</td>
<td>51&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>12&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>H-RPMI-7932-R3</td>
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<td>32&lt;sup&gt;d&lt;/sup&gt;</td>
<td>55&lt;sup&gt;d&lt;/sup&gt;</td>
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</tbody>
</table>

<sup>a</sup> Effector cells (immune lymphocytes) were prepared by in vitro sensitization of lymphocytes with tumor cell fractions. Immune lymphocytes were subsequently tested for their ability to lyse 51Cr-labeled targets. Letters, lymphocytes. Tumor cell fractions of SH-3 line sedimented at 1,000 × g, 20,000 × g, and 100,000 × g are denoted by SH-3-R1, SH-3-R2, and SH-3-R3; their supernatants are denoted by SH-3-S1, SH-3-S2, SH-3-S3, respectively. Similarly, cell fractions of RPMI-7932 tumor line are denoted as RPMI-7932-R1, RPMI-7932-R2, and RPMI-7932-R3 and supernatants are denoted as RPMI-7932-S1, RPMI-7932-S2, and RPMI-7932-S3.

<sup>b</sup> S.D. of the triplicates of the percentage of 51Cr release ranged from 1.0 to 16.0. Only 7 values exceeded 6.0.

<sup>c</sup> Mean ± S.D.

<sup>d</sup> p ≤ 0.01 for the test comparing 51Cr release from specific target by sensitized and unsensitized lymphocytes.

<sup>e</sup> p ≤ 0.01 for the test comparing 51Cr release from specific and nonspecific targets.

<sup>f</sup> 0.01 < p ≤ 0.05 for the test comparing 51Cr release from specific and nonspecific targets.

<sup>g</sup> 0.01 < p ≤ 0.05 for the test comparing 51Cr release from specific target by sensitized and unsensitized lymphocytes.
### Table 3

Reactivity of lymphocytes sensitized by SH-3 tumor cells or cell fractions

| Effector-stimulating cell or cell fraction | Source of cell or cell fraction | Target + unsensitized lymphocytes | % *¹⁰⁵ release | % specific %¹⁰⁵ release
<table>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>H-SH-3-cells (5,000)</td>
<td>SH-3 (breast)</td>
<td>SH-3</td>
<td>11 ± 2.0*</td>
<td>11 ± 1.0</td>
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<tr>
<td>H-SH-3-cells (20,000)</td>
<td></td>
<td>HT-29</td>
<td>22*</td>
<td>7*</td>
</tr>
<tr>
<td>H-SH-3-cells (80,000)</td>
<td></td>
<td></td>
<td>36*</td>
<td>13*</td>
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<tr>
<td>H-SH-3-cells (320,000)</td>
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<td></td>
<td>34*</td>
<td>12*</td>
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<tr>
<td>H-SH-3-R2 (5 µl)</td>
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<td>28*</td>
<td>10*</td>
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<td>16*</td>
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<td>H-SH-3-R2 (20 µl)</td>
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<td>H-SH-3-R3 (10 µl)</td>
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<td>1*</td>
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<tr>
<td>H-SH-3-R3 (20 µl)</td>
<td></td>
<td></td>
<td>14*</td>
<td>2*</td>
</tr>
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</table>

* Lymphocytes were sensitized with SH-3 tumor cells or fractions, and then cytotoxic activity of sensitized cells was tested against SH-3 and HT-29 tumor cells. Numbers in parentheses, concentrations of stimulating cell or cell fraction.

† S.D. of the triplicates of the percentage of %¹⁰⁵ release ranged from 1.0 to 7.2. Only 2 values exceeded 3.0.

‡ Mean ± S.D.

§ * 0.01 < p ≤ 0.05 for the test comparing %¹⁰⁵ release from specific target by sensitized and unsensitized lymphocytes.

¶ 0.01 < p ≤ 0.05 for the test comparing %¹⁰⁵ release from specific and nonspecific targets.

‖ 0.01 < p ≤ 0.05 for the test comparing %¹⁰⁵ release from specific target by sensitized and unsensitized lymphocytes.

†† p ≤ 0.01 for the test comparing %¹⁰⁵ release from specific and nonspecific targets.

### Table 4

Reactivity of lymphocytes sensitized by SH-3 tumor cells or cell fractions

| Effector-stimulating cell or cell fraction | Source of cell or cell fraction | Target + unsensitized lymphocytes | % *¹⁰⁵ release | % specific %¹⁰⁵ release
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>L-SH-3-cells (20,000)</td>
<td>SH-3 (breast)</td>
<td>SH-3</td>
<td>18 ± 2.0*</td>
<td>18 ± 1.0</td>
</tr>
<tr>
<td>L-SH-3-cells (80,000)</td>
<td></td>
<td>HT-29</td>
<td>29*</td>
<td>2*</td>
</tr>
<tr>
<td>L-SH-3-cells (320,000)</td>
<td></td>
<td></td>
<td>41*</td>
<td>15*</td>
</tr>
<tr>
<td>L-SH-3-R2 (5 µl)</td>
<td></td>
<td></td>
<td>48*</td>
<td>11*</td>
</tr>
<tr>
<td>L-SH-3-R2 (10 µl)</td>
<td></td>
<td></td>
<td>19*</td>
<td>2*</td>
</tr>
<tr>
<td>L-SH-3-R3 (10 µl)</td>
<td></td>
<td></td>
<td>22*</td>
<td>5*</td>
</tr>
<tr>
<td>L-SH-3-R3 (20 µl)</td>
<td></td>
<td></td>
<td>15*</td>
<td>8*</td>
</tr>
</tbody>
</table>

* Lymphocytes were sensitized with SH-3 tumor cells or fractions, and then cytotoxic activity of sensitized cells was tested against SH-3 and COLO 110. Numbers in parentheses, concentrations of stimulating cell or cell fraction.

† S.D. of the triplicates of the percentage of %¹⁰⁵ release ranged from 1.0 to 5.0. Only 3 values exceeded 4.0.

‡ Mean ± S.D.

§ * 0.01 < p ≤ 0.05 for the test comparing %¹⁰⁵ release from specific target by sensitized and unsensitized lymphocytes.

¶ 0.01 < p ≤ 0.05 for the test comparing %¹⁰⁵ release from specific target by sensitized and unsensitized lymphocytes.

‖ 0.01 < p ≤ 0.05 for the test comparing %¹⁰⁵ release from specific and nonspecific targets.

†† p ≤ 0.01 for the test comparing %¹⁰⁵ release from specific and nonspecific targets.
Table 5
Reactivity of lymphocytes sensitized by SH-3 tumor cells or cell fractions

<table>
<thead>
<tr>
<th>Effector-stimulating cell or cell fraction*</th>
<th>Source of cell or cell fraction</th>
<th>Target alone</th>
<th>Target + unsensitized lymphocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-SH-3-cells</td>
<td>SH-3 (breast)</td>
<td>10 ± 1.0</td>
<td>10 ± 1.0</td>
</tr>
<tr>
<td>E-SH-3-cells</td>
<td></td>
<td>50*</td>
<td>43*</td>
</tr>
<tr>
<td>E-SH-3-cells</td>
<td></td>
<td>25*</td>
<td>18*</td>
</tr>
<tr>
<td>E-SH-3-S1</td>
<td></td>
<td>14*</td>
<td>14*</td>
</tr>
<tr>
<td>E-SH-3-R1</td>
<td></td>
<td>13*</td>
<td>2</td>
</tr>
<tr>
<td>E-SH-3-R2</td>
<td></td>
<td>20*</td>
<td>9*</td>
</tr>
<tr>
<td>F-SH-3-cells</td>
<td>SH-3</td>
<td>14*</td>
<td>9*</td>
</tr>
<tr>
<td>F-SH-3-R2</td>
<td></td>
<td>10*</td>
<td>5*</td>
</tr>
</tbody>
</table>

* Lymphocytes were sensitized with SH-3 tumor cells or fractions, and then cytotoxic activity of sensitized cells was tested against SH-3, COLO 53, COLO 59, and COLO 38. COLO 53 (tumor line) and COLO 59 (lymphoid line) were established from the same patient.

Table 6
Reactivity of lymphocytes sensitized by SH-3 tumor cells or cell fractions

<table>
<thead>
<tr>
<th>Effector-stimulating cell or cell fraction*</th>
<th>Source of cell or cell fraction</th>
<th>Target alone</th>
<th>Target + unsensitized lymphocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>K-SH-3-cells</td>
<td>SH-3 (breast)</td>
<td>14 ± 1.0</td>
<td>12 ± 1.0</td>
</tr>
<tr>
<td>K-SH-3-cells</td>
<td></td>
<td>21*</td>
<td>14*</td>
</tr>
<tr>
<td>K-SH-3-R2</td>
<td></td>
<td>19*</td>
<td>27*</td>
</tr>
<tr>
<td>K-SH-3-R2</td>
<td></td>
<td>19*</td>
<td>29*</td>
</tr>
<tr>
<td>K-SH-3-R2</td>
<td></td>
<td>12*</td>
<td>10*</td>
</tr>
</tbody>
</table>

* Lymphocytes were sensitized with SH-3 tumor cells or fractions, and then cytotoxic activity of sensitized cells was tested against SH-3, RPMI-8322, COLO 8, and HT-29. RPMI-8322 and COLO 8 are tumor and normal lymphoid lines from the same patient.

RESULTS

Immunization with Tumor Cell Fractions. Various cell fractions isolated from tumor lines SH-3 and RPMI-7932 by differential centrifugations were tested for their ability to transform allogeneic lymphocytes into cytotoxic effector cells. Cell fractions sedimented at 1,000 × g (R1), 20,000 × g (R2), and 100,000 × g (R3) could immunize lymphocytes against specific tumor cells (Table 2). R1 fractions were not tested extensively because they contained a few intact cells and some cells that were not completely disrupted or lysed. R2 fractions seem to be more immunogenic than R3 fractions (P < 0.05); however, the difference between R2 fractions and intact tumor cells was not statistically significant.

Lymphocytes from the same donors were sensitized on 2 different days for determination of the variability in their response. In lymphocytes from Donor K, sensitized twice with SH-3-R3 on different days, the percentage of killing of specific tumor targets by sensitized lymphocytes was 29 and 23%, respectively. Lymphocytes from Donor G killed 25 and 21% after sensitization with SH-3-R2 in 2 different experiments, and lymphocytes from Donor S killed 23 and 44% after sensitization with SH-3-R2 on 2 different days. It appears that lymphocytes from a donor can be stimulated to become cytotoxic with tumor cell fractions on different days. Although there is variability in response, the percentage of specific killing after sensitization on each day was significant.

Although lymphocytes sensitized with cell fractions isolated from SH-3 could kill a specific target, they were also cytotoxic to cells from RPMI-7932 and RPMI-8322 (Table 2). In 7 of 24 cases, the difference between the killing of specific and nonspecific targets was significant when SH-3,
Table 7

In vitro lymphocyte stimulation with tumor cell fractions and the reactivity of stimulated lymphocytes against normal lymphoblasts

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Effector-stimulating cell fraction*</th>
<th>Source of stimulating cell fractions and tumor targets</th>
<th>% specific %^{1}Cr releaseb</th>
<th>Normal lymphoblasts</th>
<th>Normal lymphoblasts donors</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A-SH-3-S1</td>
<td>SH-3 (breast)</td>
<td>23d</td>
<td>-5</td>
<td>SK</td>
</tr>
<tr>
<td></td>
<td>A-SH-3-R1</td>
<td></td>
<td>27d</td>
<td>-11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A-SH-3-R2</td>
<td></td>
<td>31b</td>
<td>-7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A-SH-3-S2</td>
<td></td>
<td>14d</td>
<td>-10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A-SH-3-R3</td>
<td></td>
<td>21d</td>
<td>-5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A-RPMI-7932-R1</td>
<td>RPMI-7932 (melanoma)</td>
<td>17d</td>
<td>-6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A-RPMI-7932-R2</td>
<td></td>
<td>14d</td>
<td>-5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A-RPMI-7932-R3</td>
<td></td>
<td>13d</td>
<td>-4</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>D-SH-3-S1</td>
<td>SH-3</td>
<td>26d</td>
<td>-6</td>
<td>SB</td>
</tr>
<tr>
<td></td>
<td>D-SH-3-R2</td>
<td></td>
<td>51d</td>
<td>-2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D-SH-3-R3</td>
<td></td>
<td>22d</td>
<td>-10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D-RPMI-7932-S1</td>
<td>RPMI-7932</td>
<td>11</td>
<td>-3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D-RPMI-7932-R1</td>
<td></td>
<td>12d</td>
<td>-8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D-RPMI-7932-R2</td>
<td></td>
<td>16d</td>
<td>-3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D-RPMI-7932-R3</td>
<td></td>
<td>32d</td>
<td>-2</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>E-SH-3-S1</td>
<td>SH-3</td>
<td>30d</td>
<td>1</td>
<td>AK</td>
</tr>
<tr>
<td></td>
<td>E-SH-3-R2</td>
<td></td>
<td>29d</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>E-SH-3-R3</td>
<td></td>
<td>14b</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>E-RPMI-7932-R2</td>
<td>RPMI-7932</td>
<td>19d</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>F-SH-3-S1</td>
<td>SH-3</td>
<td>22d</td>
<td>-6</td>
<td>DK</td>
</tr>
<tr>
<td></td>
<td>F-SH-3-R2</td>
<td></td>
<td>28d</td>
<td>-7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>F-SH-3-R3</td>
<td></td>
<td>17d</td>
<td>-9</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>G-SH-3-S1</td>
<td>SH-3</td>
<td>19d</td>
<td>-5</td>
<td>JS</td>
</tr>
<tr>
<td></td>
<td>G-SH-3-R2</td>
<td></td>
<td>21d</td>
<td>-8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G-SH-3-R3</td>
<td></td>
<td>11d</td>
<td>-7</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>I-RPMI-7932-R2</td>
<td>RPMI-7932</td>
<td>25</td>
<td>-1</td>
<td>RH</td>
</tr>
<tr>
<td></td>
<td>I-RPMI-7932-R3</td>
<td></td>
<td>13</td>
<td>-1</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>J-SH-3-S1</td>
<td>SH-3</td>
<td>23b</td>
<td>1</td>
<td>ED</td>
</tr>
<tr>
<td></td>
<td>J-SH-3-R2</td>
<td></td>
<td>21d</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>K-SH-3-S2</td>
<td>SH-3</td>
<td>11d</td>
<td>28</td>
<td>DB</td>
</tr>
<tr>
<td></td>
<td>K-SH-3-R3</td>
<td></td>
<td>29d</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>L-SH-3-R2</td>
<td>SH-3</td>
<td>19b</td>
<td>-7</td>
<td>EJ</td>
</tr>
<tr>
<td>13</td>
<td>M-SH-3-R2</td>
<td>SH-3</td>
<td>14d</td>
<td>-5</td>
<td>ED</td>
</tr>
<tr>
<td></td>
<td>M-SH-3-R3</td>
<td></td>
<td>16d</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>N-SH-3-R2</td>
<td>SH-3</td>
<td>13</td>
<td>11</td>
<td>BB</td>
</tr>
<tr>
<td></td>
<td>N-SH-3-R3</td>
<td></td>
<td>10d</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>O-RPMI-7932-S2</td>
<td>RPMI-7932</td>
<td>14d</td>
<td>-1</td>
<td>JB</td>
</tr>
<tr>
<td>16</td>
<td>P-SH-3-S1</td>
<td>SH-3</td>
<td>10d</td>
<td>-1</td>
<td>CD</td>
</tr>
<tr>
<td></td>
<td>P-SH-3-R2</td>
<td></td>
<td>11b</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Q-SH-3-R2</td>
<td>SH-3</td>
<td>12a</td>
<td>0</td>
<td>AR</td>
</tr>
<tr>
<td>18</td>
<td>R-RPMI-7932-S1</td>
<td>RPMI-7932</td>
<td>11a</td>
<td>-8</td>
<td>LR</td>
</tr>
<tr>
<td></td>
<td>R-RPMI-7932-R2</td>
<td></td>
<td>9</td>
<td>-4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R-RPMI-7932-R3</td>
<td></td>
<td>10a</td>
<td>-3</td>
<td></td>
</tr>
</tbody>
</table>

*a Effector cells were prepared by in vitro sensitization of lymphocytes with tumor cell fractions. Immune lymphocytes were subsequently tested for their ability to lyse %^{1}Cr-labeled tumor and normal lymphoblasts. Normal lymphoblasts were prepared by incubation of the human peripheral blood lymphocytes with PHA solution as described in the text.

*b 0.01 < p < 0.05 for the test comparing %^{1}Cr release from specific target by sensitized and unsensitized lymphocytes.

*c S.D. of the triplicates of the percentage of %^{1}Cr release ranged from 1.0 to 14.0. Only 1 value exceeded 6.0.

*d p < 0.01 for the test comparing %^{1}Cr release from specific target by sensitized and unsensitized lymphocytes.
B. Sharma

Table 8
Lysis of PHA-induced blast cells by lymphocytes sensitized with allogeneic lymphocytes

<table>
<thead>
<tr>
<th>Mixed lymphocyte culture</th>
<th>Targets*</th>
<th>% ³¹Cr release*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A + Am</td>
<td>SK</td>
<td>6</td>
</tr>
<tr>
<td>A + SKm</td>
<td>SK</td>
<td>28</td>
</tr>
<tr>
<td>D + Dm</td>
<td>SB</td>
<td>15</td>
</tr>
<tr>
<td>D + SBm</td>
<td>SB</td>
<td>35</td>
</tr>
<tr>
<td>E + Em</td>
<td>AK</td>
<td>26</td>
</tr>
<tr>
<td>E + AKm</td>
<td>AK</td>
<td>34</td>
</tr>
</tbody>
</table>

* Target cells are normal lymphocytes from Donors SK, SB, and AK that have been transformed by PHA.

S.D. of the triplicates of the percentage of ³¹Cr release ranged from 2.0 to 8.0. Only 2 values exceeded 3.0.

SKm, SBm, and AKm are mitomycin C-treated stimulating lymphocytes.

These are % specific ³¹Cr release.

RPMi-7932, and RPMI-8322 were tested. In 4 of these cases, the killing of specific targets was greater than that of nonspecific targets. For determination of whether immune cells are also equally cytotoxic to cells from other tumor lines, the cytotoxic activity of lymphocytes sensitized to SH-3 was tested against that of HT-29 (coloon carcinoma), COLO 110 (ovary carcinoma), COLO 53 (malignant melanoma), and COLO 38 (malignant melanoma). In all 9 cases, the killing of the SH-3 target was significantly higher than the killing of the HT-29 target (p = 0.01 to 0.05) (Table 3). Similarly, in 5 of 6 cases, lysis of specific SH-3 target was significantly higher than lysis of COLO 110 (p = 0.01 to 0.05) (Table 4); no significant difference was noted between SH-3 and COLO 53, and in 2 of 5 cases there was significant difference between SH-3 and COLO 38 (Table 5). In 4 of 4 cases, lysis of specific SH-3 was significantly greater than lysis of HT-29; in 2 of 4 cases, lysis of the specific target was greater than that of RPMI-8322 (Table 6).

The specificity of immunization was also tested by the comparison of the cytotoxic activity of sensitized lymphocytes against tumor and normal lymphoid lines both established from the same patient (Tables 5 and 6). COLO 53 and COLO 59 were tumor and normal lymphoid lines of a patient, and RPMI-8322 and COLO 8 were tumor and lymphoid lines established from another patient. In a few of the experiments done, sensitized lymphocytes, while generally cytotoxic to tumor cells, were unable to lyse cells from the lymphoid line of the same patient (Tables 5 and 6).

The cytotoxic activity of effector cells generated against tumor cells was also tested against normal lymphocytes that have been transformed by PHA. Although lymphocytes sensitized with cell fractions lysed specific tumor targets, there was no killing of PHA-induced normal lymphoblast cells in 40 of 42 cases (Table 7).

In a test of susceptibility of PHA-transformed cells to lysis, lymphocytes from Donors A, D, and E were stimulated with allogeneic lymphocytes from Donors SK, SB, and AK, respectively. The cytotoxic activity of sensitized cells was then tested against specific lymphocytes that had been cultured for 5 days with PHA. The results given in Table 8 show that PHA-induced normal blast cells are susceptible for lysis by the cytotoxic effector cells.

**Effect of Various Concentrations of Fractions.** Lymphocytes were immunized with various concentrations of tumor cell fractions for determination of the optimal dose for inducing immunity. The maximum response was obtained with 5 to 20 µl of cell fractions (Table 9). R2 fractions induced maximum immunity with 10 µl in 4 of 5 cases. R3 fractions produced the peak response with 5 µl in 5 of 6 cases. The cell fractions at a concentration of 40 to 100 µl were able to induce only weak responses. Lymphocytes sensitized with these doses of fractions caused less than 10% lysis of tumor targets in 8 of 10 cases.

**DISCUSSION**

We previously reported that cultured cells from human tumors can be used to immunize peripheral blood lymphocytes (14–17). The results of this study demonstrate that cell fractions isolated from tumor lines SH-3 (breast carcinoma) and RPMI-7932 (malignant melanoma) are also capable of the immunization of lymphocytes from normal persons against human tumor cells. Lymphocytes cultured alone in human AB plasma were usually not cytotoxic to tumor cells. When incubated with tumor cell fractions for 5 days, these lymphocytes acquired the ability to kill tumor cells. Recently, Zielinski and Golub (19) and Ortalo and Bonnard (20) also found that lymphocytes cultured alone in human serum did not become cytotoxic to tumor cells; however, they became cytotoxic to a variety of cultured human tumor and normal target cells when incubated in fetal bovine serum.

Lymphocytes sensitized with SH-3 or RPMI-7932 tumor cell fractions were able to kill the respective tumor targets. Immunization with SH-3 cell fractions, however, produced consistent cross-immunization against RPMI-7932 and RPMI-8322 (melanoma) tumor cell lines, and immunization with RPMI-7932 produced cross-immunization to allogeneic melanoma (RPMI-8322) and breast (SH-3) tumor cells (Table 2). However, not all tumor lines were equally susceptible to lysis. Cells from RPMI-7932 seem to be most susceptible, whereas cells from HT-29 (colon carcinoma) and COLO 110 (cancer of ovary) are relatively resistant to lysis. Lysis of tumor cells other than that used in immunization is perhaps due to the presence of some common antigenic determinants. Such antigenic cross-reactivity among tumor cells of various histological types was demonstrated earlier by us (16) and other investigators (5, 12, 18). Irie et al. (5) recently reported that an antigen present on a melanoma tumor line was also found on a variety of different histological types of biopsied and cultured cancer cells. This antigen, however, was not present on biopsied normal tissue. Similar cross-reactivity, possibly due to shared antigen determinants, has been demonstrated recently in lymphoid cell lines by Corley et al. (1). Evidence presented by these investigators also indicated that histocompatibility (HL-A), blast-associated antigens, and EB viral-coded antigens were probably not the cross-reactive antigens shared by the 2 lymphoid lines. The possibility exists that effector cells generated against tumor cells are due to the difference in histocompatibility antigens. If this is the case, then effector cells generated against tumor cells should also be cytotoxic to normal lymphoblast cells. When the cytotoxic activity of sensitized lymphocytes was compared against
In vitro immunization of lymphocytes against human tumor cells with different concentrations of tumor cell fractions

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Effector-stimulating tumor cell fraction</th>
<th>Source of stimulating tumor cell fractions and targets</th>
<th>Targets alone</th>
<th>Unsensitized lymphocytes + targets</th>
<th>% specific <strong>1°Cr release</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A-SH-3-S1</td>
<td>SH-3</td>
<td>(10)</td>
<td>20 ± 1.0</td>
<td>18 ± 2.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(20)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(40)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A-SH-3-R3</td>
<td>SH-3</td>
<td>(10)</td>
<td>20 ± 4.0</td>
<td>20 ± 1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(20)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(40)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>B-SH-3-R2</td>
<td>SH-3</td>
<td>(5)</td>
<td>18 ± 2.0</td>
<td>18 ± 1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(20)</td>
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* S.D. of the triplicates of the percentage of °Cr release ranged from 1.0 to 18.0. Only 5 values exceeded 6.0.

* Concentrations of fractions in µl.

* Mean ± S.D.
those of tumor cells and normal lymphoid cells, both established from the same patient, it was found that, while effector cells were cytotoxic to tumor cells, they were not capable of lysing lymphoblast cells. Furthermore, in 40 of 42 cases these immune cells were also not cytotoxic to normal lymphocytes that had been transformed by PHA. However, the PHA-induced normal lymphoblast cells were quite sensitive to lysis by lymphocytes sensitized against allogeneic histocompatibility antigens (Table 8).

These findings suggest that antigen(s) present on tumor cells or cell fractions that provided the stimulus and the target are not likely to be HL-A, blast, or viral antigens (tumor cells were virus and Mycoplasma free). However, the possibility that histocompatibility antigens are not involved will need to be confirmed by comparing cytotoxic activity against more tumor and normal cells from the same patients and, if possible, in an autochthonous system.

Although the antigens responsible for stimulation have yet to be defined, the possibility remains that either cytotoxicity with features of natural killing may have been induced by tumor cell fractions (10) or simply that the stimulation is caused by some common tumor-associated antigenic determinants present in tumor cells of various histological types.

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REFERENCES

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Brahma Sharma


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