CONCISE REPORT

Small Cell Lung Cancer, Endocrine Cells of the Fetal Bronchus, and Other Neuroendocrine Cells Express the Leu-7 Antigenic Determinant Present on Natural Killer Cells

By Paul A. Bunn, Jr., Ilona Linnoila, John D. Minna, Desmond Carney, and Adi F. Gazdar

Small cell lung cancer is distinguished from other lung cancer histologic types by possessing a variety of neuroendocrine properties. Anti-Leu-7 is a monoclonal antibody that recognizes a 110,000-dalton molecular weight glycoprotein initially described on natural killer cells and subsequently reported on a variety of normal and malignant neural and neuroendocrine cell types. We have found intense anti-Leu-7 binding to a large number of small cell lung cancers, while other lung cancer types were negative or showed only weak and focal binding. Other antigens expressed by natural killer cells, lymphocytes, and mononuclear cells were never or less often expressed on small cell lung cancer cells. In addition, we report for the first time anti-Leu-7 binding by carcinoids, carotid body tumors, pheochromocytomas, endocrine cells of the fetal bronchus and the adult intestine, and select pancreatic islet cells. Anti-Leu-7 binding by small cell lung cancer is consistent with a derivation from pulmonary precursor cells, and anti-Leu-7 staining is clinically useful for the identification of human neuroendocrine tumors of the amine precursor uptake and decarboxylation ("APUD") type.

MATERIALS AND METHODS

Cell Lines

We have established and characterized permanent cell lines from lung cancer patients, which can be classified as "classical" small cell lung cancer (SCLC-C), "variant" small cell lung cancer (SCLC-V), and non-small cell carcinomas (NSCLC) including adenocarcinomas, epidermoid carcinomas, and large cell carcinomas. SCLC-C cell lines are distinguished by the presence of a variety of amine precursor uptake and decarboxylation (APUD) markers, including neurosecretory granules, l-dopa decarboxylase (EC 4.1.1.28), creatine kinase BB isoenzyme (CK-BB) (EC 2.7.3.2), neuron-specific enolase (NSE) (EC 4.2.1.11), and polypeptide hormones including bombesin, as well as having the unique 3p chromosomal marker. SCLC-V cell lines maintain the 3p marker, and the production of CK-BB and NSE, but have different cytologic and histologic features, a more rapid doubling time, and failure to express l-dopa decarboxylase, peptide hormone production, and neurosecretory granules. Most SCLC-V lines also have amplified expression of the c-myc oncogene. NSCLC lines usually lack all of these properties. Miscellaneous T cell or B cell lines established in our laboratory were also studied.

Monoclonal Antibodies

Anti-Leu-7, anti-Leu-11, anti-Leu-M1, anti-Leu-M2 anti-Leu-M3, and anti-Leu-12 were purchased from Becton Dickinson, (Sunnyvale, Calif.). HNK-1 was provided as purified ascites courtesy of Dr. C. Balch, Birmingham, Ala. OKT1, OKT9, and OKT10 were purchased from Ortho Diagnostics (Raritan, NJ).

Indirect Immunofluorescence Assays

Cell suspensions from cell lines were incubated with saturating concentrations of the monoclonal antibody (106 cells in 100 μL at 4°C for 30 minutes), washed two times, incubated with fluoresceinated goat-anti-mouse Ig of the appropriate class (Becton Dickinson; 1:50 dilution of 1 mg/mL in 100 μL at 4°C for 30 minutes), washed two times, and analyzed for log fluorescence intensity using an EPICS V fluorescence-activated cell sorter (Coulter Electronics,
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Strongly positive. While the SCLC-C line NCI-H209 shows reactivity only slightly above control binding.

Table 1. Immunofluorescence Analysis of Antibody Reactivity of Various Cell Lines

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Expression</th>
<th>SCLC</th>
<th>NSCLC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-Leu-7</td>
<td>Natural killer</td>
<td>24/27</td>
<td>1/6</td>
</tr>
<tr>
<td>Anti-Leu-11</td>
<td>Natural killer</td>
<td>6/24</td>
<td>0/3</td>
</tr>
<tr>
<td>Anti-OKT 10</td>
<td>Natural killer/immature lymphocytes</td>
<td>8/26</td>
<td>0/3</td>
</tr>
<tr>
<td>Anti-OKM1</td>
<td>Monocytes</td>
<td>11/26</td>
<td>2/5</td>
</tr>
<tr>
<td>Anti-Leu-M1</td>
<td>Monocytes/granulocytes</td>
<td>20/23</td>
<td>2/6</td>
</tr>
<tr>
<td>Anti-Leu-M2</td>
<td>Monocytes</td>
<td>17/20</td>
<td>0/2</td>
</tr>
<tr>
<td>Anti-Leu-M3</td>
<td>Monocytes</td>
<td>0/23</td>
<td>0/6</td>
</tr>
<tr>
<td>Anti-OKT1</td>
<td>T cells</td>
<td>0/10</td>
<td>0/6</td>
</tr>
<tr>
<td>Anti-Leu-12</td>
<td>B cells</td>
<td>0/10</td>
<td>0/6</td>
</tr>
<tr>
<td>Anti-OKT9</td>
<td>Transferrin receptor</td>
<td>23/23</td>
<td>6/6</td>
</tr>
</tbody>
</table>

Hialeah, Fla). For each cell line and antibody control, samples were stained with a nonreactive primary antibody (Litton Bionetics, Kensington, Md) and secondary antibody alone.

Immunoperoxidase Staining of Cell Lines and Paraffin-Embedded Tissues

Tissue was obtained from patients undergoing tumor biopsy or resection. Samples were fixed in formalin and processed to obtain 5-μm paraffin sections. Cell lines were cytocentrifuged (Shandon, London, England) onto glass slides and fixed in cold acetone in ethanol (4°C). The cytocentrifuge preparations and deparaffinized sections were incubated with HNK-1 ascites (1:1,000 dilution for 20 hours at 4°C) and immunoperoxidase staining was performed using the avidin-biotin technique. Controls included slides stained with non-immune mouse serum and/or omission of primary antibody.

RESULTS

Indirect Immunofluorescence of Cell Lines

Leu-7 expression by various cell lines is shown in Table 1 and Fig 1. Leu-7 was expressed on 23/27 (85%) small cell lines tested, including 17/20 classic and 6/7 variant lines. While there was some heterogeneity of binding, 75% or more of the cells were positive in 20/23 reactive lines. The antigen expression was variable, but was generally expressed in high density as evidenced by the high fluorescence intensity (mean channel number, 20) compared with controls (mean channel number, two) or with the positive non-small cell line (mean channel number, six). Weak staining was noted in 1/6 non-small cell lung cancer lines and the others were negative. Expression of the antigen was not cell cycle related as demonstrated by dual fluorescence analysis with DNA and anti-Leu-7 staining (data not shown).

The reactivity of the cell lines with other NK cell, monocyte, and lymphocyte antibodies is also shown in Table 1. Anti-Leu-11 reacted with only 6/24 SCLC lines and the cell line that had the strongest reactivity (57% positive) had little reactivity with anti-Leu-7 (10% positive). Similarly, OKT10 and OKM1 were only occasionally expressed and there was no relationship between their expression and Leu-7 expression. There was marked heterogeneity of expression with the “monocyte” antigens OKM1, Leu-M1, Leu-M2, and Leu-M3, with the majority of lines expressing Leu-M1 and Leu-M2, an intermediate number expressing OKM1, and none expressing Leu-M3. None of the

Table 2. Immunohistochemical Analysis of Cell Lines and Tissue Sections With Anti-Leu-7 Antibody

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Percentage of Positive Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10-50</td>
</tr>
<tr>
<td>Cultured cell lines</td>
<td></td>
</tr>
<tr>
<td>SCLC-C</td>
<td>14</td>
</tr>
<tr>
<td>SCLC-V</td>
<td>2</td>
</tr>
<tr>
<td>NSCLC</td>
<td>5</td>
</tr>
<tr>
<td>Miscellaneous*</td>
<td>4</td>
</tr>
<tr>
<td>Tissue sections</td>
<td></td>
</tr>
<tr>
<td>SCLC</td>
<td>20</td>
</tr>
<tr>
<td>NSCLC</td>
<td>33</td>
</tr>
<tr>
<td>Pheochromocytoma</td>
<td>10</td>
</tr>
<tr>
<td>Carcinoid tumor</td>
<td>6</td>
</tr>
<tr>
<td>Carotid body tumor</td>
<td>1</td>
</tr>
<tr>
<td>Colon carcinoma</td>
<td>2</td>
</tr>
</tbody>
</table>

*Miscellaneous tumor cell lines include two B lymphoblastoid and two colon carcinoma cell lines.
Fig 2. Photomicrographs of immunohistochemical staining of cell lines and tissue sections with anti-Leu-7. (A) A cytocentrifuge preparation of an SCLC-C cell line (NCI-H128), demonstrating positive brown immunoprecipitation on the cell membrane and cytoplasm (original magnification x 580). (B) A bottle-shaped endocrine or Kulachitzky cell positively stained, with anti-Leu-7 projecting into the lumen in a fetal bronchus lined by ciliated cells (original magnification x 1,400). (C) SCLC tissue section showing an intense immunoprecipitate in tumor cells surrounded by fibrotic tissue that is not stained (original magnification x 360). (D) Tissue section of a large cell carcinoma of the lung that remained negative after anti-Leu-7 staining (original magnification x 360). (E) Select endocrine cells in a normal pancreatic islet from an adult demonstrate a positive reaction with anti-Leu-7. The distribution of these cells corresponded to that of non-insulin-producing cells. Exocrine pancreas remains negative (original magnification x 360). (F) Peripheral nerve in lung shows a dark immunoprecipitate in longitudinal fibers on the left and in cross-section on the right (original magnification x 360).
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lines expressed OKT1 or Leu-12 and all expressed the transferrin receptor (OKT9).

**Immunoperoxidase Staining of Cell Lines and Tissue Sections (Table 2)**

The immunoperoxidase staining patterns of malignant cell lines were similar to the immunofluorescence results. The SCLC-C and SCLC-V cell lines were nearly always positive (12/14 SCLC-C and 2/2 SCLC-V), and the majority of cells were usually strongly positive (Fig 2A). In contrast, only 1/5 NSCLC lines and none of four miscellaneous tumor cell lines reacted with anti-Leu-7.

Analysis of tissue sections demonstrated that the anti-Leu-7 antigen was expressed on direct fresh human SCLC tumors (Table 2, Fig 2C). The majority of small cell tumors (16/20) were reactive. In positive sections, nearly all of the cells were positive (Fig 2C), but the intensity of staining varied between specimens. In contrast, 26/33 NSCLC tumors were unreactive (Fig 2D). The seven specimens that reacted were focally positive with staining of small clusters or occasional solitary cells. The immunoperoxidase technique was used to study other malignant neuroendocrine tumors from our pathology files as well as a variety of normal and fetal tissues. All ten pheochromocytomas, all six carcinoid tumors, and a carotid body tumor were reactive cells were non-insulin-producing (non-B) islet cells. Staining of fetal bronchus (Fig 2B) and adult intestinal mucosa also showed reactivity of select cells that also stained with antichromogranin and antibombesin antibodies. The morphology, location, and antibody reactivity patterns of these cells indicate that they correspond to the neuroendocrine cells of these organs. As reported by others, anti-Leu-7 also stained myelinated peripheral nerves (Fig 2F).

**DISCUSSION**

We have shown that an antigenic determinant present on NK cells is also expressed on SCLC cells, their putative precursor cells, and other benign and malignant neuroendocrine cells. The antibody is clinically useful in distinguishing SCLC from non-SCLC by immunoperoxidase staining of tissue sections or by immunofluorescence analysis of single cell suspensions. Anti-Leu-7 is also useful in distinguishing such neuroendocrine tumors as pheochromocytomas, carcinoids, and carotid body tumors from other epithelial tumors of the same organ. The antigen recognized by anti-Leu-7 has not been characterized from any cell lineage. Thus, whether it recognizes truly identical antigens on all cell lineages or, rather, reacts with similar epitopes on differing antigenic structures remains to be determined. However, the fact that anti-Leu-7 recognizes purified extracted myelin-associated glycoprotein suggests that this may be the antigen recognized in nervous tissues.

SCLC cells often expressed other antigens present on natural killer cells and monocytes. However, none of the antibodies we evaluated were routinely reactive with SCLC and none were useful for distinguishing SCLC from non-SCLC. Ruff and Pert recently reported that two small cell lines and several autopsy specimens had macrophage antigens and suggested the possibility that SCLC was derived from bone marrow macrophages. Our data show that SCLC tumors do not react universally with macrophage antigens. Leu-M1 was the most consistently expressed antigen and the antigen recognized by anti-Leu-M1 is the glycolipid LNFP III, which was reported to be an immunodominant antigen expressed by a variety of malignancies, including lung and colon cancers.

We have previously shown that class I histocompatibility antigens can distinguish between SCLC and non-SCLC tumors because it is weakly expressed or absent on SCLC cells.

The presence of antigens on SCLC and other cell types of diverse function and embryologic origin does not necessarily imply a common ancestry. For example, the pan-T lymphocyte antigen OKT3 is expressed on cerebellar Purkinje's cells and LNFP III is expressed on other cell types including the proximal renal tubule. Thus, we do not believe that SCLC shares a common lineage with NK cells or monocytes. Rather, the pattern of anti-Leu-7 binding with fetal bronchial endocrine cells is consistent with differentiation of normal and malignant bronchial epithelial cells along endocrine and non-endocrine pathways.

The fact that SCLC share antigens with nervous tissues may also explain the pathophysiology of well-described idiopathic paraneoplastic syndromes. Sensorimotor neuropathies and autonomic neuropathies (orthostatic hypotension and intestinal pseudo-obstruction or Ogilvie's syndrome) reported in SCLC and associated with preferential destruction of myelinated nerves may be caused by crossreactivity of endogenous cytotoxic antibodies directed against the Leu-7 antigen on SCLC tumor cells.

Other monoclonal antibodies with specificity for either SCLC or NSCLC have been reported in the past.
In our experience, neither of these has as high a specificity as anti-Leu-7 for SCLC. We are currently undertaking prospective and retrospective studies of large panels of lung cancers stained with anti-Leu-7 and these other anti-lung cancer antibodies to determine which antibodies, alone or in combination, are most effective in distinguishing histologic types and subtypes and in assigning prognosis.

ACKNOWLEDGMENT

We are indebted to Dr A. Koros for stimulating our interest in this area and for helpful discussions, and to P. Jewett and J. Norton for expert technical assistance.

REFERENCES


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