Increased Serum and Urinary Calcitonin Levels in Patients with Pulmonary Disease*

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Serum and urinary calcitonin levels were measured in patients with acute and chronic inflammatory diseases of the lung. Using both carboxyl terminal and midportion antisera, the incidence of increased immunoreactive values of this hormone was 68 percent for patients with emphysema, 59 percent for tuberculosis, and 89 percent for acute bacterial pneumonitis. In order to determine the source of the high levels of calcitonin, immunoperoxidase stains were made of sections of human lung; the hormone was found within the bronchial Kulitschzyk cell (K cell). This suggests a specific endocrine role for the K cell, and may explain not only the high calcitonin levels in patients with inflammatory lung disease, but also the high levels associated with both carcinoma and small cell carcinoma, which may originate from K cells. It is apparent that moderately high levels of calcitonin in a patient with pulmonary disease cannot always be assumed to be associated with tumor.

Previously, we reported finding detectable immunoreactive calcitonin (iCT) in the serum and urine of thyroidectomized man, suggesting that the synthesis of this hormone is not confined to the thyroid gland.1 Subsequently, we extracted iCT from human lung, and in a study performed in the monkey, we detected significant amounts of pulmonary iCT two months following total thyroidec- tomy.2,3 In the present investigation, we have determined serum and urinary iCT in patients with acute and chronic inflammatory disease of the lung, have documented elevated values in many patients, and have localized the source of the hormone to the pulmonary Kulitschzyk cell (K cell).

MATERIALS AND METHODS

Radioimmunoassay of Serum and Urine iCT

Twenty-two patients with chronic obstructive emphysema and bronchitis, 17 with bacteriologically proven tuberculosis, and 15 with acute nontuberculous bacterial pneumonitis from the University of Texas Health Center, Tyler, TX, and the Veterans Administration Medical Center, Washington, DC, were studied. All patients were normocemic with a normal blood urea nitrogen and serum creatinine levels. Fifty-one were men, and three were women. One patient, a 25-year-old man with pneumonitis, was studied sequentially. A morning fasting serum specimen was collected in nonsiliconized vacutainer tubes, as well as a second morning urinary specimen which was collected in polyethylene or soft glass receptacles containing sufficient ammonium bicarbonate to maintain a pH ≥ 7.5. All samples were stored at —20°C until assayed. Control samples included similar specimens from 20 normal men.

The radioimmunoassays of serum and urine iCT were performed as we have previously reported, using a midportion antiserum, Ab-IIIb, and a carboxyl terminal antiserum, Ab-IV.4 As little as 10 pg/mg creatinine (Ab-IIIb) and 20 pg/mg creatinine (Ab-IV) can be detected. Intraassay and interassay variences were 5 percent and 15 percent, respectively. Losses of urine iCT from alkalized urines allowed to stand at room temperature for 24 hours are generally negligible.

Immunoperoxidase Localization of Pulmonary Calcitonin

Sections of three grossly normal and of two emphysematous lungs were obtained at autopsy from adults dying from various nonneoplastic causes. They were fixed in 10 percent neutral buffered formalin and studied as previously reported, using specific rabbit antiserum at 1:1000 for 48 hours.5-10 The antiserum had been pretreated by heating at 56°C for one half hour to remove complement. Three different anticalcitonin antisera were used: Ab-II, which reacts predominantly with the midportion of calcitonin; Ab-XII, which reacts with the carboxyl terminus; and Ab-XIII, which reacts with most regions of the hormone. Adjacent sections were stained for argyrophilia by the Singh11 modification of the Bodian stain, and the toluidine-blue stain with prior acid hydrolysis as described by Solcia et al.12

RESULTS

Radioimmunoassay of Serum and Urine iCT

Figures 1 and 2 illustrate the iCT values for serum
and urine obtained for patients with inflammatory lung disease, using Ab-IIIb and IV. Table 1 summarizes the mean serum and urinary iCT values of the different groups of patients and the significance of the differences. Table 2 summarizes the percentage of patients with inflammatory lung disease who had abnormally high iCT values, utilizing serum or urinary iCT radioimmunoassay with Ab-IIIb alone,
Table 1—Serum and Urinary Calcitonin in Inflammatory Lung Disease

<table>
<thead>
<tr>
<th></th>
<th>Ab-IIIb Mean ± SD</th>
<th>P</th>
<th>Ab-IV Mean ± SD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum (pg/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>80 ± 55</td>
<td></td>
<td>57 ± 43</td>
<td></td>
</tr>
<tr>
<td>Emphysema</td>
<td>217 ± 228</td>
<td>&lt;0.015</td>
<td>63 ± 45</td>
<td>NS</td>
</tr>
<tr>
<td>Tuberculosis</td>
<td>207 ± 144</td>
<td>&lt;0.001</td>
<td>88 ± 48</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>373 ± 526</td>
<td>&lt;0.02</td>
<td>133 ± 106</td>
<td>&lt;0.007</td>
</tr>
<tr>
<td>All patients</td>
<td>257 ± 324</td>
<td>&lt;0.02</td>
<td>90 ± 72</td>
<td>NS</td>
</tr>
<tr>
<td>Urine (pg/mg creatinine)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>246 ± 63</td>
<td></td>
<td>161 ± 61</td>
<td></td>
</tr>
<tr>
<td>Emphysema</td>
<td>541 ± 419</td>
<td>&lt;0.004</td>
<td>292 ± 187</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Tuberculosis</td>
<td>359 ± 267</td>
<td>NS</td>
<td>282 ± 275</td>
<td>NS</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>400 ± 214</td>
<td>&lt;0.005</td>
<td>318 ± 216</td>
<td>&lt;0.004</td>
</tr>
<tr>
<td>All patients</td>
<td>444 ± 332</td>
<td>&lt;0.01</td>
<td>298 ± 225</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Ab-IV alone, or both antisera. When both antisera were used to assay both serum and urine iCT, 68 percent of those with emphysema, 59 percent of those with tuberculosis, and 57 percent of those with pneumonia exceeded normal. Figure 3 demonstrates the high urine and serum iCT values in a patient with pneumonitis which returned to normal after recovery.

Immunoperoxidase Localization of Pulmonary iCT

In four of the five lungs examined, there was positive immunoperoxidase staining for iCT which was localized within rare, scattered discrete cells or groups of cells of the bronchiolar epithelium (Figure 4). With the exception of the patients with emphysema, who had an average of approximately one positively stained cell per transected bronchiole, multiple sections were required to locate an occasional stained cell. All three anticalcitonin antisera gave positive results. No staining occurred in non-immune rabbit serum-treated controls or in control sections treated with immunoextracted anticalcitonin antiserum. Positive immunoperoxidase staining of the C cells of normal human thyroid tissue and medullary thyroid cancer tissue was obtained. Adjacent sections of lung, stained for argyrophilia and by toluidine blue after acid hydrolysis, revealed that the rare immunoperoxidase-positive cells had a morphologic and histochemical similarity to that described for the bronchial K cells.15-18

Discussion

Calcitonin, the hypocalcemic, hypophosphatemic polypeptide hormone, is secreted by the C cells of the mammalian thyroid.18 It has been known for a decade that levels of this hormone are high in the serum of patients with medullary thyroid cancer, and, subsequently, high levels were also found in the urine of these patients.7,17 When we and others found that patients with lung cancer and breast cancer often have high levels of serum and urine iCT, it became apparent that this hormone was not a marker exclusively for medullary thyroid cancer.8,18-21 To date, many nonthyroid tumors have been reported to be associated with high levels of serum iCT.22 In the case of lung cancer, we found that there are two types of hypercalcitonemia: the thyroid type, in which the thyroid was secreting the hormone, and the ectopic type, in which the iCT was being secreted by the tumor.23,24

Furthermore, we and others have reported that serum iCT can be elevated in various other condi-

Table 2—Percentage of Patients with Inflammatory Lung Disease who Have Abnormally High Calcitonin Values

<table>
<thead>
<tr>
<th></th>
<th>Serum</th>
<th>Urine</th>
<th>Serum Plus Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ab-IIIb &amp; Ab-IV</td>
<td>Ab-IIIb &amp; Ab-IV</td>
<td>Ab-IIIb &amp; Ab-IV</td>
</tr>
<tr>
<td>Emphysema</td>
<td>36</td>
<td>5</td>
<td>36</td>
</tr>
<tr>
<td>Tuberculosis</td>
<td>41</td>
<td>18</td>
<td>47</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>47</td>
<td>33</td>
<td>60</td>
</tr>
<tr>
<td>All patients</td>
<td>41</td>
<td>17</td>
<td>46</td>
</tr>
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</table>

CHEST, 79: 2, FEBRUARY, 1981 INCREASED SERUM AND URINARY CALCITONIN LEVELS IN PULMONARY DISEASE 213
tions, such as hypercalcemia, chronic renal failure, pregnancy, pernicious anemia, Zollinger-Ellison syndrome, and pancreatitis, thus demonstrating that not all patients who have hypercalcitonemia have cancer.4,22,29

The present study was undertaken because we had reported finding significant amounts of iCT in extracted lung tissue of 14 patients dying of various noncancerous causes.2 This iCT, which could not be distinguished immunologically, chromatographically, or electrophoretically from the iCT extracted from normal thyroid tissue, exceeded the level found in blood by an average of eighteenfold. Subsequently, we demonstrated, in the monkey, that the pulmonary iCT persisted after total thyroidectomy.3

In the present study, high serum and/or urinary iCT levels were encountered in 70 percent of patients with acute or chronic inflammatory disease of the lung. The demonstration that urine iCT is also elevated in some of these patients, and that it may be increased in patients who have normal serum levels of the hormone is consistent with our prior finding in both medullary thyroid carcinoma and lung cancer.7,8

Serum iCT exists in multiple heterogeneous forms, and different antisera to iCT detect and quantitate these forms with different avidity.5,30 In the present study, the midportion recognizing antiserum, Ab-IIIb, was more useful than the carboxyl recognizing antiserum, Ab-IV, in detecting elevations of serum iCT. In contrast to serum, the heterogeneity of urine iCT is much less, and both antisera detect increased urinary iCT.8

The levels of serum and urine iCT found in some of the patients in the present study are similar to the levels encountered in many patients with lung cancer.8,18,19,30 Previously, we have reported that serum and urine iCT can be a clinically useful marker in patients with biopsy-proven bronchogenic cancer, and that serum levels often decrease concomitantly with successful chemotherapy and/or x-irradiation.18,19,22 In view of the hypercalcitonemia which we have shown to occur in pulmonary inflammatory disease and other aforementioned conditions, it is apparent that elevated iCT in serum or urine should not be misinterpreted as being diagnostic of malignancy.

We have reported that the hypercalcitonemia associated with medullary thyroid cancer differs in its pattern of heterogeneity from that encountered in patients with bronchogenic cancer, and we have utilized different anticalcitonin antisera in order to distinguish successfully these two entities.30 The heterogeneity pattern of the serum and urinary iCT

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**Figure 4.** Bronchiolar K cells containing immunoreactive calcitonin (× 1,000).
in patients with inflammatory pulmonary disease remains to be determined.

Previously, we reported finding immunocytochemical evidence of iCT within the K cells of the bronchioles of the lungs of the human neonate, and, in the present study, we have extended our observations to the adult.\textsuperscript{10} While the iCT-containing cells are quite sparse in number in most newborns, they are even more difficult to find in the adult, and locating them requires careful study of many sections. Of the five specimens of adult lung we examined, only the patients with emphysema demonstrated positive cells which could be found with relative ease. However, no systematic attempt was made to ascertain whether any particular anatomic area of the lung exhibited more positive staining cells than others. Further studies are needed to determine the precise intrapulmonary distribution of these cells and whether their number correlates with any specific disease process.

The K cells, which have been also termed enterochromaffin, argyrophil, or Feyrter cells, are known to contain dense-cored vesicles and have been postulated to serve a secretory function.\textsuperscript{31} Histopathologic studies indicate that these cells are the cells of origin of the bronchial carcinoid tumor and small cell carcinoma of the lung, both of which are associated with high levels of serum calcitonin.\textsuperscript{18,22,32-36}

Our findings, which suggest a specific endocrine function of the K cell, may also explain the persistence of detectable serum and urinary iCT in thyroidectomized man, the significant levels of extractable iCT in human lung, and the high serum and urine levels in patients with inflammatory pulmonary disease. Further studies are needed to determine the role of iCT in human lung in both health and disease.

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Bronchoesophagology Course

Temple University (Chevalier Jackson Clinic) will present a postgraduate course in Bronchoesophagology April 6-10, under the direction of Drs. Charles M. Norris, Gabriel F. Tucker, Jr., John A. Tucker, Bernard R. Marsh and Myles G. Turtz. For information, write: Chevalier Jackson Clinic, Temple University Hospital, 3401 North Broad Street, Philadelphia 19140.

Technology Assessment Forum: Coronary Artery Bypass Surgery

The National Center for Health Care Technology, in collaboration with the National Heart, Lung and Blood Institute, National Institutes of Health, will present a Technology Assessment Forum on Coronary Artery Bypass Surgery (economic, ethical and social issues), April 21-23 at the Sheraton-Washington Hotel, Washington, D.C. For information contact Michael Eliastam, M.D., National Center for Health Care Technology, Parklawn Building Room 17A-29, Rockville, Maryland 20857.

218 BECKER ET AL

CHEST, 79: 2, FEBRUARY, 1981