Immunohistochemical Localization of Placental Alkaline Phosphatase, Carcinoembryonic Antigen, and Cancer Antigen 125 in Normal and Neoplastic Human Lung

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ABSTRACT

Human placental alkaline phosphatase (HPLAP), carcinoembryonic antigen (CEA), and cancer antigen 125 (CA 125) were localized immunohistochemically in paraffin sections of normal lung tissue from 16 patients, using monoclonal antibodies and an indirect avidin-biotin-peroxidase staining procedure. HPLAP and CEA were present in epithelial cells of respiratory bronchioles and alveolar type I pneumocytes. CEA was also observed in the tracheal, bronchial, and bronchiolar epithelium; in the trachea, bronchial, and bronchiolar glands; and in the pleural mesothelium. Normal and hyperplastic type II pneumocytes were negative for HPLAP, CEA, and CA 125 but were histochemically positive for nonspecific alkaline phosphatase. Fetal lung tissue between 11 and 15 weeks of gestation was negative for HPLAP, CEA, and CA 125. The fetal tracheal and bronchial epithelium, tracheal glands, and pleural mesothelium were positive for CA 125. For ten malignant pulmonary tumors investigated, HPLAP staining was observed in five, CEA in nine, and CA 125 in seven. The localization of HPLAP, CEA, and CA 125 in apparently normal constituents of all pulmonary specimens is in disagreement with the concept that the expression of these substances in the lung is indicative of abnormal cellular activity.

INTRODUCTION

Since the original report of Fishman et al. (1) on the ectopic production of HPLAP in a patient with bronchiogenic squamous cell carcinoma, the clinical significance of HPLAP as a tumor marker for a variety of malignant tumors has been extensively investigated by the use of different biochemical techniques. The recent introduction of monoclonal antibodies to HPLAP has made it possible to obtain a hitherto unreachable degree of specificity for HPLAP with respect to the other isoenzymes of alkaline phosphatase (AP). This has opened new perspectives for the clinical application of HPLAP in cancer, but it has also led to the discovery of trace expression of HPLAP in a number of clinically normal organs, i.e., the ovary (2, 3), testis (4, 5), cervix (2, 6), tuba (7), and lung (2, 8). The latter observation is of particular interest in view of the increased serum levels of HPLAP in cigarette smokers (9-11), for whom the increased risk for lung cancer is well documented (12). Furthermore the expression of HPLAP in the lung and other normal tissues may restrict the future application of monoclonal antibodies to HPLAP for tumor immunoscintigraphy and immunotherapy.

The link between CEA and cancer, including pulmonary cancer, has been extensively investigated. However, serum CEA levels are also increased in a number of nonneoplastic diseases and, as for HPLAP, circulating levels of CEA are slightly elevated in healthy cigarette smokers (13, 14).

Cancer antigen 125 (CA 125) is a glycoprotein derived from an ovarian serous cystadenocarcinoma. It is present in large amounts in most nonmucinous ovarian neoplasms and is recognized by the mouse monoclonal antibody OC 125 (15). Serum levels of CA 125 are strongly increased in ovarian cancer patients, in a number of nonovarian cancer patients including lung cancer, and in some benign chronic pathologies (16). Interference with cigarette smoking has not been reported.

Little or no information is available on the histological distribution of HPLAP, CEA, and CA 125 in the normal lung, although the presence of two of these substances has been demonstrated biochemically. In the present study, we used monoclonal antibodies and sensitive immunohistochemical techniques to determine the localization of these antigens on adjacent sections of normal, pathological, and neoplastic lung tissue. The distribution pattern for HPLAP was compared with that of nonspecific AP activity. The presence of HPLAP, CEA, and CA 125 in the fetal lung was also investigated.

MATERIALS AND METHODS

Patients and Tissues. The whole protocol of this study was examined and found to be ethically acceptable by the Ad Hoc Committee at the University Hospital Antwerp.

All tissues were surgical biopsies, except the samples from Patient 16, which were obtained 12 h postmortem. Normal lung tissue was obtained from organ donors (Patients 1 to 8) and at the occasion of a pulmonary Echinococcus cyst resection (Patient 9). Separate samples from the upper, intermediate, and lower pulmonary lobes were taken from Patients 3, 4, and 5. The other tissues were taken during surgical resection of a pathological lung with benign disease or a malignant lung tumor. In the latter, separate samples were taken from (a) the center of the tumor, (b) the tumor periphery including invaded lung parenchyma, and (c) macroscopically normal lung tissue distally from the tumor. Fetal lung tissue was obtained from intact fetuses within 12 h after prosta glandin induced expulsion.

The tissues were cut into 1.5-mm-thick slices, which were sequentially allotted for histology and biochemical analysis. The samples were processed for histology or frozen in liquid nitrogen within 1 h after receipt.

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The abbreviations used are: AP, alkaline phosphatase; CA 125, cancer antigen 125; CEA, carcinoembryonic antigen; HPLAP, human placental alkaline phosphatase.
Determination of HPLAP in Tissue Butanol Extracts. The amount of HPLAP in tissue samples was determined by an enzyme-antigen immunoassay, based on a mouse monoclonal antibody against HPLAP (E6) (17), after homogenization and extraction with butanol. Extraction and assay procedures have been described in detail elsewhere (8). The antibody does not cross-react with liver, bone, kidney, or intestinal AP. The assay detection limit was 0.02 unit/liter. The within-assay and between-assay coefficients of variation were 6.1 and 7.0%, respectively. Calculation of sample estimates was performed using a spline function program.

Determination of Nonspecific AP Activity in Tissue Extracts. The nonspecific AP activity in tissue butanol extracts was determined by the method of Van Belle et al. (18). One unit of enzyme activity is defined as the activity liberating 1 μmol of 4-nitrophenol (E<sub>405</sub> = 14,600 liter mol<sup>-1</sup> cm<sup>-1</sup>) from 5 mmol of 4-nitrophenyl phosphate per min in 0.1 M N-acetyl-L-cysteine buffer, pH 10.2, at 30°C.

Immunohistochemical Localization of HPLAP, CEA, and CA 125. The tissue distribution of HPLAP, CEA, and CA 125 was determined in adjacent sections. The immunohistochemical staining procedures for CEA and CA 125 were the same as described previously for HPLAP (3). Briefly the tissues were fixed during 1.5 h in buffered 4% formol (0.1 M sodium cacodylate buffer, pH 7.4, containing 1% CaCl<sub>2</sub>) and were embedded in Paraplast or in low melting point paraffin. Five-μm sections were treated with trypsin and binding of the monoclonal antibodies was revealed by an indirect avidin-biotin-peroxidase method. Staining for peroxidase was performed with 3-amino-9-ethylcarbazole. Two specific monoclonal antibodies were used for the localization of HPLAP, i.e., E6 (17) and H317 (19). E6 and H317 culture supernatant was diluted 1/50 and 1/100, respectively. The Hybritech monoclonal antibody to CEA (Lot 360791), was used at a dilution of 1/100,000. Mouse ascitic fluid containing the monoclonal antibody OC 125, recognizing the antigen CA 125 (20), was diluted 1/3000. Treatment of sections with trypsin yielded significantly stronger and more consistent staining with each of the four mouse monoclonal antibodies. The sections were counterstained with methyl green and were mounted in glycerin-gelatin. Negative control staining was performed on adjacent sections using a monoclonal antibody of irrelevant specificity or normal mouse serum. Full-term placenta, stained with methyl green and mounted in glycerin-gelatin.

RESULTS

The pertinent clinical and histopathological data of the patients are presented in Table 1. Table 2 summarizes the nonspecific AP and HPLAP content of butanol extracts from normal and nonneoplastic lung tissues, the histochemical grading for nonspecific AP, and immunohistochemical grading for HPLAP, CEA, and CA 125 in adjacent histological sections from the same tissue samples. There was a broad correlation between the extent of HPLAP staining in histological sections and the amount of HPLAP measured in extracts. Normal lung tissue contained 58.8 ± 54.2 (SD) milliunits HPLAP/g tissue (n = 16), corresponding to 4.6 ± 5.6% of the nonspecific AP content. No difference could be found between the biopsies taken from the upper, intermediate, and lower pulmonary lobes (Patients 3, 4, and 5). HPLAP and CEA staining was present in all nontumoral lung tissues, ranging from scarce to abundant.

HPLAP staining with monoclonal antibody E6 was always more intense; hence more cells were positive than when monoclonal antibody H317 was used. Staining with H317 was located in regions which were also positively stained with E6. Regions which were immunohistochemically positive for HPLAP were also histochemically positive for nonspecific AP.

Fig. 1 gives a schematic representation of the histological distribution patterns for nonspecific AP, HPLAP, CEA, and CA 125 in the normal lung. Immunostaining was absent in all tissues when the primary monoclonal antibodies were replaced by a mouse monoclonal antibody of irrelevant specificity or by normal mouse serum (negative controls). The apical trophoblastic plasma membrane of full-term placenta was strongly stained for HPLAP and was negative for CEA and CA 125; the colon carcinoma contained strong cytoplasmic staining for CEA and was negative for HPLAP and CA 125; in the ovarian carcinoma, the plasma membrane of all carcinoma cells was strongly positive for CA 125 but was negative for HPLAP and CEA (positive controls). The normal tracheal, bronchial, and bronchiolar epithelium; the tracheal and bronchial glands; and the endothelium of small blood vessels were positively stained for nonspecific AP but were negative for HPLAP. All other constituents of trachea, bronchi, and bronchioi were negative for nonspecific AP as well as for HPLAP. Intense CEA and CA 125 staining (Fig. 7A) was present in tracheal and bronchial epithelial cells. CEA staining was localized on the apical surface and in the cytoplasm of some of these cells. CA 125 staining was present in the mucus of goblet cells and also in intracellular granules and on the apical surface of some other epithelial cells. The bronchiolar epithelium stained weakly for CEA and strongly for CA 125. In addition, strong cytoplasmic CA 125 staining was observed in the tracheal and bronchial glands and their ducts (Fig. 7B).

In the terminal bronchiolus of the normal lung (Fig. 2), nonspecific AP staining was present on the surface and in the apical cytoplasm of some of the columnar epithelial cells lining the mucosal folds. Neither HPLAP nor CEA was present in these cells. Scarce positive HPLAP and CEA staining was observed in rounded or flattened cells which were occasionally present between the columnar ciliated cells and goblet cells. This staining was localized predominantly on the apical plasma membranes. Strong CA 125 staining was present in the apical cytoplasm and glycocalyx of some terminal bronchiolar epithelial cells (Fig. 2, B to D). Most of these CA 125 positive cells are goblet cells. CA 125 staining was also observed at the level of the cellular and amorphous debris in the terminal bronchiolar lumen (Fig. 2B).

In the respiratory bronchioi (Fig. 3), the columnar ciliated or unciliated epithelial cells were sometimes positive for nonspecific AP. Ciliated cells were negative for HPLAP. In contrast, the low columnar unciliated cells with bulging apical cytoplasm (Clara cells) were occasionally moderately positive for HPLAP (Fig. 3D). Cuboidal and rounded cells lining the respiratory bronchioi were heavily stained for HPLAP and for AP. Some of them were also...
positive for CEA. CEA staining in respiratory bronchiolar cells was in general less intense than HPLAP staining. HPLAP and CEA staining was localized predominantly at the luminal plasma membranes. No staining for CA 125 could be observed in the respiratory bronchiolar cells or extended areas of the alveolar surfaces and their perinuclear membranes. Positive staining was sometimes present.

The visceral pleura was negative for HPLAP and CEA, but the visceral pleural mesothelium was positive for CA 125 (Fig. 9). Lung tissue distantly from the tumors was in all aspects identical to normal lung tissue from healthy organ donors. In contrast, lung tissue at the tumoral invasion front was atelectatic and affected by retrotumoral obstructive pneumonia. It was characterized by proliferation of the alveolar interstitium and by the presence of numerous nonspecific AP positive cells lining the alveolar lumina (Fig. 6). HPLAP, CEA, and CA 125 staining was absent or very scarce in these strongly AP positive regions.

Table 3 summarizes the nonspecific AP and HPLAP content of lung tumor extracts; the histochemical grading for nonspecific AP; and the immunohistochemical grading for HPLAP, CEA, and CA 125 in adjacent histological sections from the same tissue specimens as used for biochemical analysis.

Macrophages were in general negative for nonspecific AP, HPLAP, CEA, and CA 125. However, in the respective positive regions, diffuse intracellular staining was observed. Granulocytes in alveolar capillaries and alveolar lumina were positively stained for CEA. The pulmonary interstitium and arteries and the alveolar capillary endothelium were devoid of HPLAP, CEA, and CA 125 staining, whereas a diffuse and weak nonspecific AP staining was sometimes present.

The visceral pleura was negative for HPLAP and CEA, but the visceral pleural mesothelium was positive for CA 125 (Fig. 9). Lung tissue distantly from the tumors was in all aspects identical to normal lung tissue from healthy organ donors. In contrast, lung tissue at the tumoral invasion front was atelectatic and affected by retrotumoral obstructive pneumonia. It was characterized by proliferation of the alveolar interstitium and by the presence of numerous nonspecific AP positive cells lining the alveolar lumina (Fig. 6). HPLAP, CEA, and CA 125 staining was absent or very scarce in these strongly AP positive regions.

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### Table 1

<table>
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<th>Patient</th>
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<th>S/NS</th>
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<th>Grading&lt;sup&gt;c&lt;/sup&gt;</th>
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<td>61</td>
<td>M</td>
<td>S</td>
<td>Small cell carcinoma</td>
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<sup>a</sup> S, cigarette smoker; NS, nonsmoker.
<sup>b</sup> According to the TNM (tumor-nodes-metastasis) classification (47).
<sup>c</sup> 1, well-differentiated; 2, moderately well-differentiated; 3, poorly differentiated.
TUMOR ANTIGENS IN THE HUMAN LUNG

Table 2
Normal lung tissue and benign lung pathologies

<table>
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<tr>
<th>Patient</th>
<th>Normal lung tissue</th>
<th>Normal bronchus</th>
<th>Normal trachea</th>
<th>Normal lung tissue distant from malignant lung tumor</th>
<th>Lung tissue at tumoral invasion front</th>
<th>Lung tissue from benign lung pathology</th>
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<td>HPLAP</td>
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Note: - absent; +, scarce; ++, weak; ++++, moderately strong; +++, strong; ND, not determined.

*Histological distribution: AP22.1 AP +++7.3 AP +++11.2 AP +++1.1 AP +++1.9 AP ++4.4 AP +++0.8 AP +++0.6 AP ++++1.1 AP ++++1.2 AP ++++0.9 AP +++1.1 AP ND0.4 AP +++3.4 AP ++8.2 AP +++0.2 AP +++2.7 AP ++2.8 AP +++0.2 AP +++0.8 AP +++0.6 AP ++++ND A+++ Not determined

Histological distribution: Histological distribution of AP showed polymorphic phenotype differences corresponding to those of HPLAP. The presence of HPLAP in extracts from normal lung tissue has been confirmed subsequently by the use of specific monoclonal antibodies (8, 10).

Nonspecific AP activity was demonstrable histochemically in the epithelia lining the entire respiratory system, i.e., from the trachea up to the alveoli. This is in agreement with available data (23). In contrast, HPLAP immunohistochemical staining was observed only in the peripheral respiratory lung parenchyma (i.e., respiratory bronchioli, alveolar ducts, alveolar sacs, and alveoli). The absence of HPLAP staining in the fetal lung at a pregestational age between 11 and 15 weeks, i.e., in the pseudoglandular stage, is in agreement with our data on the histological distribution of HPLAP in the adult lung, since in this stage only the conducting airways up to the terminal bronchioli are formed (24, 25). Respiratory bronchioles are observed only after the 16th week and saccules are not present before the 24th week. Further study will be necessary to determine if HPLAP is present in the canalicular and terminal sac stages. Whether these observations are due to a differential embryonic origin, i.e., a presumed mesodermal origin for the epithelium lining respiratory lung parenchyma and the entodermal origin of the epithelium in the conducting airways (25), remains an open question.

As shown in Table 4, human fetal lung tissue at a pregestational age of 11 to 15 weeks contained considerably less nonspecific AP than did adult normal lung tissue, and only small amounts of HPLAP could be detected. The fetal lung was negative for HPLAP and CEA staining. In the 11-week-old fetus, a few pulmonary tubules were moderately positive for CA 125, whereas the other fetuses were negative. The fetal visceral pleural mesothelium was positive for CA 125 (Fig. 10) but was negative for HPLAP and CEA. The apical plasma membrane of the fetal tracheal and bronchial epithelium and the tracheal glands were also positive for CA 125 (Fig. 8). Only in Fetus 5 was the tracheal epithelium moderately positive for CEA.

DISCUSSION

The link between HPLAP and the human lung dates from 1968, when Fishman et al. (1) described its presence in serum of a lung carcinoma patient. In addition to the association of HPLAP with pulmonary cancer, this enzyme has also been detected biochemically in normal lung tissue (22). Using rabbit polyclonal antisera and several inhibitors, these authors demonstrated that most of the AP activity in lung tissue extracts is of the liver-bone-kidney type. The heat-stable fraction of AP and in intracellular inclusions.

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TUMOR ANTIGENS IN THE HUMAN LUNG

Fig. 1. Schematic representation of the histochemical distribution of nonspecific AP and immunohistochemical distribution of HPLAP, CEA, and CA 125 in the adult normal lung. Thick lines or dots indicate presence of positive staining. A, alveolar sac with alveoli; AD, alveolar duct; B, bronchus; Br, bronchiolus; M, pleural mesothelium; RB, respiratory bronchiolus; HG, respiratory glands; T, trachea; TB, terminal bronchiolus.

Table 3
Malignant lung tumors

<table>
<thead>
<tr>
<th>Patient</th>
<th>AP (milliunits/g)</th>
<th>HPLAP (milliunits/g)</th>
<th>%</th>
<th>AP HPLAP CEA CA125</th>
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*The histological grading concerns only the tumoral elements in the sections. First number indicates histological distribution (% positive cells): 1, scarce (<5%); 2, focal (5–50%); 3, predominant (50–90%); 4, uniform (90–100%). Second number indicates staining intensity: 1, faint; 2, weak; 3, moderately strong; 4, strong. Letter code indicates cellular localization: c, diffuse cytoplasmic; g, intracellular granules; a, glycocalyx; m, plasma membranes.

Ovarian tissue HPLAP was confined to germinal inclusion cysts (3), considered as the origin of benign and malignant serous ovarian tumors. In contrast, the presence of HPLAP in clinically normal lung tissue was always associated with apparently normal constituents of this organ. Normal lung tissue contains more HPLAP than do most malignant lung tumors investigated. Moreover, HPLAP staining was present in all normal lung tissues from smokers as well as nonsmokers. This indicates that the expression of HPLAP in the lung is not involved in mechanisms of carcinogenesis.

The difference between histochemical nonspecific AP and immunohistochemical HPLAP staining in normal alveoli, along with the observed patterns for nonspecific AP obtained by the use of two inhibitors for AP, demonstrates the presence of two isoenzymes of AP in different celltypes of the alveolar epithelium. L-p-Bromotetramisole sensitive, L-phenylalnine resistant nonspecific AP, not recognized by the monoclonal antibodies E6 and H317 against HPLAP, is found in isolated protruding cells, compatible with type II pneumocytes. Nonspecific AP staining in type II pneumocytes has also been reported by other authors for the animal (26–28) and human lung (23). In contrast, L-phenylalnine sensitive, L-p-bromotetramisole resistant AP, recognized by the monoclonal antibodies E6 and H317 against HPLAP, is found in epithelial cells presenting cytoplasmic extensions, compatible with type I pneumocytes. CEA staining was also present exclusively in the latter type of cells. These results show that type II pneumocytes in normal lung tissue are negative for HPLAP, CEA, and also CA 125. They also demonstrate that histochemical staining for nonspecific AP in the presence of L-phenylalnine can be used to distinguish type II pneumocytes at the light microscopic level.

Moderate interstitial fibrosis in atelectatic lung parenchyma is accompanied by alveolar type II pneumocyte proliferation and hypertrophy (29, 30). The peritumoral lung tissue presents this type of histological alteration. Histochemical nonspecific AP
staining of individual cells was more intense than in nonproliferating type II pneumocytes in histologically normal alveoli. However, again these cells were negative for HPLAP, CEA, and CA 125 staining.

The finding that type I pneumocytes contain the tumor associated antigens HPLAP and CEA whereas their progenitor cells, the type II pneumocytes (31, 32), are negative is intriguing. One explanation could be that HPLAP and CEA, which are observed on the luminal surface of type I pneumocytes, are of bronchial origin, reaching the alveoli via the bronchioalveolar surface fluid. However, the presence of strong HPLAP and CEA staining on well defined portions of the alveolar septa, adjacent to negative zones, is a strong argument against this hypothesis. A second hypothesis could be that some (if not all) type I pneumocytes are descendants from HPLAP and CEA producing bronchiolar epithelial progenitor cells instead of type II pneumocytes. The continuity of HPLAP positive staining in the respiratory bronchiolar epithelium, from the uniliated low columnar epithelial cells with bulging apical cytoplasm (Clara cells) over the cuboid and rounded cells to the neighboring flattened bronchioalveolar cells adds further support to the latter hypothesis. This hypothesis is, however, in contradiction with the currently accepted hypothesis that the elevated serum levels of CA 125 in patients with benign chronic liver disease (16) are probably of extrapulmonary origin and are caused by a reduced hepatic clearance of CA 125.

Our data indicate that HPLAP is a poor marker for lung carcinomas. This is in agreement with available serological data (19, 40, 41), using polyclonal and monoclonal antibodies and heat stability criteria. The observed CEA staining patterns for the different histological types of pulmonary carcinomas are in agreement with available data (34, 42, 43). CA 125 staining, present in seven of the ten pulmonary neoplasms, was in general scarce. These findings confirm the limited value of serum CA 125 levels for the detection of lung cancer (20).

The observed HPLAP immunohistochemical staining patterns are almost surely due to the presence of HPLAP because of the well-documented specificity of the two monoclonal antibodies used with respect to the other isoenzymes of AP and since nonspecific AP activity could always be demonstrated histochemically in the HPLAP positive sites. For CEA and CA 125, however, it cannot be excluded that cross-reacting substances with closely related or shared epitopes may be responsible for the observed staining (44–46).

In conclusion, the present study demonstrates that three tumor associated antigens, HPLAP, CEA, and CA 125, are present in clinically normal lung tissue. The immunohistochemical staining patterns for these three antigens were not correlated. HPLAP was localized exclusively in the peripheral airways, CEA both in the peripheral and the central airways, and CA 125 only in the central airways, the respiratory glands, and the pleural mesothelium. Fetal lung tissue at the age of 11 to 15 weeks was negative for HPLAP, CEA, and CA 125, except the pleural mesothelium which was positive for CA 125. The presence of these tumor associated antigens in all normal lung tissues from smokers and nonsmokers and its association with apparently normal pulmonary constituents do not support the hypothesis that the expression of these antigens is indicative for precancerous alterations.

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TUMOR ANTIGENS IN THE HUMAN LUNG

REFERENCES


Fig. 2. Normal terminal bronchiolus. A, AP staining, x 160. B, C, and D, CA 125 staining. B, x 160; C, x 200; D, x 500.

Fig. 3. Normal respiratory bronchiolus. A, C, and D, HPLAP staining. D, continuity of positive HPLAP staining from the low columnar unciliated cells over the cubic and rounded cells to the flattened bronchioloalveolar cells; arrow, HPLAP positive Clara cell. A, x 160; C, x 500; D, x 500. B, CEA staining, x 160.
Fig. 4. Normal alveoli. Comparison of nonspecific AP staining on adjacent sections. Arrows, nonspecific AP positive type II pneumocytes; arrowheads, AP positive type I pneumocyte. A, without inhibitors. × 250. B, with 1 mM L-p-bromotetramisole. × 250. C, with 50 mM L-phenylalanine. × 250.

Fig. 5. Normal alveoli. A and B, comparison on adjacent sections of nonspecific AP staining and immunohistochemical HPLAP staining, respectively. Arrows, type II pneumocytes; arrowheads, type I pneumocytes. A and B, × 250. C, HPLAP staining, × 250. D, CEA staining, × 250.

Fig. 6. Atelectatic alveoli. AP staining, × 250.
Fig. 7. Normal bronchus with bronchial glands. CA 125 staining. A, × 96. B, detail of bronchial glands, × 230.

Fig. 8. Fetal trachea with respiratory glands. CA 125 staining, × 160.

Fig. 9. Normal visceral pleura. CA 125 staining. Arrows, nuclei of mesothelial cells, × 250.

Fig. 10. Fetal visceral pleura. CA 125 staining, × 285.

Fig. 11. Poorly differentiated bronchiolar-alveolar carcinoma (Patient 18). Arrows, intracellular CA 125 positive staining. A, CEA staining, × 260. B and C, CA 125 staining, B, × 260; C, × 340.

Fig. 11A, 11B, 11C.
Fig. 12. Moderately well-differentiated epidermoid carcinoma (Patient 11). Arrows, regions of positive staining. A, AP staining, × 150. B, HPLAP staining, × 150. C, CEA staining, × 150. D, CA 125 staining, × 150.

Fig. 13. Poorly differentiated bronchiogenic adenocarcinoma (Patient 16). A, AP staining, × 100. B, HPLAP staining, × 100. C, CEA staining, × 100. D, CA 125 staining. Arrow, intracellular positive staining. D, × 100.
Immunohistochemical Localization of Placental Alkaline Phosphatase, Carcinoembryonic Antigen, and Cancer Antigen 125 in Normal and Neoplastic Human Lung


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