Immunoreactive Prolactin in Epithelial Cells of Normal and Cancerous Human Breast and Prostate Detected by the Unlabeled Antibody Peroxidase-Antiperoxidase Method

Dallas M. Purnell, Elizabeth A. Hillman, Barry M. Heatfield, and Benjamin F. Trump

Department of Pathology, School of Medicine, University of Maryland, Baltimore, Maryland 21201

ABSTRACT

A rabbit antiserum to ovine prolactin was used with the peroxidase-antiperoxidase method to search for immunoreactive prolactin in normal and pathological human breast and prostate. We first substantiated that the heterologous antiserum could recognize human prolactin. When sections of human anterior pituitary were exposed to the anti-prolactin serum, groups of angular-shaped cells were selectively stained. The immunostaining of pituitary cells appeared specific for prolactin since both ovine and human prolactin abolished immunostaining when they were added to the anti-prolactin serum. In contrast, human growth hormone and human chorionic gonadotropin did not eliminate immunostaining. Using this anti-prolactin serum that was capable of selective recognition of prolactin-containing cells in human tissue, we stained sections of normal breast (five cases) and normal prostate (three cases). Numerous epithelial cells in these tissues were immunostained by the antiserum. The immunostaining was abolished by deletion of the prolactin antiserum and also by use of prolactin antiserum absorbed previously with human prolactin rather than the heterologous antiserum alone. Immunostaining was not eliminated by absorption of antiserum with human growth hormone or human chorionic gonadotropin. These results suggested that the immunostaining of normal breast and prostate was related to the presence of immunoreactive prolactin in epithelial cells of these organs. In prostate, immunostaining was confined to the secretory cell population; the basal cells were not stained with anti-prolactin serum. In breast, immunostaining did not appear to be confined to secretory cells but seemed to occur throughout the breast parenchyma. The immunostaining of normal breast epithelium was markedly heterogeneous with cells lacking immunoreactivity intermixed with cells having positive reactivity. This was less pronounced in normal prostate epithelium where most secretory cells were immunostained. However, marked variation in staining intensity between acini was commonly observed in normal prostate.

Sixty-four % (14 of 22) of the primary prostate adenocarcinomas which were examined showed strong staining reactions with anti-prolactin serum. Two of three metastatic tumors of prostate origin had similar strong staining reactions. Most primary tumors of the prostate with strong staining reactions were poorly differentiated infiltrating adenocarcinomas (11 of 14; 79%). By contrast, 25% (3 of 12) of the well-differentiated prostate adenocarcinomas and 11% (one of nine) of the benign prostate hyperplasias which were tested showed strong staining reactions when treated with anti-prolactin serum. Seven of 12 (58%) infiltrating duct carcinomas of the breast were strongly stained with anti-prolactin serum, but most breast fibroadenomas were not stained (six of eight; 75%). None of seven adenocarcinomas of the colon tested as controls were stained by anti-prolactin serum. As was the case with normal breast and prostate, absorption of anti-prolactin serum with human prolactin but not human growth hormone or human chorionic gonadotropin abolished the immunostaining of malignant breast and prostate. These results suggested that the immunostaining of breast and prostate carcinomas was related to immunoreactive prolactin. The results reported here emphasize the value of the peroxidase-antiperoxidase method in the study of hormone target organ interactions as well as in retrospective studies of pathological materials.

INTRODUCTION

Endogenous prolactin has been detected in lactating rat breast (22, 23) and in cells of the corpus luteum and adrenal cortex of lactating rats (21) by immunoperoxidase histochzymy. Immunocytochemical evidence of prolactin in hyperplastic and neoplastic lesions of dog prostate and breast was reported by El Etreby and Mahrous (7), although they failed to substantiate their observations by means of absorption studies. Binding of prolactin to rat (30, 32), dog (7), and human prostate epithelium (31) has also been reported using the PAP method (29). However, immunohistochemical evidence for endogenous prolactin in human breast and prostate epithelial cells has not been reported previously. In this paper, we report the results of our examination of normal and pathological human breast and prostate for prolactin using the PAP method.

MATERIALS AND METHODS

Tissue. Reduction mammoplasty specimens (5 cases) provided the source of normal human breast epithelium. Normal human prostate (3 cases) was obtained at the immediate autopsy of young male trauma victims. Specimens of malignant breast (12 cases), malignant prostate (26 cases), benign prostate hyperplasia (9 cases), breast fibroadenoma (8 cases), and colon adenocarcinoma (7 cases) were obtained through the Division of Surgical Pathology at the University of Maryland Hospital.

The anterior pituitary gland obtained at the autopsy of an 18-year-old woman was used in studies to characterize the prolactin antiserum. Pituitary tissue from a female rat was also used for the same purpose.

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2 To whom requests for reprints should be addressed.
3 American Cancer Society Professor of Clinical Oncology.
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The abbreviations used are: PAP, horseradish peroxidase-anti-horseradish peroxidase; PBS, phosphate-buffered saline (0.1 M NaCl; 0.01 M Na2HPO4; 0.003 M KH2PO4, pH 7.4); DAB, 3,3'-diaminobenzidine tetrahydrochloride.
Pieces of normal breast were fixed in Bouin's solution and in 4% formaldehyde:1% glutaraldehyde. Samples of normal prostate were fixed in 4% formaldehyde:1% glutaraldehyde and one specimen was fixed in Bouin's solution as well. Specimens of pathological breast, prostate, and colon were fixed in 4% formaldehyde and stored in paraffin from 4 to 8 years. Human pituitary was fixed in neutral phosphate-buffered formalin. Rat pituitary was fixed in Bouin's solution. For the experiments, multiple 5-μm sections were cut from each block of tissue, cleared of paraffin in xylene, and placed in absolute methanol containing 3% H2O2 for 30 min to ablate endogenous peroxidase activity. Tissue sections were then rehydrated by passage through a series of alcohol solutions of decreasing concentration and finally placed in PBS.

Reagents. Rabbit antiserum to ovine prolactin was used as the primary antiserum in the PAP method. Anti-prolactin serum was obtained from Calbiochem-Behring Corp., La Jolla, Calif., as a lyophilized preparation. According to the supplier, the antiserum had been purified by ammonium sulfate precipitation and was developed for the measurement of serum prolactin by radioimmunoassay. It had a potency of 500 radioimmunoassay tubes/ml and had <0.1% cross-reactivity with growth hormone by radioimmunoassay. Additional data concerning the specificity of the antiserum were not available. Therefore, the prolactin antiserum was further characterized in our laboratory using immunohistochemical techniques.

Goat antiserum to whole rabbit serum (Research Products International Corp., Elk Grove Village, III.) or rabbit IgG (Sternberger-Meyer, Jarrettsville, Md.) was used as the secondary antiserum in the PAP method. PAP produced in rabbit was obtained from Sternberger-Meyer and also from Polysciences, Inc., Warrington, Pa. Normal rabbit serum was obtained from Sternberger-Meyer. Normal goat serum, Tris, and DAB were obtained from Polysciences. DAB was also obtained from Sigma Chemical Co., St. Louis, Mo. Lypophilized rabbit antisera to estradiol, progesterone, and testosterone were used as substitutive controls in certain experiments. These antisera were obtained from Polysciences. Ovine prolactin (38.9 IU/mg) and human growth hormone (2.1 IU/mg) were obtained from Sigma. Human prolactin and human choric gonadotropin (≥3000 IU/mg) were obtained from Calbiochem-Behring Corp. Lypophilized antisera were reconstituted with PBS (1 ml/vial). Aliquots (0.05 ml) of antisera were then prepared and stored at −70°C until used in experiments. Other immunological reagents were also stored in aliquots at −70°C.

Immunostaining Procedure. Tissue sections were treated with undiluted normal goat serum (30 min) and then exposed sequentially at room temperature or at 4°C to the following reagents: (a) rabbit antiserum to ovine prolactin (1:250 to 1:500 dilutions) for 18 to 22 hr; (b) goat antiserum to rabbit whole serum (1:10 dilution) or goat anti-rabbit IgG (1:40 dilution) for 30 min; and (c) PAP (1:50 to 1:100 dilutions) for 30 min. All controls, anti-prolactin serum was replaced with similar dilutions of prolactin-absorbed antiserum, normal rabbit serum, or PBS.

All dilutions were made in PBS containing 3% normal goat serum. After each incubation, tissue sections were washed 3 times with PBS (100 ml, 5 min each) except following PAP when 0.05 M Tris buffer (pH 7.6) was used in place of PBS. Antibody localization in tissue sections was detected by treating the sections with a filtered freshly prepared solution of DAB (0.05% in 0.05 M Tris buffer, pH 7.6, containing 0.06% H2O2 for 5 to 10 min. This method results in an unsoluble reddish-brown reaction product at sites of peroxidase activity. After treatment with DAB, the sections were washed in tap water, dehydrated, and mounted in Coverbond (Scientific Products, Inc.). Prior to dehydration, sections were then rehydrated with PBS. Binding of exogenous prolactin was indicated by intensification of the immunostaining reaction in comparison to tissue not so treated.

Absorbed Antiserum. Anti-prolactin serum containing 36 μg of ovine prolactin or 200 ng of human prolactin per ml (1:250 and 1:400 antiserum dilution, respectively) were prepared and refrigerated overnight or for 4 to 6 hr at 4°C. Anti-prolactin serum (1:400 dilution) containing human growth hormone (50 μg/ml and 200 ng/ml) or human choric gonadotropin (165 μg/ml and 200 ng/ml) were prepared at the same time. In order to evaluate the effects of absorption, solutions of 1:200 and 1:400 anti-prolactin serum containing no added hormones were also prepared and refrigerated in parallel with those containing added hormones.

The effect of substituting absorbed antiserum for anti-prolactin serum in the PAP procedure was ascertained using both normal and neoplastic breast and prostate tissue and human anterior pituitary gland. Anti-prolactin serum (1:200) absorbed with 400 ng human prolactin, 5 μg human growth hormone, or 10 μg human choric gonadotropin per ml were also evaluated using pituitary tissue.

RESULTS

Immunostaining of Pituitary. First, we determined if the anti-prolactin serum was able to detect prolactin-containing cells in anterior pituitary. In preliminary experiments, we showed that selected cells in rat anterior pituitary gland were immunostained by the anti-prolactin serum (Fig. 1a). Such cells (Fig. 1e) had the characteristic morphology of rat lactotrophs (25). These experiments were repeated using human pituitary tissue. Sections of human anterior pituitary were stained using absorbed prolactin antiserum or antiserum absorbed with ovine prolactin, human prolactin, human growth hormone, or human choric gonadotropin. Anti-prolactin serum selectively stained groups of angular-shaped cells in the anterior pituitary gland (Fig. 1b), some of which had a morphology similar to that observed in the rat anterior pituitary (Fig. 1f, arrows). The distribution of the immunostained cells was similar to that of acidophilic cells in adjacent hematoxylin and eosin-stained sections (Fig. 1c), one type of which produces prolactin (10). Immunostaining of pituitary cells was abolished when antiserum absorbed with human or ovine prolactin was substituted for anti-prolactin serum (Fig. 1, g and h). In contrast, addition of human growth hormone or human choric gonadotropin had little or no effect on the immunostaining of human pituitary (Fig. 1, d and l). These results indicate that the selectively stained cells of the pituitary are lactotrophs and provide evidence that the antiserum was capable of recognizing human prolactin.

Immunostaining of Normal Breast and Prostate. In preliminary experiments, representative sections of normal breast and prostate were treated with DAB alone, PAP followed by DAB, goat anti-rabbit IgG or goat anti-rabbit whole serum followed by PAP followed by DAB, and anti-prolactin serum followed by PAP followed by DAB. Epithelial staining was not observed under any of these conditions. This eliminated the possibility of nonspecific staining of breast or prostate tissue. In each of the cases which were examined with the complete PAP protocol, the epithelium of normal breast (5 cases) and normal prostate (3 cases) was stained with anti-prolactin serum (Figs. 2 to 4). The immunostained epithelial cells of the breast were usually intermixed with unstained cells (Fig. 4, a and c). This type of heterogeneity was not observed in normal prostate tissue. However, variation in staining intensity between acini was noted (Fig. 2, b and c).
In the prostate, immunostaining was limited to secretory cells, while basal cells remained unstained (Fig. 3a). This differential staining pattern was not present in breast, since secretory cells as well as basal or myoepithelial cells appeared to be immunostained (Fig. 4, a and c). In the epithelial cells of both prostate and breast, the immunostaining was located primarily in the cytoplasm. Microscopically, the cytoplasmic staining had a granular and/or net-like appearance (Figs. 2 to 4).

When breast and prostate were immunostained under conditions where the prolactin antiserum was replaced with normal rabbit serum (diluted 1:250 to 1:500) or with similar dilutions of rabbit antiserum to other hormones (estradiol, progesterone, or testosterone), epithelial staining did not occur or was negligible in comparison to tissue treated with anti-prolactin serum. These results showed that the immunostaining of breast and prostate was dependent on rabbit anti-prolactin serum and not on rabbit serum or rabbit immunoglobulin per se.

Evidence that immunostaining of breast and prostate was dependent on prolactin antibodies was obtained in additional experiments. Sections of normal breast and prostate were immunostained using either antiserum absorbed with prolactin or unabsorbed antiserum. In every instance, addition of human prolactin (200 ng/ml) to the anti-prolactin serum (1:400 dilution) completely abolished the immunostaining of normal breast and prostate (Figs. 3b and 4b). Admixture of anti-prolactin serum (1:250 dilution) with ovine prolactin (36 µg/ml) also resulted in abolition of immunostaining (Fig. 2f).

These results showed that the immunostaining of breast and prostate was dependent on prolactin antibodies. Evidence for the specificity of the immunostaining was also obtained. Samples of normal breast and prostate were treated with anti-prolactin serum (1:400 dilution) to which either human growth hormone (50 µg/ml or 200 ng/ml) or human chorionic gonadotropin (165 µg/ml or 200 ng/ml) had been added. The results were compared to those obtained when the same tissue was immunostained using unabsorbed antiserum or antiserum absorbed with human prolactin (200 ng/ml). At the concentrations studied, neither human growth hormone nor human chorionic gonadotropin eliminated immunostaining (Fig. 3, c and d).

Immunoreactive Prolactin in Human Breast and Prostate

<table>
<thead>
<tr>
<th>Pathological diagnosis</th>
<th>No. of specimens with indicated staining reaction for prolactin over total examined</th>
<th>Patient age (yr)</th>
<th>Time tissue in paraffin (yr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostate, adenocarcinoma, well differentiated</td>
<td>Strong positive: 3/12 (25%) Weak positive: 6/12 (50%) Negative: 3/12 (25%)</td>
<td>64-87</td>
<td>6-7</td>
</tr>
<tr>
<td>Prostate, adenocarcinoma, poorly differentiated, infiltrating</td>
<td>11/11 (100)</td>
<td>40-79</td>
<td>4-8</td>
</tr>
<tr>
<td>Prostate, adenocarcinoma, metastatic</td>
<td>2/3 (67)</td>
<td>52-85</td>
<td>4-6</td>
</tr>
<tr>
<td>Prostate, benign hyperplasia</td>
<td>1/9 (11)</td>
<td>60-85</td>
<td>7-8</td>
</tr>
<tr>
<td>Breast, infiltrating duct carcinoma</td>
<td>7/12 (58)</td>
<td>54-65</td>
<td>4-6</td>
</tr>
<tr>
<td>Breast, fibroadenoma</td>
<td>2/8 (25)</td>
<td>24-43</td>
<td>4-6</td>
</tr>
<tr>
<td>Colon, adenocarcinoma</td>
<td>7/7 (100)</td>
<td>58-67</td>
<td>6</td>
</tr>
</tbody>
</table>

* Refer to text figures for examples of strong, weak, and negative staining reactions.

**Numbers in parentheses, percentage.

Exposure of prostate tissue to ovine prolactin (4 to 36 µg/ml) was found to increase the intensity of immunostaining compared to tissue not so treated (Fig. 2d). This result suggested that prostate epithelial cells contained prolactin-binding sites in addition to immunoreactive prolactin. As in tissue not exposed to exogenous prolactin, the immunostaining was abolished when antiserum absorbed with prolactin was used in place of unabsorbed antiserum (Fig. 2e).

An unexplained yet consistent observation was the absence of immunostaining in breast tissue which had been fixed in 4% formaldehyde:1% glutaraldehyde. This was in contrast to the positive immunostaining obtained with specimens of the same tissue which had been fixed in Bouin’s solution. This was a peculiarity of breast, since no difference in immunostaining related to fixation was observed in prostate. The immunostaining of Bouin-fixed breast was not artificial since it was specifically ablated when antiserum absorbed with human prolactin was used in the PAP method (Fig. 4b).

Immunostaining of Pathological Breast and Prostate. Malignant tissue from breast and prostate and tissue from benign conditions of these organs were also studied using anti-prolactin serum (Table 1). A total of 12 primary breast carcinomas, 23 primary prostate carcinomas, 3 metastatic prostate carcinomas, 9 prostate hyperplasias, and 8 breast fibroadenomas were studied. All of the breast cancers were infiltrating duct carcinomas from postmenopausal women. The prostate neoplasms included 12 well-differentiated adenocarcinomas and 11 poorly differentiated infiltrating adenocarcinomas. Two metastatic prostate tumors were well-differentiated adenocarcinomas and the third was a poorly differentiated adenocarcinoma. In addition, several colon adenocarcinomas (7 cases) were also examined as controls.

Poorly differentiated prostate adenocarcinomas tended to be strongly stained with anti-prolactin serum (Fig. 6a), in contrast to well-differentiated prostate adenocarcinomas only some of which were similarly stained (Fig. 5e). Two benign prostate hyperplasias were moderately stained by anti-prolactin serum (Fig. 7a), but most were weakly stained or unstained. Variation in staining intensity was common among the cells of prostate carcinomas. Areas of nonneoplastic prostate within tumor specimens were often unstained in contrast to the surrounding tumor tissue (Fig. 6c). Such areas were sometimes atrophic; thus, lack of immunostaining may reflect the absence of secretory cells since basal cells of the prostate were not stained by anti-prolactin serum. Similar findings were observed in the case of benign prostatic hyperplasia (Fig. 7b).
Most of the breast carcinomas were stained strongly with anti-prolactin serum (Fig. 8). As with prostate tumors, variation in the staining intensity of neoplastic breast cells was a common finding (Fig. 8). Nonneoplastic breast tissue in tumor specimens was often unstained in contrast to the cancerous breast tissue (Fig. 9, inset). Most breast fibroadenomas were not stained with anti-prolactin serum (Fig. 10).

The immunoreactivity of neoplastic cells of breast and prostate was located in the cytoplasm. Microscopically, the immunostaining pattern did not differ from that of normal epithelial cells of these organs.

The immunostaining of malignant breast and prostate was abolished by addition of human prolactin to the anti-prolactin serum (Figs. 5b and 8, inset). In contrast, human growth hormone and human chorionic gonadotropin did not eliminate immunostaining (Fig. 5, c and d). These results point to the presence of immunoreactive prolactin in some tumors of breast and prostate.

None of the colon carcinomas were stained by anti-prolactin serum (Fig. 11).

DISCUSSION

An antiserum developed in rabbits to ovine prolactin was used to search for endogenous prolactin in normal and neoplastic human breast and prostate. By means of several experiments, we substantiated that this antiserum could recognize human prolactin.

We showed that the antiserum selectively recognized prolactin-containing cells in human, as well as rat, pituitary. Groups of angular-shaped cells were selectively stained when sections of anterior pituitary were treated with anti-prolactin serum. Evidence for the specificity of immunostaining was obtained in that both ovine and human prolactin abolished immunostaining of pituitary cells when they were admixed with anti-prolactin serum. In contrast, human growth hormone and human chorionic gonadotropin did not eliminate immunostaining of pituitary cells even when added to the anti-prolactin serum in higher concentrations.

These results confirm those of several other investigators who have used antiserum to ovine prolactin to detect prolactin in pituitary cells of heterologous species including human (24, 33), ape and monkey (12), rat (17), and dog (8). This success appears related to the extensive molecular and immunological overlap between ovine prolactin and the prolactins of human and other species (16, 18).

These experiments showed that the antiserum to ovine prolactin recognized the presence of prolactin in human tissue. Other investigators have also detected human prolactin in tissue and in serum and other fluids by means of antiserum to ovine prolactin (14, 15, 17, 24). We next showed that the immunostaining of breast and prostate was related to the presence of immunoreactive prolactin in these tissues. We found that the immunostaining of normal and abnormal breast and prostate was abolished by admixture of the antiserum with human prolactin as well as by admixture with ovine prolactin. In contrast, addition of human growth hormone or human chorionic gonadotropin to the anti-prolactin serum did not eliminate immunostaining. These observations satisfy the generally accepted criteria for immunological specificity. Therefore, our results are consistent with the presence of immunoreactive prolactin in normal and malignant breast and prostate. Nonetheless, our results as well as those of any other immunohistochemical study must be presented with a proviso. It could always be postulated that some unidentified nonprolactin cross-reactive material might explain the staining. This was the case in an immunocytochemical study of the localization of follicle-stimulating hormone in rat testis (13). Yet, the fact that both breast and prostate are known prolactin target organs with cellular receptors for this hormone (1, 3, 19, 27) lends strength to our conclusion. Immunoreactive prolactin has been detected in some breast carcinomas by radioimmunoassay (6). In addition, El Etreby and Mahrous (7) using immunohistochemistry reported the presence of prolactin in hyperplastic and cancerous lesions of dog prostate and breast, although their study did not include the important absorption control.

The results which we obtained with human prostate require comment in relation to those of an earlier study. Witorsch (31) reported only trace staining of human prostate with anti-prolactin serum unless the tissue had been treated with prolactin in vitro. In contrast, we found that prostate epithelium was immunostained with anti-prolactin serum without exposure to exogenous prolactin. He suggested that his results were due to the use of antisera diluted beyond the point of detecting the presumably small amounts of endogenous hormone in the prostate. This could explain the difference between the 2 studies since we used relatively concentrated antisera. However, differences in the titer and/or nature of the antisera used in the 2 studies could also explain the different results.

Like Witorsch (31) and El Etreby and Mahrous (7), we found that exposure of fixed prostate to prolactin prior to anti-prolactin serum resulted in intensification of immunostaining. This result was compatible with the presence of unoccupied prolactin-binding sites in prostate epithelium.

Our examination of prostate carcinomas for immunoreactive prolactin appears to be the most extensive yet published. We found that poorly differentiated prostate adenocarcinomas were strongly positive for immunoreactive prolactin. In contrast, well-differentiated prostate adenocarcinomas were less often strongly positive. While the role of prolactin in the pathogenesis of prostate cancer is unclear (2, 4, 5, 9, 11, 20), our results suggest that an inverse relationship between immunoreactive prolactin and the degree of differentiation of prostate adenocarcinomas may exist. Additional studies will be necessary to confirm this interesting possibility.

In this context, it is not without interest that Bartsch et al. (4) found that plasma prolactin levels were significantly elevated in patients diagnosed as having prostate adenocarcinoma of cribriform and/or solid growth patterns compared to prolactin levels measured in patients with well-differentiated prostate adenocarcinomas.

To our knowledge, there are no previous immunohistochemical studies of human breast carcinomas for the presence of prolactin. However, biochemical evidence for immunoreactive prolactin in some breast tumors has been published (6). Castro et al. (6) measured the prolactin content of cytosol extracts of human breast carcinomas by radioimmunoassay. In most instances, the prolactin content of the extracts was significantly higher than could be accounted for by serum contamination. Our immunohistochemical data for intracellular immunoreactive prolactin in some breast carcinomas are consistent with their results. Our data are also compatible with a recent study (28).
which showed that prolactin is taken up in vitro by 2 human breast cancer cell lines (MCF-7 and T47D) and one normal breast cell line (HBL-100). The present study suggests that this process may occur in vivo as well.

Shafie and Brooks (26) showed that prolactin stimulates de novo formation of estrogen receptors in the MCF-7 human breast cancer cell line. Their observations are intriguing in relation to our evidence for immunoreactive prolactin in some human breast carcinomas. It is possible that a correlation might be found between the estrogen receptor status of some breast carcinomas and their content of immunoreactive prolactin. However, Castro et al. (6) found no relationship between the content of prolactin by radioimmunoassay and the estrogen receptor status of the breast carcinomas in their study.

The role of hormones and their receptors in the normal and pathological physiology of various tissues is, at present, an area of intense interest and investigation. Data such as the above make it reasonable to suppose that immunohistochemical techniques will be an important tool in such endeavors. The utility of the PAP method in retrospective (using formalin-fixed tissue) as well as prospective studies suggests that data such as the above represent only the threshold of possibilities for the method.

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REFERENCES

Fig. 1. Immunostaining of pituitary. In a, cells of rat anterior pituitary gland (right) are selectively stained by anti-prolactin serum (1:200). Immunostained cells are shown at higher power in e. At upper left is pars nervosa (PN). No counterstain, × 17. In b, cells of human anterior pituitary are selectively stained by anti-prolactin serum (1:200). No counterstain, × 35. In c, the distribution of acidophilic cells in the pituitary is similar to that of the immunostained cells. H & E, × 35. In d, immunostaining of human pituitary was not eliminated with anti-prolactin serum (1:200) preabsorbed with human growth hormone (5 µg/ml). No counterstain, × 140. In e, cells of rat adenohypophysis selectively stained by anti-prolactin serum (1:200) have an angular morphology characteristic of lactotrophs. No counterstain, × 140. In f, the cells of human pituitary immunostained by anti-prolactin serum (1:200) have an angular morphology similar to that of rat adenohypophysis (arrows). No counterstain, × 140. In g, immunostaining of human pituitary was abolished by addition of human prolactin (200 ng/ml) to anti-prolactin serum (1:400). Identical results were obtained by absorbing 1:200 anti-prolactin serum with 400 ng of human prolactin per ml. No counterstain, × 140. In h, immunostaining of human pituitary was abolished with anti-prolactin serum (1:250) preabsorbed with ovine prolactin (36 µg/ml). No counterstain, × 140. In i, staining of pituitary was not eliminated with anti-prolactin serum (1:200) preabsorbed with human chorionic gonadotrophin (10 µg/ml). No counterstain, × 140.

Fig. 2. Immunostaining of normal prostate. In a, anti-prolactin serum (1:250) stains the cytoplasm of secretory epithelial cells (arrows). Dark staining at apical epithelial surfaces is probably related to accumulation of antigen in this region. In b, variation in the intensity of immunostaining by anti-prolactin serum (1:250) between acini was a common finding. Acinus on left shows weak staining, whereas that on right shows moderate staining. In c, anti-prolactin serum was replaced with PBS. No immunostaining is present in cytoplasm of secretory cells. In d, immunostaining was intensified by treatment of prostate with ovine prolactin (36 µg/ml) prior to anti-prolactin serum (1:250). Contrast with a which is the vehicular control. In e, substitution of absorbed antiserum (36 µg ovine prolactin per ml of 1:250 antiserum) abolished immunostaining of tissue pretreated with prolactin. Contrast with d. In f, immunostaining was abolished with anti-prolactin serum (1:250) preabsorbed with ovine prolactin (36 µg/ml). Contrast with a. All panels hematoxylin counterstain, × 140.
Fig. 3. Effect of absorbed antiserum on immunostaining of normal prostate. In a, prostate secretory cells were stained with 1:400 anti-prolactin serum (control for remaining panels). No counterstain, × 140. In b, absorption of anti-prolactin serum (1:400) with human prolactin (200 ng/ml) abolished immunostaining. No counterstain, × 140. In c, absorption of anti-prolactin serum (1:400) with human chorionic gonadotropin (200 ng/ml) did not abolish immunostaining. No counterstain, × 140. In d, absorption of anti-prolactin serum (1:400) with human growth hormone (200 ng/ml) did not abolish immunostaining. No counterstain, × 140. Dark staining of luminal surfaces in a and d is probably related to accumulation of prolactin near apical epithelial surface.

Fig. 4. Immunostaining of normal breast. In a and c, the immunostaining of human breast by anti-prolactin serum (1:400) is markedly heterogeneous. Note relatively dense staining of small acinus (arrow) in c and the absence of staining in many of the cells lining the mammary! ducts in both a and c (thick arrows). Staining reaction appears to involve all cells of the breast parenchyma. No counterstain, × 140. In b, absorption of anti-prolactin serum (1:400) with human prolactin (200 ng/ml) abolished immunostaining of breast. No counterstain, × 140.

Fig. 5. Immunostaining of prostate carcinoma. In a, well-differentiated prostate adenocarcinoma shows a strong staining reaction with anti-prolactin serum (1:400) (control for other panels). In b, immunostaining of tumor was abolished by preabsorption of prolactin antiserum (1:400) with human prolactin (200 ng/ml). No counterstain, × 70. In c, absorption of anti-prolactin serum (1:400) with human growth hormone (50 µg/ml) did not abolish staining of tumor. No counterstain, × 70. In d, absorption of anti-prolactin serum (1:400) with human chorionic gonadotropin (165 µg/ml) did not eliminate immunostaining. No counterstain, × 70. In e, tumor immunostained by anti-prolactin serum (1:250) appearance with hematoxylin counterstain. Arrow points toward immunostained tumor acinus. × 70.
Fig. 6. In a, poorly differentiated prostate adenocarcinoma shows a strong staining (arrow) reaction with anti-prolactin serum (1:250). Hematoxylin counterstain, x 140. In b, no immunostaining is present in tumor treated with normal rabbit serum (1:250) in place of antiserum (arrow). Hematoxylin counterstain, x 140. In c, nonneoplastic prostate epithelium near tumor shows no staining (arrow) with anti-prolactin serum (1:250). Hematoxylin counterstain, x 140.

Fig. 7. In a, specimen of benign prostatic hyperplasia was treated with anti-prolactin serum (1:250). Some secretory cells (arrow) are immunostained, while others are not. Hematoxylin counterstain, x 140. In b, intact secretory epithelium (arrow) in benign prostatic hyperplasia shows positive staining reaction with anti-prolactin serum (1:250), while immunostaining is not present in adjacent atrophic glands. Hematoxylin counterstain, x 70. In c, there is no immunostaining in section treated with normal rabbit serum (1:250) in place of antiserum. Hematoxylin counterstain, x 140.

Fig. 8. Infiltrating duct carcinoma of breast shows strong staining reaction (arrow) with anti-prolactin serum (1:400). Note that some of the carcinoma cells are not immunostained. No counterstain, x 140. In the inset, absorption of anti-prolactin serum (1:400) with human prolactin (200 ng/ml) abolished immunostaining of carcinoma. No counterstain, x 140.

Fig. 9. Breast carcinoma with a moderately strong cytoplasmic staining reaction with anti-prolactin serum (1:250) (arrow). Hematoxylin counterstain, x 70. In the inset, normal breast epithelium near carcinoma shows no cytoplasmic staining (arrows). Hematoxylin counterstain, x 70.

Fig. 10. Breast fibroadenoma shows no cytoplasmic staining (arrows) with anti-prolactin serum (1:250). Hematoxylin counterstain, x 70.

Fig. 11. Colon adenocarcinoma treated with anti-prolactin serum (1:250). No immunostaining is present. No counterstain, x 140.
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