Immunohistochemical Analyses of Estrogen Receptor in Endometrial Adenocarcinoma Using a Monoclonal Antibody


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ABSTRACT

Immunohistochemical localization of estrogen receptor (ER) using specific monoclonal anti-human estrogen receptor antibody, H222, with an immunoperoxidase technique was performed on fresh frozen tissue derived from 100 endometrial adenocarcinomas. Immunohistochemical evaluation incorporated both intensity and distribution of staining. In all cases, H222 localized in the nucleus of target cells. A significant quantitative relationship was shown between histological score (H-Score) and the biochemical analysis of ER content in tissue homogenates (r = 0.65, P = 0.00001). Excellent sensitivity (92%) and specificity (93%) were observed for the comparison of H-Score to the biochemical assay. Significant ER localization was present in stromal and myometrial elements, component H-Score of which correlated weakly with component H-Scores of malignant epithelial elements. Divergent receptor localization in stromal and myometrial verus malignant epithelial elements suggests that biochemical assays of endometrial carcinoma specimens may not reflect cancer-relevant receptor content. The data presented here suggest that the immunoassay of ER using H222 monoclonal antibody provides additional histochemical information to complement conventional analyses of endometrial adenocarcinomas.

INTRODUCTION

While sex steroid receptor analysis plays a pivotal role in the evaluation of patients with breast cancer, its clinical application has not become as well established for gynecological neoplasms (1, 2). The standard quantitative biochemical methods for steroid receptor analysis, dextran-coated charcoal assay, and sucrose density gradient assay do not consistently predict hormonal responsiveness and prognosis in endometrial carcinomas even though high concentrations of specific receptor are often present. Uterine tissue components, such as stroma and myometrium, are frequently included in specimens submitted for estrogen receptor analysis, are known to contain substantial quantities of ER,2 and thus may obscure detection of the cancerspecific ER component most relevant to response (3). By contrast, normal nonepithelial components of the breast do not contribute significantly to receptor levels, thus simplifying interpretation of receptor content in breast cancer specimens. The dextran-coated charcoal and sucrose density gradient analyses are accurate and reliable methods but are subject to certain limitations. Inherent in biochemical assays of tissue homogenates is an inability to distinguish the source or distribution of cells which have binding activity. Numerous attempts have been made at developing histochemical methods for receptor analysis using fluorescein steroid conjugates (4, 5) or anti-estrogen antibodies (6, 7), but none heretofore has correlated well with biochemical assays or clinical outcome (8–12).

The recent development by Greene et al. (13–15) and Miller et al. (16) of highly specific and sensitive monoclonal antibodies directed against human estrogen receptor provides a new approach to histochemical receptor localization. The availability of well-characterized reagents and a sensitive immunoperoxidase technique allow detection and amplification of the few receptor molecules present in individual cells (17, 18). We report the results of a study utilizing the monoclonal anti-human estrogen receptor antibody H222 to assess the relative contribution of cancer and noncancerous components of endometrial adenocarcinomas to total biochemical estrogen receptor content and compare the estrogen receptor content to tumor differentiation.

MATERIALS AND METHODS

Patient Population. Tissues were evaluated from 173 patients with endometrial adenocarcinoma seen at Duke University Medical Center from January 1, 1980, to December 31, 1983. All tissues were submitted for pathological examination and analyses of steroid receptor content. The histological criteria for inclusion are those defined by the International Federation of Gynecologists and Oncologists (19). One hundred consecutive cases had adequate tissue submitted for complete evaluation.

Of the 173 consecutive cases of endometrial adenocarcinoma, 73 cases were excluded from the study for the following reasons: in 32 cases there was inadequate tissue for complete analysis (included in this group were patients in whom diagnosis was by preoperative endometrial curettage); in 30 no residual tumor was seen on cryostat sections prepared from the remaining tissue available for immunohistochemical analysis (see below); 5 cases contained only necrotic tissue; and in 6 cases the tissue had undergone significant desiccation. The 100 remaining cases comprise a study group of 90 primary adenocarcinomas of the endometrium and 10 cases in which tissue was derived from metastatic foci.

The patients ranged in age from 30 to 92 years. Eighty patients were 56 years of age or older, 12 patients were between 50 and 55 years of age, and 8 patients were in the age range of 30 to 49 years.

Histological Classification and Grading. Histological grading was completed according to the International Federation of Gynecology and Obstetrics Cancer Committee. Grade 1 consisted of highly differentiated adenomatous carcinoma in which 100 to 75% of the tumor cells are differentiated with 0–25% of cells undifferentiated. Grade 2 is a differentiated adenocarcinoma with partly solid areas and Grade 3 is a predominantly solid growth pattern with poorly differentiated cells. Well-differentiated carcinoma was distinguished from atypical hyperplasia by the loss of polarity of the glandular epithelium, by irregular gland profiles with cribriform pattern and intraglandular bridging, and by relatively scant stroma in the areas of tumor.

Biochemical Estrogen Receptor Analyses. Estrogen receptor content of the tissues was analyzed using modifications of the methods described by Schrader et al. (20). Tissue was obtained fresh immediately, washed in buffer (10 mM Tris-HCl-5 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid-HCl, pH 7.5, 1 mM EDTA-1.0 mM thiglyglycerol-0.02% NaN3) at 4°C, quick frozen in liquid nitrogen, and maintained at −80°C in airtight liquid nitrogen capsules until assayed. The frozen tissue was pulverized in liquid nitrogen using a Spex freeze mill (Spex Industries, Inc., Metuchen, NJ) at three-forths power with...
a stainless steel impeller for 15 s (five 3-s cycles). The tissue powder was homogenized at a sample:buffer volume of 1:4 at 4°C using a Polytron (Brinkman Instruments, Westbury, NY) (setting 3) for 60 s (four 15-s cycles with 30-s cooling periods). Cytosol was prepared from the homogenate by centrifugation at 1,000 x g for 10 min followed by 145,000 x g for 1 h, both at 4°C. Endogenous unbound steroid was removed by 0.75% (w/v) Norit A (Matheson, Coleman and Bell Manufacturing Chemists, Norwood, OH) in 0.0025% dextran. The supernatant protein content was analyzed by the method of Bradford (21) and adjusted to 4–6 mg protein/ml. Sucrose density gradient and dextran-coated charcoal analyses were performed.

Sucrose Density Gradient Analysis. Aliquots of 250 μl of cytosol were incubated for 4 h at 4°C with 1.6 pmol [3H]estradiol (New England Nuclear, Boston, MA) containing a 100-fold excess of cold testosterone. Parallel control incubations also contained a 250-fold excess of cold diethylstilbestrol preincubated with cytosol for 5 min prior to incubation with the radioactive hormone. Two hundred μl were layered on 10–25% isokinetic sucrose gradients and centrifuged to a ω2 of 157,417 in a Beckman SW 60 rotor (Beckman Instruments, Inc., Palo Alto, CA) at 4°C (polycarbonate tubes). Gradients were fractionated by upward displacement with 75% glycerol into 40 fractions. Radioactivity was determined in an LS-4000 Intertechnique liquid scintillation counter (IN/US, Fairfield, NJ) using 10 ml Biofluor (New England Nuclear). Counting efficiency was determined by external standardization used for computer calculations. Sedimentation coefficients were determined by the use of [14C]-labeled bovine serum albumin (4.6S) or γ-globulin (7S) standards.

Dextran-coated Charcoal Analysis. The cytosol was diluted to 1–2 mg protein/ml, and 200-μl aliquots were incubated for 16 h at 4°C in eight Kahn tubes containing 1.6–0.125 pmol [3H]estradiol and 100-fold concentration of cold testosterone. Parallel control incubations at each concentration, when adequate samples were available (or a single control incubation at 0.8 pmol [3H]estradiol when the sample was limited), contained a 250-fold excess of cold diethylstilbestrol, added immediately prior to the radioactive steroid. Non-protein-bound free steroid was removed by 0.75% (w/v) Norit A (dextran-treated) and bound radioactivity (not removed by the charcoal) was determined. Calculations of KT (dissociation constant) and bound receptor were made by the methods of Woosley and Muldoon (22) and Scatchard (23) by means of an on-line LEM computer in an LS-4000 Intertechnique liquid scintillation counter.

Immunohistochemical Estrogen Receptor Analysis. Portions of the tissue specimens which had been fresh frozen in liquid nitrogen for biochemical assays were prepared as serial 4- to 6-μm cryostat sections mounted on poly-L-lysine-coated slides. The initial section was stained with hematoxylin-eosin for tissue diagnosis. Histological grade of the tumor was evaluated according to Federation of International Gynecologists and Oncologists criteria (19). Assignment of tumor-type and human breast cancer by several criteria (16, 17, 24). H222 recognizes a stable and well-conserved determination that is close to the steroid-binding site. The peroxidase-antiperoxidase method of Sternberger (18) for immunohistochemical localization was performed as

1. Cryostat sections were placed in 3.7% formaldehyde-phosphate-buffered saline for 10 min. They were then transferred to a PBS bath until formalin fixation had been completed for all sections in a given run.
2. Slides were placed in cold absolute methanol at −15°C to −20°C for 4 min and then cold acetone at −15°C to −20°C for 1 min.
3. Slides were washed in PBS twice for 5 min each at ambient temperature.
4. Sections were treated with 2% normal goat serum in PBS for 15 min in a humidified chamber to reduce the nonspecific binding of bridging antibody (see below).
5. Sections were incubated with primary antibody (H222, 5 μg/ml; 33 pmol/ml in PBS) for 30 min at ambient temperature. Negative control slides of adjacent cryostat sections from each of the same tissues were incubated with control antibody (normal nonimmunized rat immunoglobulin in PBS) in place of the primary antibody for 30 min. Control slides of estrogen receptor-rich MCF-7 human breast cancer cells were incubated with primary control antibody.
6. Slides were washed in PBS twice for 5 min each.
7. Sections were incubated with bridging antibody (goat anti-rat immunoglobulin in PBS) for 30 min.
8. Slides were washed in PBS twice for 5 min each.
9. Peroxidase-antiperoxidase complex horseradish peroxidase-rat anti-horseradish peroxidase in PBS was applied to the sections for 30 min.
10. Slides were washed in PBS twice for 5 min each.
11. Slides were flooded with 8 mg diamobenzidine and 16 ml hydrogen peroxide in PBS for 6 min in the dark. The diaminobenzidine-H2O2 solution had been prepared using minimal light and was used within 30 min.
12. Sections were rinsed in gently running tap water for 5 min, dehydrated in serial alcohols to xylene, and coverslipped with Permount without counterstaining.

In the presence of hydrogen peroxide, the tissue-bound peroxidase converts the diaminobenzidine chromagen to an insoluble brown reaction product which can be visualized with a light microscope. By this procedure, the minute quantity of estrogen receptor-monoclonal antibody complex is amplified through the use of the bridging antibody, peroxidase-antiperoxidase complex, and enzyme reaction products.

Scoring of Assays. Biochemical assays were summarized as fmol of specific estrogen binding per mg of tissue protein.

The immunohistochemical localization was scored in a semiquantitative fashion incorporating both the intensity and distribution of specific staining. Evaluations were recorded for each observed tissue component, i.e., myometrium, stroma, benign epithelium, and malignant epithelium, as percentage of cells in each of four intensity categories. The intensity of specific staining was characterized as not present (0), weak but detectable above control (1+), distinct (2+), and very strong (3+). For each observed tissue component, a summary value we refer to as H-Score was calculated. This consists of a sum of the percentages of positively stained cells multiplied by a weighted intensity of staining.

\[
H\text{-Score} = \sum P_i \times (i + 1),
\]

where \(P_i\) is the percentage of stained cells in each intensity category, and \(i\) is the intensity for \(i = 1, 2, 3\). A total H-Score for the tissue section was derived as the sum of the component H-Scores weighted by the fraction of each component observed in the tissue section.

Total H-Score = Cancer H-Score \times \frac{\% Cancer}{100} + Stroma H-Score \times \frac{\% Stroma}{100} + Myometrial H-Score \times \frac{\% Myometrium}{100} + Benign epithelial H-Score \times \frac{\% benign epithelium}{100}

Biochemical assays were compared to the total tissue histological score as well as to its components.

Sections were evaluated independently by two observers and reevaluated by the initial observer. Intraobserver (\(r = 0.983, P = 0.00001\)) and interobserver differences for total H-Score were resolved by consensus evaluation (\(r = 0.984, P = 0.00001\)) for primary observer versus consensus total H-Scores.

Data Management and Analysis. Biochemical assay values and immunohistochemical assay values were coded separately in a blinded fashion and maintained as independent files in the Time Oriented
IMMUNOHISTOCHEMICAL ANALYSES OF ER IN ENDOMETRIAL ADENOCARCINOMA

RESULTS

Immunohistochemical localization of estrogen receptor identified with monoclonal anti-human estrogen receptor H222 antibody by indirect immunoperoxidase technique resulted in specific staining in the nuclei of target cells (Figs. 1 and 2). Specific cytoplasmic staining was not observed. Control slides treated with normal nonimmunized rat immunoglobulin in place of primary antibody demonstrated only minimal background staining.

Estrogen receptor localization occurred in stromal and myometrial elements as well as the epithelial component. When the epithelial component bound antibody, a heterogeneous pattern was generally observed. Few cases were uniform in their staining pattern. Among stromal and myometrial elements, however, staining was usually uniform (Fig. 2).

Cancer was present in every specimen retained in the study whereas stroma was present in 83% and myometrium in 26% of cases. The distribution of component percentages in specimens is shown in Fig. 3, which shows broad variation in component distributions despite macroscopic “trimming” of the specimen on receipt to “maximize” tumor epithelial components. Ten % of specimens contained benign epithelial elements; no specimen contained over 30% benign epithelial tissue. Nine of the 10 were positive with H222 ranging from 125 to 370 in component H-Score. The tenth value was 70. Because of their low frequency, benign epithelial elements were not further analyzed.

The relationship between the logarithm of the quantitative biochemical assay of tissue homogenate extract (fmol of radio-labeled estradiol bound per mg of protein) and the H-Score of the total tissue section is shown in Fig. 4 (r = 0.65). Tables 1 and 2 compare total tissue H-Score to the biochemical assay. A biochemical receptor content of ≥10 fmol/mg protein was considered positive when its dissociation constant was less than or equal to 1.0 × 10^-9. An H-Score of ≥75 was the threshold value for a “positive” immunohistochemical assay. Sensitivity of the total tissue H-Score was 92% and specificity was 93% relative to the biochemical assay. The overall accuracy of the H-Score was 92%.

Correlations of individual tissue component H-Scores to biochemical ER appeared to reflect the percentages of tissue components present. Thus the malignant epithelial component, which was the predominant portion of most specimens, correlated with the biochemical value merely as well as did the total tissue H-Score. Overall accuracy of the malignant H-Score to biochemical ER dropped to 87%. Myometrial and stromal elements had a much weaker relationship to biochemical ER. Sensitivity and specificity were likewise degraded when considering individual tissue components (Table 2).

The relationship between the normal stromal and myometrial H-Score components and malignant H-Score is shown in Fig. 5. The myometrial component was moderately correlated with the cancer component (r = 0.56) but stromal component was only weakly correlated (r = 0.30).

Table 3 depicts the proportion of Grade I, Grade II, and Grade III tumors which were determined to be ER positive by each of the assays. Twelve % of Grade III tumors were ER positive by immunohistochemical evaluation of the malignant component only, whereas 35% were positive by biochemical ER assay. Twenty-three of 37 (62%) Grade II tumors were ER positive and 34 of 37 (92%) Grade I tumors were positive by malignant component H-Score compared to 76% of Grade II and 92% of Grade I tumors positive by biochemical ER.

The cumulative distribution function curves for biochemical ER in Fig. 6 shows an overlap in values between Grades I and II (P = 0.50), particularly for values above 50 fmol/mg protein, whereas Grade III values are shifted to significantly lower values.

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Fig. 1. Immunohistochemical localization of estrogen receptor using monoclonal anti-human estrogen receptor antibody H222 with the peroxidase-antiperoxidase technique. Sections were prepared from fresh frozen tissue. A, endometrial carcinoma with well-differentiated glands demonstrating intense nuclear localization of estrogen receptor. Staining is relatively weak in the stroma. B, control slide for A treated with normal nonimmunized rat immunoglobulin in place of the monoclonal anti-receptor antibody. Minimal background staining with absence of specific staining in target cells is observed. No counterstain, × 170.
IMMUNOHISTOCHEMICAL ANALYSES OF ER IN ENDOMETRIAL ADENOCARCINOMA

Fig. 2. Immunohistochemical localization of estrogen receptor-stromal-myometrial localization. In A, note the relatively homogeneous nuclear staining of the myometrium and stromal cells, which in contrast to the case in Fig. 1 are more intensely stained than the majority of the epithelial elements (see Fig. 5). Also note the heterogeneity of staining intensity in the glandular epithelium. In B, again, substituting control antibody for the primary antibody yields only minimal background staining. No counterstain, × 170.

Fig. 3. Proportion of tissue components found in endometrial carcinoma specimens. Line 1, cancer component; Line 2, stromal component; Line 3, myometrial component. Cancer was present in all cases with only 37% of the specimens being composed of <50% cancer. Some degree of benign epithelial elements were present in 10% of specimens.

Fig. 4. Comparison of biochemical estrogen receptor content to total tissue histological score (H-Score). A linear relationship is observed between the logarithm of the quantitative biochemical assay of tissue homogenates (fmol of radiolabeled estradiol bound per mg protein) and the immunohistochemical H-Score of the total tissue section (intensity and proportion of cells showing positive localization of estrogen receptor). Six data points are at 0,0 (r = 0.65).

(P = 0.00002, I versus III; P = 0.0003, II versus III). By contrast, the curves for the malignant epithelium (Fig. 7) show a highly significant separation between each of the curves (P = 0.00002, I versus II; P = 0.00002, II versus III).

In contrast to the relationship found between tumor grade and cancer component H-Score, the stromal and myometrial component H-Scores bore no relationship to the histological grade of the accompanying tumor (Fig. 8).

Histological patterns of the 100 cases of endometrial carcinomas included 77 adenocarcinomas with no special features, 15 papillary adenocarcinomas, 4 adenosquamous carcinomas, 2 adenoacanthomas, and 2 clear cell carcinomas. Positive localization of ER was not observed in the squamous elements of

Table 1 Comparison of H-Score to total tissue homogenate extract biochemical ER content*

<table>
<thead>
<tr>
<th>Component</th>
<th>Biochemical ER</th>
<th>H-Score</th>
<th>+</th>
<th>-</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total tissue</td>
<td>65</td>
<td>2</td>
<td>6</td>
<td>27</td>
<td>33</td>
</tr>
<tr>
<td>Malignant epithelium</td>
<td>59</td>
<td>1</td>
<td>12</td>
<td>28</td>
<td>40</td>
</tr>
<tr>
<td>Myometrium</td>
<td>19</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Stroma</td>
<td>22</td>
<td>4</td>
<td></td>
<td></td>
<td>26</td>
</tr>
</tbody>
</table>

* Biochemical assay positive if >10 fmol/mg protein; immunohistochemical assay positive if H-Score >75.

Table 2 Comparison of immunohistochemical assay to biochemical ER assay*

<table>
<thead>
<tr>
<th>Component</th>
<th>N</th>
<th>Accuracy (%)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Predictive value (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total tissue</td>
<td>100</td>
<td>92.0</td>
<td>91.5</td>
<td>93.1</td>
<td>97.0</td>
</tr>
<tr>
<td>Malignant epithelium (H-Score &gt;75)</td>
<td>100</td>
<td>87.0</td>
<td>83.1</td>
<td>96.6</td>
<td>98.3</td>
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<tr>
<td>Myometrium (H-Score &gt;75)</td>
<td>26</td>
<td>80.8</td>
<td>86.4</td>
<td>50.0</td>
<td>90.5</td>
</tr>
<tr>
<td>Stroma (H-Score &gt;75)</td>
<td>83</td>
<td>54.2</td>
<td>44.6</td>
<td>88.9</td>
<td>93.5</td>
</tr>
</tbody>
</table>

* Biochemical assay positive if >10 fmol/mg protein; immunohistochemical assay positive if H-Score >75.
**IMMUNOHISTOCHEMICAL ANALYSES OF ER IN ENDOMETRIAL ADENOCARCINOMA**

**Fig. 5.** Comparison of immunohistochemical scores (H-Score) for normal uterine component tissues to malignant epithelial component. A, cancer component versus myometrium. Three data points are at 0, 0 (n = 26, r = 0.56). B, cancer component versus stroma. Eight data points are at 0, 0 (n = 83, r = 0.30).

**Table 3 Comparison of biochemical and immunohistological ER assays to tumor differentiation**

<table>
<thead>
<tr>
<th>Assay</th>
<th>Grade I (N = 37)</th>
<th>Grade II (N = 37)</th>
<th>Grade III (N = 26)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biochemical ER (≥10 fmol/mg protein)</td>
<td>34 (92)</td>
<td>28 (76)</td>
<td>9 (35)</td>
</tr>
<tr>
<td>Total tissue (H-Score ≥75)</td>
<td>35 (95)</td>
<td>26 (70)</td>
<td>6 (23)</td>
</tr>
<tr>
<td>Malignant epithelium (H-Score ≥75)</td>
<td>34 (92)</td>
<td>23 (62)</td>
<td>3 (12)</td>
</tr>
</tbody>
</table>

* Biochemical assay positive if ≥10 fmol/mg protein; immunohistochemical assay positive if H-Score ≥75.
* Numbers in parentheses, percentage.

**Fig. 6.** Biochemically determined estrogen receptor content by degree of tumor differentiation. Line 1, Grade I; Line 2, Grade II; Line 3, Grade III. Ordinate, cumulative proportion of tumors for each Grade which have receptor contents less than or equal to the value on the abscissa (1 versus 2, P = 0.00002; 1 versus 3, P < 0.00001; 2 versus 3, P = 0.000003).

**Fig. 7.** Malignant epithelial H-Score by degree of tumor differentiation. Line 1, Grade I; Line 2, Grade II; Line 3, Grade III. Ordinate, cumulative proportion of tumors for each Grade which have H-Scores less than or equal to the value on the abscissa (1 versus 2, P = 0.00002; 1 versus 3, P < 0.00001; 2 versus 3, P = 0.000003).

**Fig. 8.** H-Score of normal uterine tissue components by degree of tumor differentiation. Line 1, Grade I; Line 2, Grade II; Line 3, Grade III. A, myometrial component (1 versus 2, 0.05 < P < 0.1; 1 versus 3, 2 versus 3, P > 0.1). B, stromal component (1 versus 2, 1 versus 3, 2 versus 3, p > 0.1).

**DISCUSSION**

Immunohistochemical analysis of estrogen receptor in endometrial adenocarcinoma, using H222 monoclonal anti-human estrogen receptor antibody with an immunoperoxidase technique, resulted in localization of ER principally in the nuclei of target cells. This nuclear localization of estrogen receptor is in agreement with other reports using monoclonal variants.

These tumors. There were no other characteristic differences in intensity, pattern, or distribution of immunohistochemically localized estrogen receptor observed among other histological variants.
antibody immunohistochemical techniques (24, 27, 28). Earlier studies had also suggested that receptor is predominantly located in the nucleus (29–31). Welshons et al. (32) used a novel approach in which, after estrogen receptor-rich rat pituitary GH-3 cells had been fractionated with cytochalasin B, the vast majority of unoccupied estrogen receptor was associated with nucleoplasm rather than cytoplasm fraction. These findings suggest modification of the “two-step” mechanism of estrogen action (33, 34). What may be measured as cytosol receptor by standard biochemical assays may represent relative extraction from the nuclear compartment as a result of cell disruption during tissue homogenization (28, 29). It is likely that small amounts of receptor are present in the cytoplasm at sites of receptor synthesis which are not detected by immunohistochemical methods, but it seems reasonable from the data that the vast majority of receptor resides in the nucleus. Although modification of the standard model for estrogen receptor action seems to be indicated, its fundamental premise still holds; i.e., activated steroid-receptor complexes exert their effects through interaction with the nuclei of target cells (35–37).

Comparison of biochemical assays of tissue homogenates for estrogen receptor values to the immunohistochemical assay on total tissue sections of endometrial carcinoma resulted in a correlation coefficient, accuracy, sensitivity, specificity, and predictive values with respect to biochemical ER superior to those observed by comparison to individual tissue components. While stromal and myometrial components were strongly related to total H-Score (as they must be since they entered into its calculation), they were only weakly correlated to the biochemical ER. This observation suggests that the normal tissue contribution to biochemical ER may be quantitatively less than that from the cancer component, while still significant, particularly in which the epithelium was ER poor.

A smaller proportion of Grade III tumors were shown to be ER positive by immunohistochemical analysis of the cancer component only than by biochemical assay of tissue homogenates, again reflecting the potential of stromal and myometrial elements to confound the biochemical assay of the tissue homogenates. Thus the cancer-specific H-Score might be anticipated to provide a discriminant of prognostic and therapeutic utility complementary to that of the biochemical assay.

A critical finding of this study is the poor correlation of receptor localization scores (H-Score) in stromal-myometrial elements to malignant epithelial element H-Score (Fig. 5). This observation emphasizes the suggestion that biochemical assays of total tissue homogenates may not accurately reflect estrogen receptor content of the cancer component, which is the principal element relevant to treatment response and prognosis. Inasmuch as nonmalignant components often may be significant in quantity, they may be expected to confound the prediction of biological response of the malignant component from biochemical receptor assays.

The correlation observed between biochemical assays and the immunohistochemical assay for estrogen receptor (Fig. 4) underscores the importance of incorporating both the percentage of positively stained cells and the intensity of individual cell staining in the evaluation of any histological receptor localization method. The intensity of staining appears to reflect the number of receptor sites, which may vary from 1,000 to 20,000 sites per cell (38). Fig. 4 and Tables 1–3 reflect the excellent sensitivity and specificity of this scoring technique at relatively low levels of estrogen receptor binding (10 fmol/mg protein).

An observation of particular note is the heterogeneity of staining in malignant epithelium compared to the relatively homogeneous localization of ER in benign epithelium, stroma, and myometrium. Similar heterogeneity in breast cancers was noted by others (27). Whether this heterogeneity in staining reflects polyclonal origin of the tumors or asynchrony of receptor expression due to physiological factors requires further investigation. Press et al. (28) demonstrated that immunohistochemical localization of ER in human endometrium varied with cell type and with the menstrual cycle. They also noted the absence of specifically stained mitotic figures, suggesting that histological detection of ER may not be possible during some phases of the cell cycle.

Immunohistochemical localization of estrogen receptor using H222 monoclonal antibody appears to circumvent some of the complicating factors of benign epithelial, stromal, and myometrial contribution to biochemical receptor analyses of tissue homogenates. Such analyses may provide information that complements quantitative biochemical receptor analyses, pathological features, and tumor staging in the evaluation of endometrial adenocarcinomas including study of stromal-epithelial relationships in the biology of these tumors. The present study will serve as a data base for establishment of the possible significance of the differences observed in the behavior of these tumors with long term clinical follow-up. The observations reported should serve to alert others to the probable need to discriminate component contributions to total receptor in such studies.

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Immunohistochemical Analyses of Estrogen Receptor in Endometrial Adenocarcinoma Using a Monoclonal Antibody
