Immunohistological demonstration of progesterone receptor in breast carcinomas: correlation with radioligand binding assays and oestrogen receptor immunohistology

D D GIRI, J R GOEPEL, K ROGERS,* J C E UNDERWOOD

*From the Departments of Pathology and *Surgery, University of Sheffield Medical School, Sheffield*

SUMMARY The validity of determining the progesterone receptor status of breast carcinomas with a monoclonal antibody was investigated by comparison with data from a radioligand binding assay on adjacent cryostat sections of 103 tumours. Significant nuclear staining for progesterone receptor was observed in 37 (36%) of the tumours studied and this showed a close correlation with the results of radioligand binding assays for progesterone receptor. In three progesterone receptor positive tumours there was an apparent paradoxical absence of oestrogen receptor; progesterone receptor normally depends on the presence of oestrogen receptor, but these rare tumours may be essentially progesterone receptor positive.

It is concluded that this monoclonal antibody is an appropriate reagent for use in the immunohistological determination of progesterone receptor status of breast carcinomas; that it advantageously identifies both the occupied and unoccupied receptor sites; and that it provides information about tumour cell heterogeneity with respect to receptor status.

Progesterone receptor assays are a useful adjunct to oestrogen receptor assays in the management of breast cancer. Progesterone receptor is a post-receptor marker of oestrogen receptor, and its presence in a tumour indicates that the oestrogen receptor is functioning and that the patient is more likely to benefit from endocrine treatment.¹

Biochemical assays for progesterone receptor using radioligand binding techniques and tissue homogenates suffer from drawbacks (alluded to previously) with respect to oestrogen receptor assays²; there is no confirmation that viable tumour is present in the assayed sample; dilution by stromal elements produces falsely low values which cannot be easily corrected; there is no information about cellular heterogeneity with respect to receptor status; and, as routinely performed, only the unoccupied form of the receptor is assayed. Monoclonal antibodies to oestrogen receptor have been validated as appropriate reagents for immunohistological analysis, thus obviating the disadvantages of radioligand binding assays,³

but monoclonal antibodies to progesterone receptor have been subjected to only limited validation.⁴

We correlated the results of immunohistological analysis for progesterone receptor using a monoclonal antibody claimed to have that specificity⁵ with radioligand binding assay data on a series of breast carcinomas. Because such comparisons of two methods on separate tissue samples of possibly dissimilar cellular composition may lead to discrepancies on account of tumour heterogeneity, we performed progesterone receptor immunohistology and radioligand binding assays on adjacent cryostat sections from the same tissue block.⁶

Material and methods

Tissue blocks from 103 breast carcinomas were sampled and stored as described previously.³

Cryostat sections were cut as described previously.³ Adjacent sections were cut at 4-6 μ m for immunohistology and quantitative estimation of cellularity by point counting,⁶ and at 40 μ m for radioligand binding assays.

PROGESTERONE RECEPTOR IMMUNOHISTOLOGY

Cryostat sections were fixed in a picric acid-paraformaldehyde fixative (pH 7.4)⁷ for 15 minutes at 4°C and then washed in phosphate buffered saline (PBS; pH 7.4) for 45 minutes at 4°C. Endogenous peroxidase was blocked by incubation in 0.5% hydrogen peroxide in PBS for 15 minutes at room temperature followed by two washes in PBS, each of 10 minutes' duration. Sections were then successively incubated with the monoclonal murine antibody to mammalian progesterone receptor (mPRI; 10 µg/ml; Transbio Sarl, Paris, France)⁵ for 16–18 hours at 4°C, rabbit antimouse IgG (1/80; Dako Ltd, UK) for 45 minutes at room temperature, and mouse peroxidase-antiperoxidase (1/100; Miles Laboratories, UK) for 45 minutes at room temperature. Each incubation was followed by three 10 minute washes in PBS containing 0.05% Tween 80. After the final wash sections were incubated in 0.05M Tris-hydrochloric acid buffer (pH 7.6) containing 0.5 mg/ml 3,3'-diaminobenzidine tetrahydrochloride (DAB) and 0.01% hydrogen peroxide for 10 minutes at room temperature. Sections were then washed in PBS, lightly counterstained with Harris's haematoxylin for 4–6 minutes, washed, dehydrated, and mounted in DPX.

Sections of breast cancer known to be progesterone receptor positive and sections incubated with non-immune mouse serum were included as positive and negative controls, respectively, in each batch of staining.

The intensity of the nuclear staining was graded: 0 = no staining; + = weak staining; ++ = intermediate; +++ = strong staining. In a previous study in which staining indices were computed for oestrogen receptor immunohistology³ it was observed that at least 25% of the tumour cells were required to have ++/+++ staining intensities to achieve a significant correlation between the immunohistological and biochemical assay results. In this study we therefore regarded sections showing ++/+++ staining intensity in over 25% of the tumour cells as positive immunocytochemically for progesterone receptor.

OESTROGEN RECEPTOR IMMUNOHISTOLOGY

Oestrogen receptor was shown immunohistochemically using a monoclonal antibody (Abbott Laboratories Ltd, UK) and the protocol described previously.³

RADIOLIGAND BINDING ASSAY FOR PROGESTERONE RECEPTOR

Radioligand binding assay was performed as described previously.⁸ In summary, progesterone receptor was eluted from cryostat sections by immersion in 200 µl buffer (5 mM KH₂PO₄, 2 mM mercaptoethanol,

10% W/V glycerol, 0.05% W/V bovine serum albumin, 0.02% W/V NaN₃; pH 7.4) in small plastic tubes, to which was added an equal volume of the same buffer containing 2×10^{-9} M ³H-ORG2058 (2.14 TBq/mM; Amersham International plc). After incubation for two hours with intermittent agitation and centrifugation the clear supernatant was treated with dextran-coated charcoal. After a further short incubation followed by centrifugation the protein concentration in a 50 µl aliquot was determined by a modified Lowry's method.⁹ Duplicate 100 µl aliquots were then subjected to isoelectric focusing to separate the receptor-bound ligand from that which was non-specifically bound.

Each 100 µl aliquot was pipetted into a perspex sample application frame on the surface of the polyacrylamide gel (245 × 110 × 2 mm; 2.4% W/V ampholine, pH 3.5–9.5; LKB). The gel was focused for 120 minutes after which each sample track was cut into 5 mm slices. The radioactivity in each slice was determined by liquid scintillation counting. Values greater than 5 fmol/mg protein were regarded as positive.

RADIOLIGAND BINDING ASSAY FOR OESTROGEN RECEPTOR

The oestrogen receptor content of cryostat sections was assayed by radioligand binding and isoelectric focusing as described previously.⁶

The χ^2 test was used for all the comparative analyses.

Results

The monoclonal antibody used in this study produced consistent staining of tumour cell nuclei (figure). No staining of stromal cell nuclei or staining of the cytoplasm of either the tumour or stromal cells was seen. Three patterns of staining were noted: uniform staining of all tumour cell nuclei; cell to cell heterogeneity—some nuclei stained, others unstained; regional heterogeneity—for example, staining of the nuclei of the intraduct component, but no staining of the nuclei of the surrounding invasive tumour cells.

There was a high degree of correlation between the immunohistological staining grades and the assayable progesterone receptor in adjacent sections ($p < 0.0001$) (table 1).

There was good correlation between progesterone receptor and oestrogen receptor staining grades in adjacent sections from the same tumour ($p < 0.001$) (table 2). Three tumours however, assigned 0/+ grades for oestrogen receptor immunohistology, were regarded as ++/+++ when stained for progesterone receptor; of these three tumours, one showed a complete absence of staining for oestrogen receptor.

Fig Uniform staining of breast cancer cell nuclei by monoclonal antibody to progesterone receptor. (Immunoperoxidase with weak nuclear counterstain.)

Staining patterns, whether homogeneous or heterogeneous, were similar for both oestrogen receptor and progesterone receptor in most tumours. In about 40% of tumours oestrogen receptor positive cells seemed to be more abundant than progesterone receptor positive cells. Only in less than 10% of tumours did progesterone receptor positive cells exceed those that were oestrogen receptor positive.

Discussion

The high degree of correlation between progesterone receptor immunohistological analysis and the radioligand-binding assay results on this series of tumours confirms the suitability of the monoclonal

Table 1 Correlation between staining grades obtained with progesterone receptor monoclonal antibody and progesterone receptor content of adjacent cryostat sections assayed by radioligand binding

Immunohistological staining grades	No of tumours	
	Radioligand binding assay	
	Positive	Negative
0/+	2	64
++/+++	25	12

$p < 0.0001$

Table 2 Correlation between progesterone and oestrogen receptor immunohistological staining grades

Progesterone receptor immunohistological staining grades	No of tumours	
	Oestrogen receptor immunohistological staining grades	
	++/+++	0/+
0/+	32	34
++/+++	34	3

$p < 0.001$

antibody used in this study for determining the progesterone receptor status of breast carcinomas.

The nuclear location of staining was consistent with current concepts of sex-steroid receptor metabolism and was identical with that observed with monoclonal antibodies to oestrogen receptor protein. We noted that the nuclear staining was more dense and sharply defined than that for oestrogen receptor, corresponding to the ultrastructural distribution observed on immunoelectron microscopy, in which progesterone receptor is associated with condensed chromatin.¹⁰ The antibody also showed intratumoural heterogeneity of progesterone receptor expression, similar to that observed in human breast carcinomas stained for oestrogen receptor.³

Progesterone receptor in breast epithelium is usually considered to be a "post-receptor" marker of a functioning oestrogen receptor. In defiance of currently accepted sex-steroid receptor mechanisms a few tumours in this study stained strongly for progesterone receptor but, unexpectedly, negatively or only weakly for oestrogen receptor with concordant results by radioligand binding assays. It is unlikely that this discrepancy could be due to interference of the immunostaining for oestrogen receptor by endogenous oestrogens because the monoclonal antibody reacts with an epitope which is neither occluded nor conformationally distorted by oestrogen binding. These tumours may therefore be constitutively progesterone receptor positive. This phenomenon has been reported previously in meningiomas: oestrogen receptor has been shown to be absent biochemically⁸ and immunocytochemically (Ironside JW, personal communication 1987), but progesterone receptor has been found in a considerable proportion of these tumours by radioligand binding assay⁸ and immunocytochemistry (Giri DD, *et al*, unpublished observations).

Immunohistological analysis will also show progesterone receptor positive breast cancers that give false negative results for oestrogen receptor by radioligand binding assay because endogenous oestrogens have saturated the available binding sites.¹¹ The only study comparable with ours comprised 27 cases, of which

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just 19 were appropriately examined by the application of monoclonal antibodies to adjacent sections; discrepancies between oestrogen and progesterone receptor staining were not observed but this may well have been accounted for by the absence of tumours exhibiting this rare but interesting phenomenon—that is, constitutive progesterone receptor positivity—from that relatively small series.⁴ Whether these constitutively progesterone receptor positive tumours respond to appropriate endocrine treatment awaits evaluation.

Twelve tumours in this study were progesterone receptor negative by radioligand binding assay but positive by immunohistological analysis. Of these, four were from premenopausal women in whom endogenous progestational steroids may have occupied the receptor thus rendering it inaccessible to assay, and three tumours, including two colloid carcinomas, were of unusually low cellularity (<5% compared with about 20% for the whole group). Ten of these 12 tumours gave a radioactive peak on isoelectric focusing at a pI appropriate for progesterone receptor, but the calculated assay value was <5 fmol/mg and therefore regarded as negative. One of the two tumours positive by radioligand binding assay but negative by immunohistology had unusually high cellularity (35%).

Immunohistological analysis for progesterone receptor is a useful adjunct to oestrogen receptor immunohistology, thus permitting histological identification of tumours most likely to benefit from currently available endocrine treatment. The development of breast screening programmes will yield a greater proportion of small carcinomas for which traditional biochemical steroid receptor assays would be unsuitable because there may be insufficient tissue. The monoclonal antibodies to steroid hormone receptors yield results which correlate closely with traditional biochemical methods and will be especially appropriate for tumours detected by screening.

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References

- McGuire WL, Clark GM. The prognostic role of progesterone receptors in human breast cancer. *Semin Oncol* 1983;10:2-6.
- Underwood JCE. Oestrogen receptors in human breast cancer: review of histopathological correlations and critique of histochemical methods. *Diagn Histopathol* 1983;6:1-22.
- Giri DD, Dangerfield VJM, Lonsdale R, Rogers K, Underwood JCE. Immunohistology of oestrogen receptor and D5 antigen in breast cancer: correlation with oestrogen receptor content of adjacent cryostat sections assayed by radioligand binding and enzyme immunoassay. *J Clin Pathol* 1987;40:734-40.
- Perrot-Applanat M, Groyer-Picard M-T, Lorenzo F, et al. Immunocytochemical study with monoclonal antibodies to progesterone receptor in human breast tumours. *Cancer Res* 1987;47:2652-61.
- Logeat F, Hai MTV, Fournier A, Legrain P, Buttin G, Milgrom E. Monoclonal antibodies to rabbit progesterone receptor: crossreaction with other mammalian progesterone receptors. *Proc Natl Acad Sci USA* 1983;80:6456-9.
- Underwood JCE, Dangerfield VJM, Parsons MA. Oestrogen receptor assay of cryostat sections of human breast carcinomas with simultaneous quantitative histology. *J Clin Pathol* 1983;36:399-405.
- Stefani M, De Martino C, Zamboni L. Fixation of ejaculated spermatozoa for electron microscopy. *Nature* 1967;216:173-4.
- Ironside JW, Battersby RDE, Dangerfield VJM, Parsons MA, Timperley WR, Underwood JCE. Cryostat section assay of oestrogen and progesterone receptors in meningiomas: a clinicopathological study. *J Clin Pathol* 1986;39:44-50.
- Hartree EF. Determination of protein: a modification of the Lowry method that gives a linear photometric response. *Anal Biochem* 1972;48:422-7.
- Perrot-Applanat M, Groyer-Picard M-T, Logeat F, Milgrom E. Ultrastructural localization of the progesterone receptor by an immunogold method: effect of hormone administration. *J Cell Biol* 1986;102:1191-9.
- Kiang DT, Kollander R. Breast cancers negative for oestrogen receptor but positive for progesterone receptor, a true entity? *J Clin Oncol* 1987;5:662-6.

Requests for reprints to: Professor J C E Underwood, Department of Pathology, University of Sheffield Medical School, Beech Hill Road, Sheffield S10 2RX, England.

DD Giri is a Commonwealth medical research scholar for whom the Association of Commonwealth Univer-