Patterns of basement membrane deposition in benign, premalignant, and malignant endometrium

P N Furness, E W H Lam

From the Department of Pathology, Queen’s Medical Centre, University Hospital, Nottingham

SUMMARY Immunocytochemical staining for laminin, an intrinsic basement membrane component, was used to show and quantify the distribution of basement membranes in endometrium. In normal endometrium, glands which are not mechanically disrupted have almost entirely continuous basement membranes, even in the menstrual phase. This is also seen in benign cystic hyperplasia. In atypical hyperplasia a small proportion of glands show small breaks in basement membrane staining in the absence of invasion. The number of breaks increases with more severe cytological changes, and this abnormality may persist even when a second biopsy specimen shows an apparent return to normal morphology. Invasive tumours show a strikingly different pattern of basement membrane staining, even when very well differentiated.

In normal tissues the basement membrane forms a continuous sheet that demarcates the epithelium from the stromal compartments, and this is essential for the normal organisation and differentiation of tissues. Penetration of the basement membrane has to occur before an epithelial neoplasm can be described as invasive; indeed, the definition of carcinoma in situ hinges on this point. Recently there have been considerable advances in the understanding of the composition and morphology of basement membrane.

Several glycoproteins have been identified which seem to be found almost exclusively in basement membranes; these include type IV collagen, laminin and heparan sulphate proteoglycan. This has allowed accurate and specific immunohistochemical techniques for basement membrane localisation to be developed. Even very small (10 μm) breaks in the basement membrane can be visualised. Application of multiple staining techniques to individual sections indicates that all the basement membrane components mentioned above invariably colocalise at light microscope level, so any one can be used as a reliable marker for the presence of a basement membrane. Laminin is particularly suitable, as it can readily be shown on conventionally processed paraffin sections.

This technique has repeatedly shown that normal tissues and benign epithelial proliferations have continuous basement membranes; fragmented or absent basement membranes are seen in invasive carcinoma. Few exceptions have been reported; some benign skin appendage tumours apparently have discontinuous basement membranes, and “linear” basement membranes have been described in adenoid cystic carcinoma, though the illustrations showed numerous distinct breaks. It has been suggested that this method of basement membrane staining may have diagnostic application, particularly in breast disease. A recent report found the intensity of laminin staining in rectal carcinoma to be a better prognostic indicator than tumour grade.

Reports of basement membrane continuity in epithelial dysplasia and carcinoma in situ have varied; some have described continuous basement membranes; others have found numerous small breaks in the absence of evidence of invasion. These discontinuities might be interpreted as a “first step” towards the development of invasion. It is not known whether they are due to defective production or increased removal of basement membrane material.

Immunocytochemical localisation of basement membrane components has not previously been applied to endometrium, where the fragmented nature of the curettage specimen often makes direct observation of invasion impossible. We therefore studied the pattern of basement membrane deposition in benign, premalignant, and malignant conditions of the endometrium. As the endometrium undergoes repeated proliferation and degeneration we also studied normal endometrium in each phase of the cycle to confirm that continuity of glandular basement membranes is a normal feature.

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Material and methods

We studied one hundred and fifty five uterine curettage specimens submitted to the pathology department of the University Hospital, Nottingham. Each diagnostic category represented a sequential series, though specimens were excluded if: review of the haematoxylin and eosin stained section did not confirm the reported diagnosis; pronounced inflammatory changes could be seen; sufficient tissue was not available for study. The table details the categories studied. Atypical hyperplasia was subdivided by subjective grading by one observer on the basis of the severity of cytological atypia alone.

Polyclonal antilaminin antiserum was obtained from EY laboratories. The technique was slightly modified from that of Ekblom et al.\(^9\) Paraflin sections were dewaxed and incubated in a 0-4% solution of pepsin (Sigma) in 0-01M hydrochloric acid for two and a half hours at 37°C. A routine indirect immunoperoxidase technique was then used, with a 1/75 dilution of primary antiserum for half an hour at room temperature.

Sections stained for laminin were scanned by an individual observer. Intact glands only were counted, with a maximum of 300 and minimum of 50 glands per case. The number of glands showing any discontinuity in basement membrane staining was recorded. To check precision one case (from the atypical hyperplasia group) was assessed in this manner 10 times on separate days.

As the data recording the number of breaks per gland were non-parametric, significance was assessed using Mann-Whitney's U test.

In every case of cystic hyperplasia, atypical hyperplasia, or well differentiated adenocarcinoma a search was made of the pathology department files for any subsequent curettage or hysterectomy specimens. Where found, these were reviewed for concordance with the original report. In selected cases sections of the second specimens were stained for laminin and assessed as above.

Results

Immunostaining for laminin produces extremely sharp definition which readily permits identification of small basement membrane discontinuities. The table shows the average number of breaks per 100 glands. In normal endometrium very few glands showed any discontinuity in basement membrane staining (mean 0-34%), though with menstrual endometrium only morphologically intact glands were counted (fig 1). Post menopausal endometrium showed a slightly higher mean number of breaks (0-51%), though this was not significant. No increase was found in benign cystic hyperplasia (mean 0-22%). Atypical hyperplasia, however, showed a considerably increased proportion of glands with basement membrane discontinuities (mean 6-53%) (fig 2), which was significant (p < 0.0001). These breaks were not confined to areas showing morphological features of atypical hyperplasia. Within the subdivisions of atypical hyperplasia, a significant difference was also found between mild atypia (5-28%) and severe atypia (8-41%, p < 0.05).

![Fig 1 Normal proliferative endometrium showing continuous basement membranes. (Immunoperoxidase method for laminin.)](http://jcp.bmj.com/content/40/11/1321)
compared subjected were abnormalities are defined break had who any two of which three came apparently representing an invasive lesion. The specimen, complete specimens, which showed any evidence of invasive neoplasm and two showed an apparently complete return to normal. Of the specimens showing a return to normal morphology, these came from patients treated by repeat curettage who had received progestogens in the interval; patients treated by hysterectomy had received no other treatment.

Follow up of the cases of adenocarcinoma confirmed invasive neoplasm in 11 hysterectomy specimens.

The cases showing an apparent return to normal were subjected to laminin staining of the endometrium. In every case, although the number of basement membrane discontinuities was reduced when compared with that of the first biopsy specimen, it remained considerably higher than the mean of the control group (2.2%, 2-2%, 5%, 6-4% and 8-9%; control mean 0-34%).

The assessment of numbers of breaks was found to have a coefficient of variation of 4-75%, indicating an acceptable level of precision.

Discussion

The pattern of basement membrane deposition in premalignant epithelial lesions is the subject of conflicting reports, even within one tissue. Our results indicate that in the endometrium non-invasive lesions have almost continuous basement membranes; but with the development of progressively more atypical features there is an increasing tendency for small, well defined breaks in basement membranes, without invasion. This may reasonably be attributed to a progressive change towards the development of invasion, but it is not clear whether this is the result of defective production of basement membranes, or the induction of degradative enzymes, or both.

The development of an invasive neoplasm seems to be associated with a much more sudden change in basement membrane deposition. Severe fragmentation is seen, even in very well differentiated adenocarcinoma. This change is easily recognisable and invariable, and therefore has potential as a diagnostic tool. The findings on follow up in this series suggest that the distinction between atypical hyperplasia and adenocarcinoma can be made accurately in most cases, using only conventional haematoxylin and eosin stained sections, but difficulty can occasionally arise, particularly if the specimen is small. At the other end of the spectrum, our findings support the belief that cystic hyperplasia and atypical hyperplasia are biologically distinct entities, and that cystic hyperplasia has no premalignant potential. We would not advocate the use of basement membrane staining as a diagnostic tool in this area, however, as the technique is laborious and there is slight overlap between the two groups.

Where atypical hyperplasia morphologically returned to normal on second biopsy, we found a persistent increase in basement membrane discontinuities. This suggests that an apparently normal morphology may be masking a continuing biological abnormality. We have only a small number of such cases and further study is obviously required, but we would suggest that a single report of normal endometrium following one of atypical hyperplasia should not be taken as evidence of "cure"; continuing follow up, with repeated curettage or hysterectomy, is still indicated.

In conclusion, we have shown that normal endometrial glands have almost completely continuous basement membranes, even in the menstrual phase—if mechanically fragmented glands are excluded. The
development of atypical hyperplasia is associated with increasing numbers of small, well defined breaks in basement membranes, which do not necessarily return to normal even when normal morphology is otherwise restored. The development of invasive neoplasms is associated with a sudden change to a fragmented pattern of basement membrane deposition, a finding which is of potential diagnostic use.

References


Requests for reprints to: Dr PN Furness, Department of Pathology, University Hospital, Queen’s Medical Centre, Nottingham NG7 2UH, England.