Expression of epithelial membrane and 3-fucosyl-N-acetyllactosamine antigens in cervix uteri with particular reference to adenocarcinoma in situ

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SUMMARY The staining patterns obtained with antiepithelial membrane antigen (anti-EMA) and the monoclonal antibody to 3-fucosyl-N-acetyllactosamine (AGF 4:48) in the uterine cervix in intraepithelial and invasive neoplasia were compared to determine a possible role in differential diagnosis of reactive and neoplastic conditions. Both early invasive and in situ adenocarcinoma stained equally intensely with both agents and both antibodies stained diffusely tubal metaplasia, endometrial lined glands, and even occasional areas of normal endocervical mucosa.

It is concluded that these agents are unlikely to be of use in the routine histological differentiation of glandular and squamous cervical dysplasia or neoplasia, but immunostaining with anti-EMA may help differentiate between reactive and metaplastic changes in endocervical glands and adenocarcinoma in situ.

Previous studies by other workers,1 using a monoclonal antibody related to Dako-EMA (anti-human milk fat globulin 1), have suggested that differentiation antigens and neoplastic markers using monoclonal antibodies against carbohydrate determinants of the above type, and previous studies of human breast tissue have indicated that clinically important changes in fucose containing glycoconjugates occur in malignant disease.14

The carbohydrate group believed to be detected by AGF 4:48 is widely distributed in human tissue.2 An association has recently been shown between certain differentiation antigens and neoplastic markers using monoclonal antibodies against carbohydrate determinants of the above type, and previous studies of human breast tissue have indicated that clinically important changes in fucose containing glycoconjugates occur in malignant disease.14

It has also been shown that in formalin fixed normal endocervical tissue considerable changes in staining with AGF 4:48 may be achieved by prior treatment with neuraminidase, which may be due to antigen unmasking by removal of neuraminic acid residues.

We compared the staining patterns obtained with antiepithelial membrane antigen (anti-EMA) and AGF 4:48 in the uterine cervix in intraepithelial and invasive neoplasia to determine whether these monoclonal antibodies could be used in the differential diagnosis of reactive and neoplastic conditions, with particular reference to intraepithelial glandular neoplasia.

Material and methods

Anti-EMA (Dako-EMA, Dako Ltd, High Wycombe, Buckinghamshire) is a monoclonal antibody raised against a preparation of human milk fat globule membrane protein.5 6 The determinant recognised by this antibody is thought to be carbohydrate in nature. AGF 4:48 is a monoclonal antibody which has been shown to detect the carbohydrate group 3-fucosyl-N-acetyllactosamine7 also detected by the monoclonal antibodies VEPF, VEP9, My-1, SSEA1, and IG10.8 9

All of the tissues used were fixed in Bouin’s solution. All sections were dewaxed and endogenous peroxidase blocked with 0.5% hydrogen peroxide in methanol for 30 minutes. After rinses in distilled water and phosphate buffered saline (PBS) at pH 7.5, containing 1% normal swine serum, sections for AGF 4:48 staining were incubated with hybridoma culture supernate, diluted 1 in 3 with PBS, in a damp chamber for one and a half hours. Sections for staining with anti-EMA were

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rinsed in distilled water and left in distilled water for 10 minutes at 37°C before immersing in 0.1% trypsin (Difco) in 0.1% calcium chloride for 20 minutes at 37°C. They were then washed in running tap water for 15 minutes, twice in PBS with 1% normal swine serum for five minutes and then incubated with anti-EMA, diluted 1 in 50 with PBS, in a damp chamber for one hour.

For both antibodies, treatment from this stage was identical and as follows: sections were washed twice in PBS with 1% normal swine serum, incubated with rabbit antimouse immunoglobulins (Dako, diluted 1 in 40 with PBS) for 30 minutes, washed twice in PBS, incubated with swine antirabbit immunoglobulin (diluted 1 in 40 with PBS) for 30 minutes, washed twice in PBS with 1% normal swine serum, incubated with PAP (Dako, diluted 1 in 40 with PBS) for 30 minutes, washed twice in PBS with 1% normal swine serum followed by a PBS wash and incubated with diaminobenzidine-hydrogen peroxide solution in PBS for eight minutes. After rinsing in distilled water sections were counterstained with Mayer's haematoxylin for one and a half minutes, blued in tap water, dehydrated, cleared and mounted.

Prior treatment with neuraminidase was performed before blocking endogenous peroxidase by incubating sections rinsed with acetate buffer at pH 5.5 with a solution of one unit per ml neuraminidase (Type V, Sigma Chemical Co Ltd, Poole, Dorset) and 1% calcium chloride for one hour and 16 hours at 37°C, followed by washing with tap water. Trypsinisation before staining with AGF 4:48 was performed as described for EMA staining. Control sections were incubated in buffer.

Sections used were all from cone biopsy specimens and were chosen to permit internal controls on each section—for example, cervical intraepithelial neoplasia, grade III (CIN III), adenocarcinoma in situ and immature metaplasia in one section. Twenty one cases were studied including six cases of adenocarcinoma in situ.

Results

Normal Cervix

Both anti-EMA and AGF 4:48 stained the ectocervical squamous mucosa variably within and between cases. Occasional areas showed intense staining usually with relative sparing of the suprabasilar zone, but more commonly staining was weak or absent.

Both antisera stained the squamous side of the squamo-columnar junction more intensely than the rest of the squamous mucosa, and this seemed to be a consistent finding.

The normal endocervical surface and glandular mucosa typically stained only on its free (luminal)
AGF 4:48 and anti-EMA expression: association with adenocarcinoma

Again more weakly of intensity cinoma made it positively with anti-EMA endosalpingeal-type epithelium both stained this EMA This was more clearly non-dysplastic diffuse, usually anti-EMA but with surface gave a "pencilled" margin to the cells (fig 1). This was seen consistently and intensely with anti-EMA but with both antibodies occasional foci of diffuse, usually weak cellular staining were seen in clearly non-dysplastic cells.

NON-NEOPLASTIC CERVICAL CONDITIONS
Immature metaplastic squamous epithelium and areas of reserve cell hyperplasia generally stained positively with both anti-EMA and AGF 4:48. This staining was not as intense as that of adenocarcinoma in situ but did approach the intensity of staining of some areas of CIN III. Clearly this finding makes it unlikely that either agent would be of use in the cytological differentiation of these cellular changes from neoplasia. Microglandular hyperplasia did not stain with either antibody.

Glands in the endocervix lined by endometrial or endosalpingeal-type epithelium both stained positively with anti-EMA and AGF 4:48. With anti-EMA this staining was weak but diffuse, the intensity made it generally easy to distinguish from adenocarcinoma in situ. In the case of AGF 4:48 (fig 2), the intensity of staining was similar to that seen in the more weakly stained areas of adenocarcinoma in situ. Again this clearly limits the usefulness of this antibody in differentiating these conditions, which can occasionally be a source of diagnostic difficulty.

Areas of reactive hyperchromasia in glandular epithelium and squamous epithelium did not stain positively with either antibody.

CERVICAL INTRAEPITHELIAL NEOPLASIA (SQUAMOUS)
All cases of CIN III studied stained positively with anti-EMA, though staining was variable and patchy. Gland cleft tissue stained similarly to surface mucosa. Findings with AGF 4:48 were similar but more variable; some areas of CIN III stained intensely and others unstained even in the same section.

ADENOCARCINOMA IN SITU
Anti-EMA stained areas of adenocarcinoma in situ (both gland cleft and surface epithelial) intensely and consistently (fig 3). In areas where a mixed pattern of mucin secreting cells and squamous cells was seen staining was equally intense and well defined. Unlike the normal glandular cells, the neoplastic cells stained diffusely throughout the cytoplasm, though some surface accentuation was seen in a few cases. In almost all areas the clear cut division between neoplastic and non-neoplastic glandular cells was reflected in the anti-
EMA staining (fig 4). In an occasional focus some increased staining of the adjacent non-neoplastic cells was seen but this appeared to be only on the free border and was consistent with excess glycocalyx production by the adjacent neoplastic cells.

AGF 4:48 stained adenocarcinoma in situ in a very similar manner to anti-EMA (fig 5), but the staining was generally less intense. Occasional areas of adenocarcinoma in situ did not stain at all.

**INVASIVE SQUAMOUS, ADENOSQUAMOUS, AND ADENOCARCINOMA**

All three disease states stained intensely with anti-EMA (fig 6), and positively, though more variably with AGF 4:48.

**EFFECT OF NEURAMINIDASE AND PRETREATMENT WITH TRYPSIN ON AGF 4:48 STAINING**

Neuraminidase treatment for either one hour or 16 hours produced no definite increase in staining intensity or change in the staining pattern. Trypsinisation also produced no increase in staining. In fact, both pretreatments seemed, in some cases, to lead to a reduction in staining generally.

**Discussion**

Adenocarcinoma in situ of the endocervix was first described over 30 years ago and until recently was considered to be relatively rare, though 160 cases had been reported up until 1984. Clearly, in the diagnosis of this condition the most important prognostic feature must be the presence or absence of invasion. This is often extremely difficult to determine and assessment of architectural changes, stromal response, and gland depth may all be problematic. Unfortunately, this study does not offer hope for the use of the antibodies investigated in differentiating early invasive from in situ lesions as both conditions stain strongly with both agents.

As stated, it has been suggested that glandular atypia of lesser degrees of severity than adenocarcinoma in situ can be recognised in the endocervix and that a monoclonal antibody raised to human milk fat globulin might be useful as a marker for this atypia. In our studies neither anti-EMA nor AGF 4:48 clearly defined areas of glandular dysplasia of lesser severity than adenocarcinoma in situ adjacent to this condition, though this conclusion is based on subjective interpretation of what degree of cellular pleomorphism constitutes “atypia”. The results cannot be regarded as evidence against the existence of glandular dysplasia which should be expected to occur on an empirical basis, and which, we believe, does exist. What does seem to be clear is that in Bouin’s fixative both anti-EMA and AGF 4:48 may be used preferen-
Glandular adenocarcinoma to younger and the invasive immature metaplasia in invasive zone. of staining fixed with analogy neuraminidase non-neoplastic from discrepancy (and cervical Whether this is the EMA. possibility mucin after treatment with both antibodies were seen in glands. The fact that lesser differentiate entirely endometrial lined inflammatory reactive case occur, patchy staining metaplasia “unmasking” of weaker but anti-EMA, may differentiate adenocarcinoma in situ, and AGF 448 is unlikely to be of diagnostic use in the cervix. Our grateful thanks go to Mrs S Smith for her excellent secretarial assistance and to Mr Alan Cooper for photographic help.

References
1 Brown LJR, Griffin NR, Welle M. Cytoplasmic reactivity with the monoclonal antibody HMF1 as a marker of cervical glandular atypia. J Pathol 1987;151:203–8.


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