Virchows Arch [Pathol Anat] (1985) 407:59–67

Virchows Archiv A

Expression of MAM-3 and MAM-6 antigens in endometrial and endocervical adenocarcinomas

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Summary. Expression of milkfat globule membrane antigens (MAM-3, MAM-6) was investigated by an indirect immunoperoxidase method on normal and malignant glands of the endocervical and endometrial tissues. MAM-3 antigens, as detectable with monoclonals 67D11 and 115G3, were not detected in normal endometrial glands, but they were found in the majority of endometrial adenocarcinomas. In the endocervix, MAM-3 antigens were present in almost all of the normal glands and in the cancerous glands. MAM-6 antigens, as detectable with monoclonals 115D8 and 115F5, were observed in majority of normal and neoplastic glands within the endometrium and endocervix.

The staining appearance of the antigens was generally heterogenous in the neoplastic tissues but more homogenous in normal glandular tissues. These results are discussed and compared with the grade of histological differentiation, mucin production and positivity for CEA.

Key words: Uterine adenocarcinoma – Monoclonal antibody – Immunoperoxidase technics

Introduction

Monoclonal antibodies raised against the antigens present on milkfat globule membranes, derived from the apical site of the lactating mammary gland cells, are useful in the field of cancer research, because such epithelial antigens are usually detected not only in mammary glands but also in many other glandular tissues (Hilkens et al. 1984b). Of various antigenic groups present in functional epithelial cells of the mammary gland, two groups seem to be very useful for distribution studies in normal and tumour tissues, because they are either not at all or hardly destroyed by routine histological procedures (Rasmussen et al. 1984). Since it is notoriously difficult to distin-

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guish between reactive and neoplastic changes in the endometrial glands and between the tumours of endometrial and endocervical origin, an attempt was made to use these monoclonal antibodies in immunohistological examinations on material from hysterectomized women. This was previously be done on the same material with more conventional methods, such as routine mucin and carcinoembryonic antigen (CEA) staining (Ueda et al. 1983).

Materials and methods

Monoclonal antibodies against purified membranes of milkfat globules were described previously (Hilkens et al. 1981, 1984a, b). Two antibodies (67D11 = MAM-3a and 115G3 = MAM-3c) are against the MAM-3 antigen and two others (115D8 = MAM-6a and 115F5 = MAM-6c) against the MAM-6 antigen. These antigens are characterized by the fact that they are pronase and $NaOI_4$-sensitive but formalin-insensitive. It has been reported by Rasmussen et al. (1984), however, that the antigens detected with MAM-3c or MAM-6c were affected to some extent by routine fixation procedures. The antigens detectable by MAM-3a and -6a are not affected by routine histological procedures at all. The biochemical characterization of these antigens is incomplete. In the case of the MAM-6 group the various antigens may be on the same molecule, a glycoprotein of about 400 kD identical to the so-called PAS-O molecule isolated by Shimizu and Yamauchi (1982). The 115D8 and 115F5 monoclonals react with this purified molecule (Hilkens et al. 1984c), but this does not imply that these antigens would not be present on glycolipids as well (Hilkens, personal communication), because the antigenic determinants are on the carbohydrate moieties of the glycoprotein. In the case of the MAM-3 group of antigens, the determinants are most likely on the carbohydrate moieties of another glycoprotein, but could be also present on glycolipids (Hilkens et al. 1984b).

A total of 61 samples were collected from 36 patients. These included 16 with normal endocervical glands, 23 with normal endometrial glands, 6 endocervical adenocarcinomas and 16 endometrial adenocarcinomas. Fourteen normal samples of endocervical and endometrial glands were taken from cases recorded as having a regular menstrual cycle. The material come from hysterectomies for either leiomyoma (7 patients) or endometriosis (7 patients). In addition, two samples of normal endocervical glands from endocervical cancer cases and 9 samples of normal endometrial glands from endometrial cancer cases were studied, as the material included large areas of adjacent normal glandular tissue. Of the normal women, 3 were in proliferative phase, 7 in secretory phase, 2 were pregnant and 13 were postmenopausal. The cancer samples were composed of 6 anatomically distinct endocervical tumours and 16 macroscopically obvious endometrial tumours. Histological observations revealed a common adenocarcinoma of endocervical or endometrial origin in various degrees of differentiation. Neither squamous cell carcinoma nor mesonephroid adenocarcinoma were included in the present series.

Formalin-fixed, paraffin-embedded sections, 4 μm, were incubated for 10 min with 5% normal goat serum, followed by 3 washes in 0.05 M Tris buffer, pH 7.6, and incubated in each monoclonal antibody for 1 h in a moist chamber at room temperature. Blocking for endogenous peroxidase was carried out in 0.1% H_2O_2 in methanol for 10 min, followed by 3 washes in buffer. Peroxidase conjugated goat anti-mouse immunoglobulin (Cappel Laboratory, Malvern, PA, USA) was used subsequently for 30 min. After 4 washes in the buffer, DAB was applied as a chromogenic substrate for 3 min. Counterstaining was carried out with Gill haematoxilin.

Results

Normal endocervical and endometrial glands

Antibodies, 67D11 and 115G3, detecting antigens of the MAM-3 group, showed a positive reaction in the glandular epithelium of most endocervical glands, but they did not show any positivity in the glandular epithelium

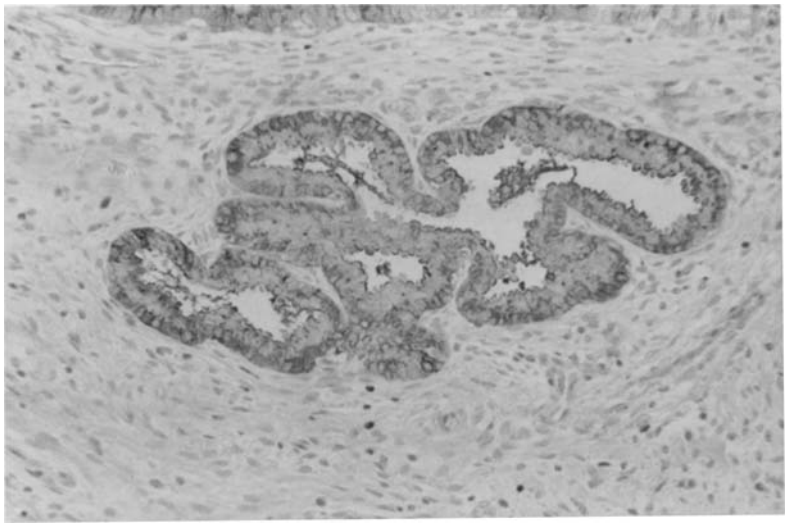


Fig. 1. Reaction of antibody 67D11 in the normal endocervical glands. Note diffuse intracytoplasmic staining, mainly perinuclear and on apical luminal borders. ×200

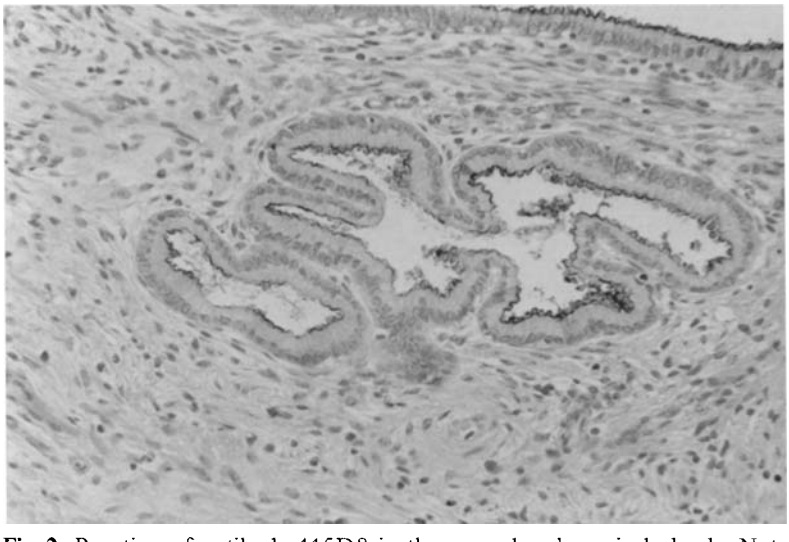


Fig. 2. Reaction of antibody 115D8 in the normal endocervical glands. Note strong apical staining along the luminal borders and some in the secreted material, but no intracytoplasmic staining. ×200

of the endometrium. The staining appearance was homologous in these positive glands. The reaction products were located circumferentially around the cell or on the apical surface of the gland cells, and hardly present in the lumen (Fig. 1).

Antibodies, 115D8 and 115F5, detecting 2 different epitopes of the

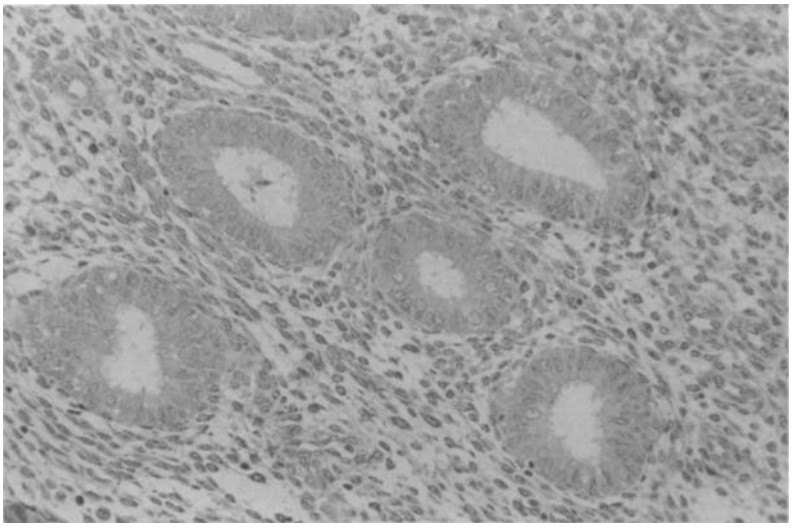


Fig. 3. Negative reaction of antibody 67D11 in the normal endometrial glands. ×400

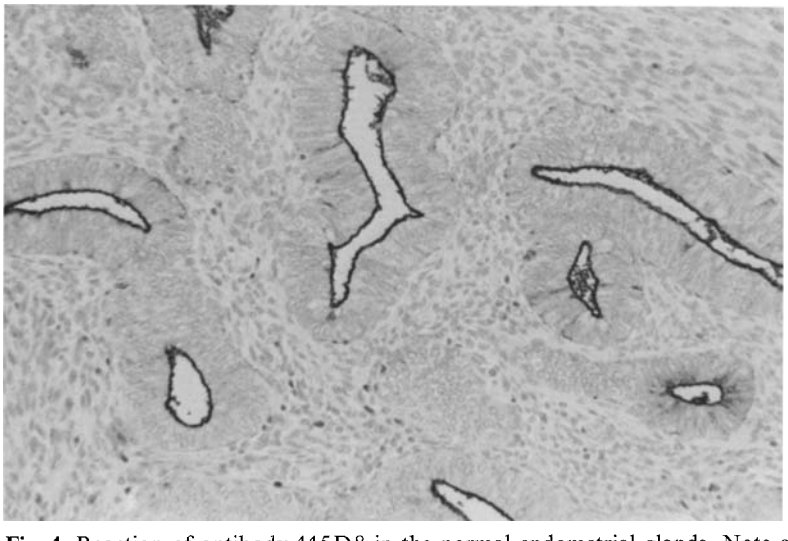


Fig. 4. Reaction of antibody 115D8 in the normal endometrial glands. Note apical staining. ×400

MAM-6 antigen, stained all of the endometrial glands observed homologously. The MAM-6a antigen was found in all of the endocervical glands tested, while MAM-6c was detected in only approximately 40% of the examined cases. MAM-6 antigens were found at the luminal surface of glandular cells and in the secreted materials within the endocervix (Fig. 2). The staining with 115F5 was invariably weaker than that with 115D8.

Figures 3 and 4 show that MAM-3 antigens are not detected in the

Table 1. Frequence of positivity for MAM antigens in tumour and normal glandular cells of the endometrium and endocervix

Tissue	Endometrium				Endocervix			
Antigen	MAM-3		MAM-6		MAM-3		MAM-6	
Antibody	67D11	115G3	115D8	115F5	67D11	115G3	115D8	115F5
Carcinoma	10/16	12/16	16/16	16/16	4/6	5/6	6/6	5/6
well and mod. diff.	8/14	10/14	14/14	14/14	3/5	4/5	5/5	4/5
poorly diff.	2/2	2/2	2/2	2/2	1/1	1/1	1/1	1/1
Normal gland	0/23	0/23	23/23	23/23	14/16	15/16	16/16	6/16

endometrium, while MAM-6 antigens are found only as a sharp line along the apical surface but not on intracytoplasmic vacuoles during the secretory phase. They are absent from the lateral and basal membranes of the glandular cells.

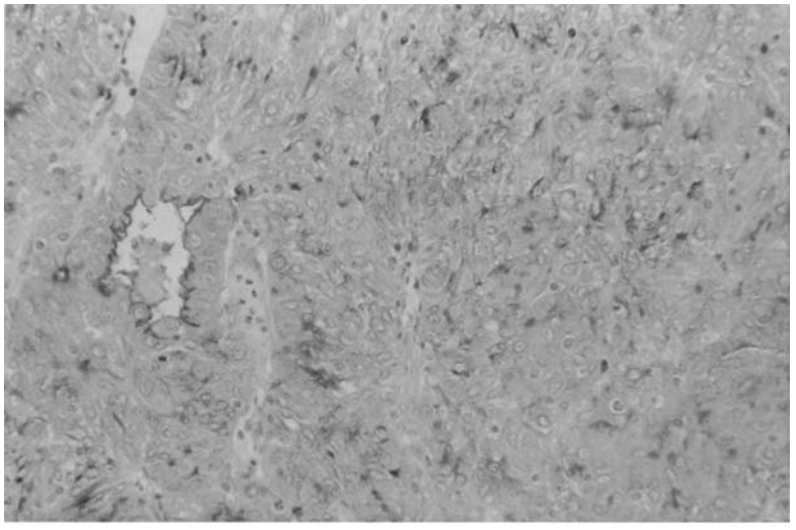
Endometrial and endocervical adenocarcinomas

The staining appearance of these antigens in cancer tissues showed different distribution patterns. Staining was very heterogenous and varied between adjacent regions of cells in the same specimens. The frequencies of antigen expression in the various groups of these cases examined are shown in Table 1.

The majority of endometrial adenocarcinomas were positive for all of the tested antibodies. This was wholly unexpected for the MAM-3 antigens, because normal endometrial glands were completely negative for this antigen. Staining with 67D11 or 115G3 was diffuse or even in the tumour cells, and there did not seemed to be a clear relation with the grade of histological differentiation. The staining pattern of MAM-6 antigens was more apical in the neoplastic cells of the highly differentiated endometrial adenocarcinoma, and was more circumferential in the poorly differentiated endometrial adenocarcinoma (Fig. 5). The distribution of positivity was very heterogenous in the same case, but the frequency of positive cases with 115G3 was a little higher than that with 67D11.

As expected, the endocervical adenocarcinomas revealed the highest degree of positivity for all of the tested antibodies in much the same way as described for the endometrial carcinomas. More circumferential staining patterns were observed in the poorly differentiated areas, more apical in the well differentiated areas (Fig. 6).

Two cases of endocervical adenocarcinomas and one of endometrial adenocarcinoma had some metastatic foci, and the staining pattern of the antigens in the metastases did not differ from that in the primary tumours.



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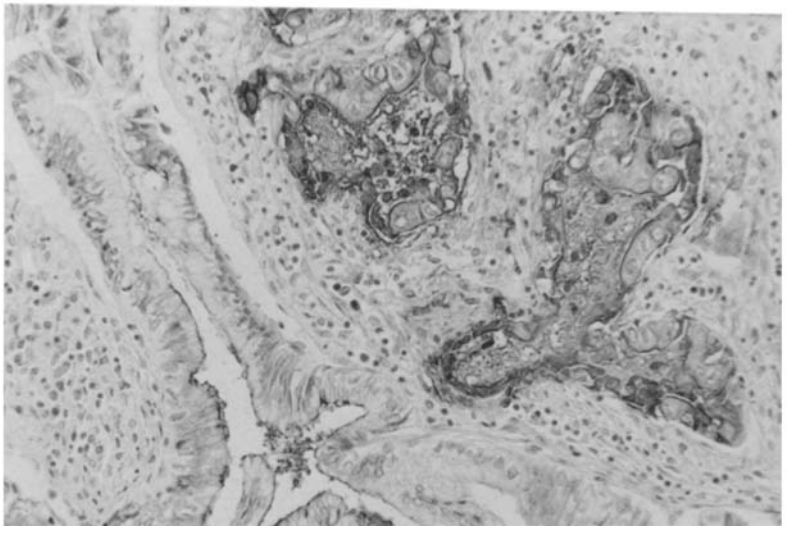

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Figs. 5 and 6. Reactions of 115D8 in the endometrial and endocervical adenocarcinomas, respectively. Note apical staining in the well differentiated areas and intracytoplasmic staining in the poorly differentiated foci of these tumour tissues. ×400

Discussion

Mouse monoclonal antibodies against milkfat globule membrane antigens can stain the epithelial cells of lactating mammary glands and some but not all glandular cells of the resting mammary gland (Arklie et al. 1981; Hilkens et al. 1981; Foster et al. 1982). Some antigens of the normal mammary gland are also found in mammary tumours, notably the HMFG-1 and -2 antigens (Arklie et al. 1981) and MAM-3 and -6 antigens (Hilkens

et al. 1984a). These antigens are, however,not specific for the mammary gland, and they are in fact detected in epithelial cells of different organs, such as the salivary and sweat glands (Hageman et al. 1983) and many other epithelia (Hilkens et al. 1984b). The reactivity pattern of these antigens shows characteristic differences for the individual determinants. For example, in the salivary glands, MAM-3 antigens are found in mucinous acini only, while MAM-6 antigens are not detected in serous or mucinous acini. The reactivity with the ducts and various capillaries in the salivary glands are also very characterisic for each antigenic specificity (Hageman et al. 1984). The presence of some MAM group antigens had been reported on endometrial cancer by Rasmussen et al. (1982), while Hilkens et al. (1984b) demonstrated them in uterine cervical cancers. Here we investigated whether the staining pattern of these antigens would be helpful in solving the difficult problem of the differential diagnosis of uterine adenocarcinomas.

Staining for MAM antigens, especially using of monoclonal antibody, 115G3, may be very suitable for the differential diagnosis of endometrial cancers. The reaction for MAM-3 antigens with 115G3 was completely negative in normal endometrial glands, while it was markedly positive in some tumour cells of endometrial adenocarcinomas. Apparently MAM-3 antigens are not expressed highly in normal glands, although it has been reported recently (Koldovsky et al. 1985) that these antigens can be detected during the short proliferative phase of the endometrial epithelium. Therefore MAM-3 is not a "tumour-antigen", but an antigen characteristic of proliferation in epithelia like that of the endometrium.

It appears that monoclonal antibodies against MAM-6 are not so useful for the differential diagnosis of uterine adenocarcinomas. In the endometrium and endocervix, there is no distinction in the positivity and staining patterns between normal and tumour tissues. The presence of these antigens in an extremely high percentage of the cases and in such high quantities within the glandular tissues might render them more suitable for targeting purposes, for the localization of uterine adenocarcinomas and their metastasis in vivo. Such studies have been carried out with HMFG-1 antibodies (Epenetos et al. 1982), which are very similar to 115D8, because they react with an overlaping epitope on the MAM-6 molecule (Hilkens et al. 1984b).

Diagnosis of uterine adenocarcinoma after curettage is sometimes difficult, as the uterine glandular cell reacts quickly and prominently to hormonal stimulations and often reveals atypical hyperplasia. The distinction between tumours from endocervical and endometrial origin is not always easy to make by routine histological examination, and mucin staining has been used for this purpose. Many endocervical adenocarcinoma cells as well as normal endocervical gland cells are stained strongly with PAS, Alcian blue and metachromatic with Toluidine blue, indicating intracytoplasmic mucin, but in the poorly differentiated type, the mucin staining is only present on luminal borders, implying that the cytoplasm contains no mucin. In cases with normal and neoplastic glands within the endometrium, mucin is never seen in the cytoplasm, but weak mucin reactions can be seen on the luminal surfaces, while most of mucin is found in the secretory products.

Table 2. Staining patterns of antigens and mucin in normal and neoplastic glandular cells of the endometrium and endocervix

	MAM-3	MAM-6	CEA	Mucin
Normal endometrial gland	–	A	A	A
Endometrial adenocarcinoma				
Well differentiated	D	A	–	A
Poorly differentiated	D	D	–	–
Normal endocervical gland	D	A	–	D
Endocervical adenocarcinoma				
Well differentiated	D	A	A	D
Poorly differentiated	D	D	D	A

A: Apical staining; D: Diffuse cytoplasmic staining; –: Negative

Poorly differentiated adenocarcinomas of endometrial origin finally loose the capacity to produce mucin completely. Neither the presence of intracytoplasmic mucin, nor the absence of mucin production could be taken as an absolute criterion for the differential diagnosis of uterine adenocarcinomas.

As additional means to distinguish between these adenocarcinomas of endometrial and endocervical origin, the presence of CEA has been studied on the same material as used the present series. This antigen could not be demonstrated in normal endocervical glands, but it was present in the normal endometrial glands in all phases of the menstrual cycle. In the tumour tissues the situation was completely reversed; endocervical adenocarcinomas were positive and endometrial adenocarcinomas were negative (Ueda et al. 1983).

The comparison of mucin production and antigen localization in uterine adenocarcinomas of endometrial origin from those of endocervical origin are listed in Table 2. The underlying mechanism for the appearance of MAM-3 antigens in endometrial carcinomas and disappearance of CEA in such a cancers is still obscure.

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Accepted March 24, 1985