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Immunohistochemical study of possible changes in keratin expression during neoplastic transformation of the uterine mucosa

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Summary. The present study aimed to examine possible changes in keratin expression during neoplastic transformation of the uterine mucosa and possible differences in keratin expression between endocervical and endometrial adenocarcinomas. Routinely processed specimens with normal morphology or neoplastic changes were stained immunohistochemically using 5 commercial antibodies to keratinfilaments of molecular weight 39–58 kD: CAM 5.2, RCK 102, MCA 144, PKE and PRE.

We generally found a change in keratin expression during the neoplastic transformation, consisting of pronounced heterogeneity compared with normal epithelia. In distinguishing koilocytic atypia from CIN, RCK 102 (52.5, 58 Kd) may prove helpful as it stains neoplastic cells strongly and shows no reaction in koilocytes. Staining with the antibody CAM 5.2 (reactive with 39, 43, 50 kD filaments) may aid in distinguishing between cervical and endometrial adenocarcinomas. The former is stained uniformly; the latter shows a more variable staining.

Key words: Cervix – Endometrium – Keratins – Immunohistochemistry

Introduction

Keratin polypeptides are a group of intermediate filaments characterized by their molecular weight and isoelectric pH, and form part of the cytoskeleton of all kinds of epithelial cells (Moll et al. 1982; Sun et al. 1984). Immunohistochemical staining with antikeratins has become a well established diagnostic tool in surgical pathology to differentiate between epithelial and non-epithelial tumours. In the human female genital tract the expression of the various keratin polypeptides in normal and pathological specimens have been described biochemically by two dimensional gel electrophoresis (Moll et al. 1983).

Several immunohistochemical studies have focused on keratin expression in normal and pathological lesions of the ecto- or endocervix (Puts et al. 1985; Gigi-Leitner et al. 1986; Weikel et al. 1987; Levy et al. 1988). Few studies have dealt with the keratin expression in normal and neoplastic endometrium and in adenocarcinomas of the endocervix and endometrium (Hurlimann and Gloor 1984; Dabbs et al. 1986). We have examined the keratin expression in the ectocervix (normal, koilocytosis, cervical intraepithelial neoplasia (CIN) and invasive squamous carcinoma) the endocervix (normal, squamous metaplasia, adenocarcinoma in situ and invasive adenocarcinoma) and the endometrium (normal proliferative and secretory phase, simple hyperplasia, complex hyperplasia and adenocarcinoma). Our aim was to examine possible changes in keratin expression during neoplastic transformation and possible differences in keratin expression between adenocarcinomas of the endocervix and the endometrium.

Material and methods

The material was obtained from biopsies and gyneacological operations, consisting of normal and neoplastic lesions as shown in Table 1. Histological changes were classified according to the CIN terminology (Richart 1967). Endometrial lesions were classified as discribed by Blaustein (1985). The tissues were fixed at room temperature for 18–24 h in 4% phosphate buffered formaldehyde pH 7, dehydrated in ethanol and isopropanol and embedded in paraffin at 59° C. Sections of 5 μm were cut and placed on uncoated slides.

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Table 1. Lesions and number of cases examined

Sample	No. of cases
Ectocervix	
Normal	8
Koilocytosis	2
CIN	
I	2
II	3
III	5
Invasive squamous carcinoma	
Keratinizing	4
Non-keratinizing	7
Endocervix	
Normal	8
Squamous metaplasia	2
Adenocarcinoma in situ	2
Adenocarcinoma	8
Endometrium	
Normal proliferative phase	2
Normal secretory phase	2
Simple hyperplasia	3
Complex hyperplasia	3
Adenocarcinoma	11

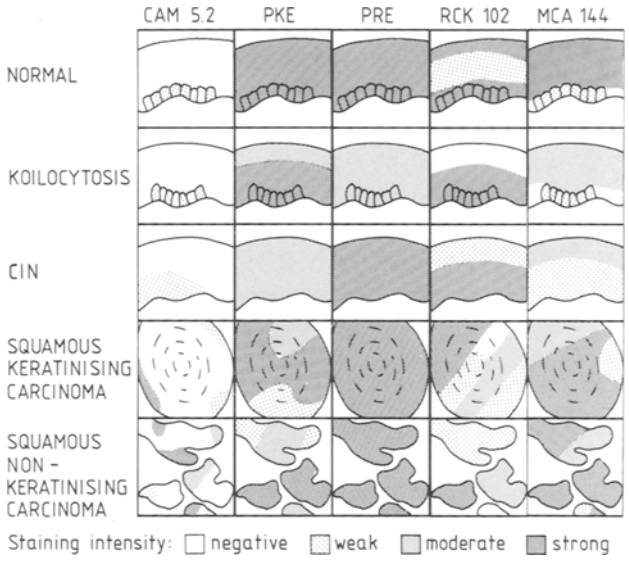


Fig. 1. Ectocervix: The keratin expression of all samples examined is summarized in each drawing. Different shadings in the same drawing reflect a variability from region to region and/or from sample to sample

The following antibodies, covering the spectrum of polypeptides with molecular weights 39–58 kD were used: Monoclonal anti-cytokeratin CAM 5.2 (Becton-Dickinson, Cat. 7650) identifying keratin polypeptides with molecular weights 39, 43, and 50 kD, polyclonal antikeratin PKE (PKE) (Eurodiagnostics) identifying 43–58 kD keratin polypeptides, polyclonal prekeratin (PRE) (DAKO Z622) reacting with keratin polypeptides of molecular weight 48–58 kD, monoclonal antikeratin RCK 102 (RCK 102) (Euro diagnostics) identifying 52.5 and 58 kD keratin polypeptides, monoclonal antikeratin MCA 144 (MCA 144) (Serotec) identifying 55–57 kD keratin polypeptides.

Before immunoperoxidase staining with antikeratins PRE, PKE, RCK 102 and CAM 5.2, deparaffinized sections were treated at 37° C with 0.05% trypsin (Sigma No T-8128) in 0.05% calcium chloride adjusted to pH 7.8 with 0.1 M sodium hydroxide for 20 min. Before staining with MCA 144, sections were treated at 37° C with 0.4% pepsin (Sigma No P-S147) in 0.01 M hydrochloride for 20 min.

An indirect immunoperoxidase technique was used with both monoclonal and polyclonal antibodies. The staining procedure comprised the following steps: Methanol 0.5% H_2O_2 for 10 min, phosphate buffered saline pH 7.2 with 10% swine serum for 10 min, incubation with primary antikeratins for 30 min in the following dilutions: PRE 1:100, PKE 1:25, MCA 144 1:100, RCK 102 1:5, CAM 5.2 1:10. As secondary antibody, peroxidase conjugated swine antirabbit (DAKO P217) in dilution 1:20 was used for polyclonal antibodies and peroxidase conjugated rabbit antimouse (DAKO P260) diluted 1:20 was used for monoclonal antibodies.

The reaction product was visualized by development with 0.04% 3-amino-9-ethylcarbazole (Sigma), 0.01% H_2O_2 in 0.05% M sodium acetate/acetic acid buffer pH 5 for 10 min. The tissues were counterstained with Mayer's haematoxylin and mounted with Aqua Mount.

When using polyclonal antibodies the primary antibody was replaced by immunoglobulinfraction of serum from non-immunized rabbits in controls. When using monoclonal antibodies the primary antibody was replaced by phosphate buffered saline.

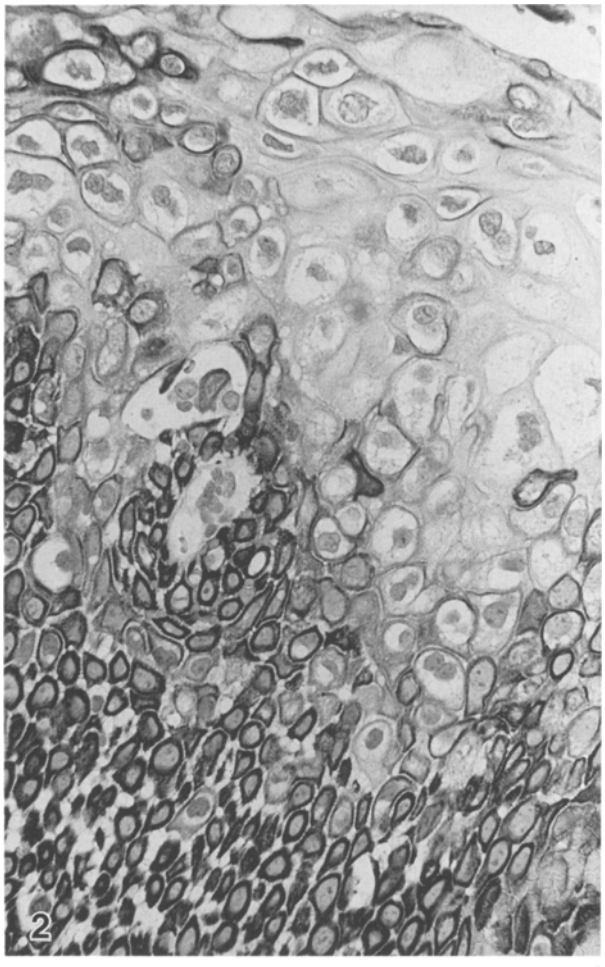
For the evaluation of the specific staining reactions a semi-quantitative scoring system was used: + weak positive reaction, + + moderate positive reaction, + + + strong positive reaction, − no reaction.

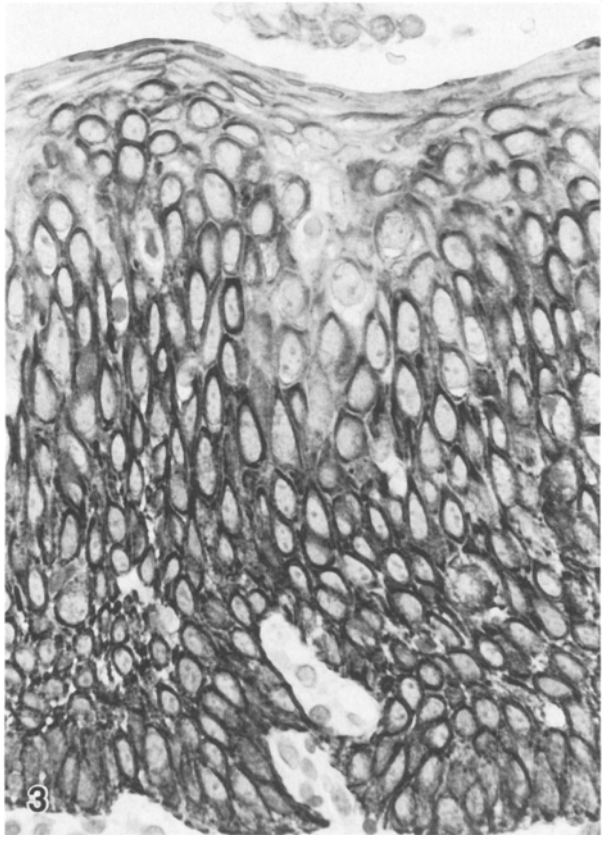
Results

The staining pattern in the various lesions is shown schematically in Figs. 1, 4 and 8. Each drawing summarizes the keratin expression of several samples of each lesion. Different shadings in the same drawing reflect a variable keratin expression from region to region and/or from sample to sample.

In the normal ectocervix (Fig. 1) the low molecular weight antikeratin CAM 5.2 only stains a few basal cells in the squamous epithelium weakly. PKE and PRE, covering a broad spectrum of molecular weights of keratin polypeptides, stain all layers strongly. RCK 102 stains basal and superficial cells strongly, and intermediate cells weakly. The high molecular weight antikeratin MCA 144 stains all suprabasal cells strongly, and basal cells weakly.

The koilocytes are localized in the superficial layers of the epithelium. The staining intensity in





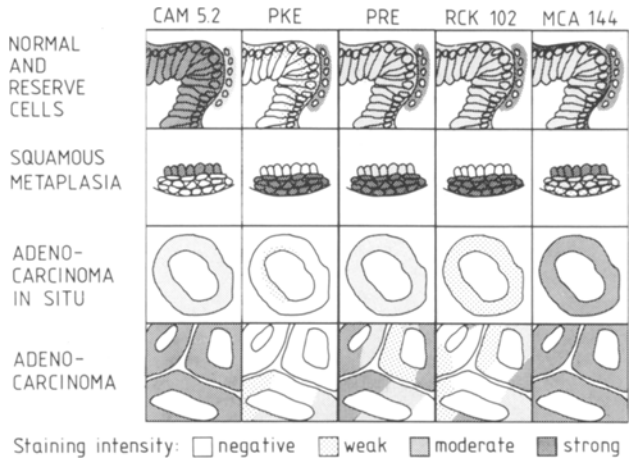


Fig. 4. Endocervix. The keratin expression of all the samples examined is summarized in each drawing (see Fig. 1)

the koilocytotic areas is generally slightly weaker than in normal epithelia, most notably with RCK 102 where koilocytes are completely negative (Fig. 2).

There is no notable difference in the keratin expression of normal squamous epithelium and of CIN I, II and III with PKE and PRE. When stained with CAM 5.2, RCK 102 and MCA 144 the neoplastic cells show the same keratin expression as the basal cells in normal ectocervix (Fig. 3).

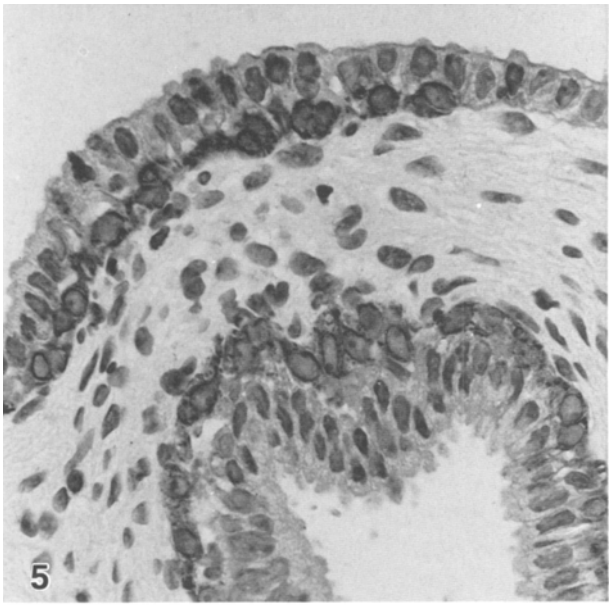
Two invasive carcinomas are completely negative when stained with CAM 5.2, the other two show a focal strong reaction. Otherwise the carcinomas show the same expression of keratins as normal squamous epithelium and CIN. The keratin expression of non-keratinising carcinomas differs from the keratinizing carcinomas only by a higher content of low molecular weight keratins.

The columnar cells of the normal endocervix (Fig. 4) stain strongly with CAM 5.2, moderately with PRE, RCK 102 and MCA 144, and weakly with PKE. Reserve cells appear in a few of the "normal" cervical glands as a single layer of cuboidal cells beneath the columnar cells. These reserve cells are labelled strongly with PKE (Fig. 5), PRE, RCK 102 and MCA 144, and moderately with CAM 5.2.

The metaplasia examined in the endocervix is from the transformation zone and is of the mature

Fig. 2. Ectocervix with koilocytosis, stained with RCK 102. Koilocytes show no reaction. × 250

Fig. 3. Ectocervix with CIN III, stained with RCK 102. Neoplastic cells stain strongly. × 250



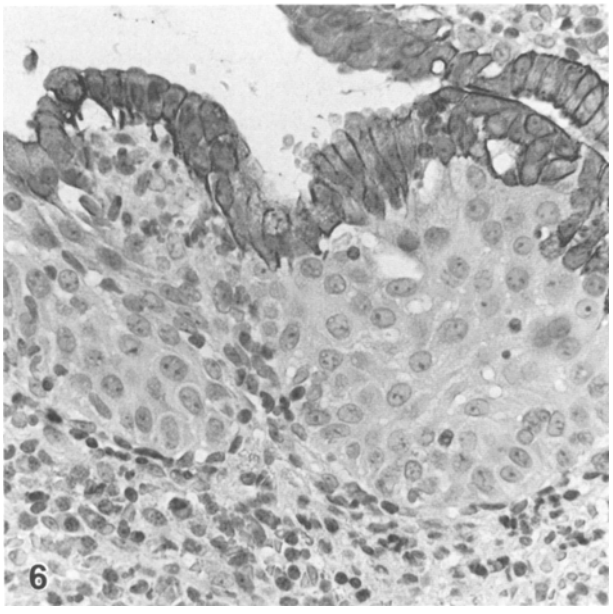


Fig. 5. Endocervix stained with PKE. Reserve cells stain strongly. ×250

Fig. 6. Endocervix with metaplasia, stained with CAM 5.2. Metaplasic squamous cells show no reaction. ×250

type, where residual endocervical epithelial cells at the luminal aspect of the metaplasia stain strongly with CAM 5.2 and MCA 144, similar to the expression in normal endocervix. The keratin expression of the metaplastic squamous cells is similar to that of ectocevix, though there is a reduction of low molecular weight keratins, as they do not stain with CAM 5.2 (Fig. 6).

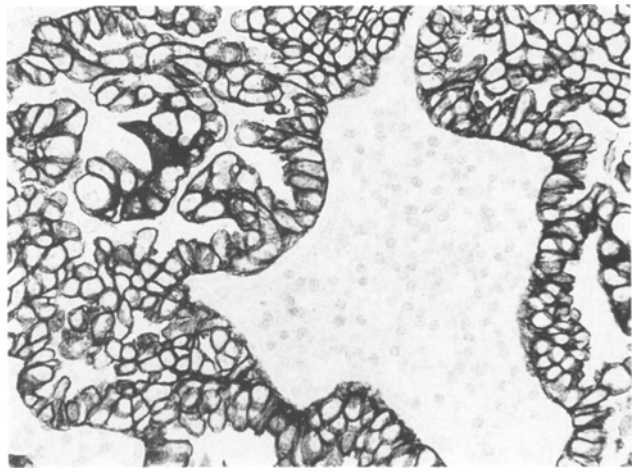


Fig. 7. Cervical adenocarcinoma showing a diffuse strong labelling with CAM 5.2. ×250

Adenocarcinoma in situ (ACIS) shows the same labelling as normal endocervix for most of the antibodies used. However, staining with MCA 144 was more pronounced in ACIS. No reserve cells appear in association with ACIS.

All of the cervical adenocarcinomas show a diffuse strong labelling with CAM 5.2 (Fig. 7) and MCA 144 as in ACIS. Staining with the other antikeratins varies from sample to sample and within each tumour being mainly negative with PKE and varying from negative to strongly positive with PRE and RCK 102. No morphological difference is observed between stained and unstained areas.

In the normal proliferative phase the endometrium shows that CAM 5.2, PRE and MCA 144 label the glands diffusely with moderate to strong staining intensity. Focal and weak staining is seen with PKE and RCK 102.

Compared with proliferative endometrium glands show a stronger staining with CAM 5.2 and RCK 102 in the secreting phase, an unchanged staining pattern with MCA 144 and slightly weaker staining with PKE and PRE.

The cases of simple hyperplasia examined shows no atypia. The keratin expression is mainly the same as in normal proliferation, though there is a slight variation in the staining intensity within each sample. The three cases of complex hyperplasia examined have areas of mild, moderate and severe atypia. The staining pattern is similar to simple hyperplasia.

The keratin expression of the endometrial adenocarcinomas varies greatly. One adenocarcinoma shows diffuse strong staining with CAM 5.2. The remaining show areas of negative to strong stain-

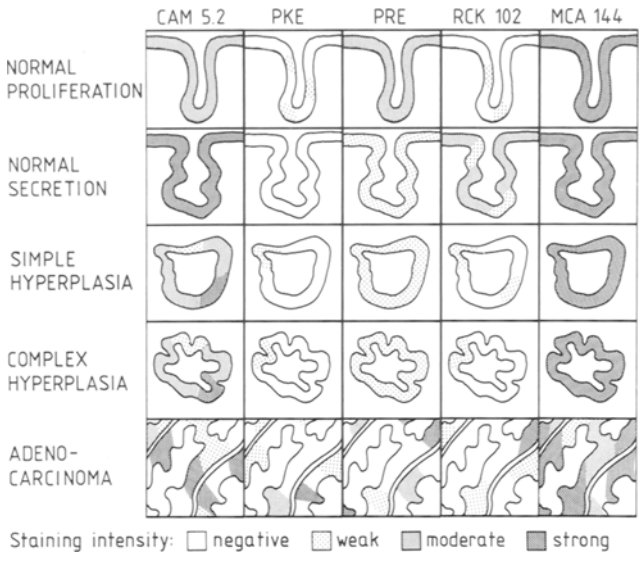


Fig. 8. Endometrium. The keratin expression of all samples examined is summarized in each drawing (see Fig. 1)

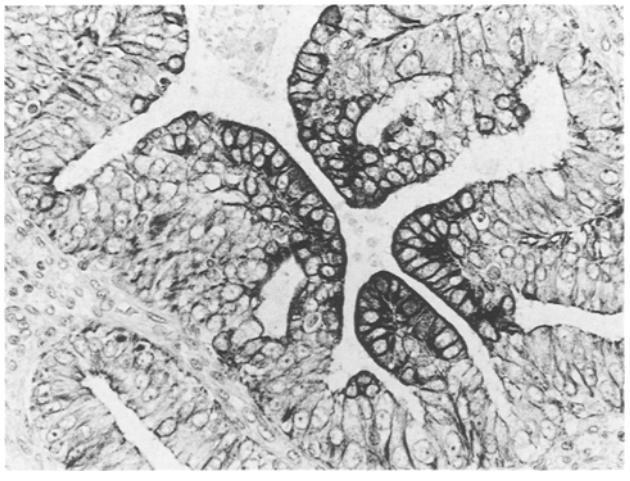


Fig. 9. Endometrial adenocarcinoma showing a variable staining intensity with CAM 5.2. ×250

ing within each tumour (Fig. 9). MCA 144 stains the major part of the tumours strongly, whereas staining with PRE, PKE and RCK 102 show a mainly weak or negative reaction. No morphological difference is observed between stained and unstained areas.

Discussion

The biochemical composition of keratins in normal ectocervix has been studied by Moll et al. (1983), who found substantial amounts of high molecular weight keratins (m.w.k.) and minor amounts of low m.w.k. in the major portion of the ectocervix. Previous immunohistochemical studies have mainly been concerned with CAM 5.2. Some of these find normal ectocervix completely negative when stained with CAM 5.2 (Bobrow et al. 1986; Raju 1988; Angus et al. 1988). Two other studies do show labelling of the basal cell layer with a 40 kD antikeratin (Dixon and Stanley 1984; Gigi-Leitner et al. 1986), which is consistent with our results, as we find occasional weak staining of basal cells with CAM 5.2. In contrast to Teglbjaerg et al. (1985), who describe a variable staining pattern with high m.w.k., we find a more homogeneous keratin expression.

In one of the few studies on keratin expression in epithelium with koilocytosis, Bychkov and Chejfec (1984) found koilocytes to be keratin-free. Syrjänen et al. (1988) described the neoexpression of SK 60–61 (specific for 45 and 52.5 kD) in human papillomavirus lesions, but did not describe the staining reaction in koilocytes. We found that ectocervix with koilocytosis generally shows the same keratin expression as normal ectocervix, except in the koilocytes themselves, which contains less keratin, and is completely negative when stained with RCK 102 (specific for 52.5 and 58 kD). In distinguishing koilocytic atypia from CIN, RCK 102 could prove helpful, as it is negative in koilocytes and strongly positive in neoplastic cells.

In accordance with Lehto and Virtanen (1986) we find that areas with CIN I to III retain the keratin expression of normal basal cells. This supports the view that basal cells in normal ectocervix are involved in the development of CIN (Puts et al. 1985).

Positive staining with CAM 5.2 has been described as a marker for invasive potential in CIN (Bobrow et al. 1986; Raju 1988). As we find areas of weak positive staining in the majority (8/10) of samples with CIN, and find 4 of 11 invasive squamous carcinomas negative with CAM 5.2, we can not support this view, in accordance with Wells et al. (1986).

The keratin expression in invasive squamous carcinomas is previously described as very complex (Moll et al. 1983; Bychkov and Chejfec 1984; Moll 1987). We also find a very heterogeneous keratin expression in the invasive squamous carcinomas. The content of low m.w.k. corresponds to the degree of differentiation, as we find a larger amount of low m.w.k. in the less differentiated invasive squamous carcinomas, in accordance with Moll (1987). The heterogenic keratin expression in the carcinomas may reflect the presence of many

clones of cells with different stages of maturation/differentiation.

The keratin profile of subcolumnar reserve cells is in dispute. Weikel et al. (1987) using broadspectrum antibodies as well as antibodies to specific cytokeratin types concluded that the subcolumnar reserve cells contain cytokeratin no. 5 and 17 (with other cytokeratins) which are markers of squamous epithelia. Levy et al. (1988) using antibodies to keratin no. 13 and 18 and the antibody KS 2.1 (not electrophoretically characterized) find strong reactivity for keratin no. 13 in all reserve cells and KS 2.1 in some of them.

The antibodies used in our investigation react with a broad spectrum of keratin polypeptides and cannot distinguish between the subtypes of keratins. The reserve cells react with all five antibodies, most intensely with high molecular weight keratins. This is in accordance with Weikel et al. (1987) and Levy et al. (1988) and supports the view that reserve cells contain keratin polypeptides mainly found in squamous epithelia and to a lesser degree in cervical columnar cells.

The keratin profile in squamous metaplasia is characterized by its content of keratin polypeptide combinations different from cervical columnar cells (Gigi-Leitner et al. 1986). Levy et al. (1988) found pronounced staining for keratin no. 13 in metaplastic squamous epithelium while they did not find any staining in cervical columnar epithelium.

In squamous metaplastic epithelium we found keratin expression corresponding to that found in squamous epithelium and no reaction with low molecular weight keratin CAM 5.2. We are therefore tempted to support the view (Weikel et al. 1987; Levy et al. 1988) that reserve cells have a bipotent nature as they are able to differentiate into columnar cells – loosing keratins with high molecular weight and expressing a higher content of low molecular weight keratins. Differentiation into metaplastic squamous epithelium is followed by loss of low molecular weight keratins and a higher content of high molecular weight keratins.

The literature on keratin expression in ACIS and invasive adenocarcinoma of the cervix is sparse. The keratin staining patterns of ACIS does not differ markedly from normal endocervix. However, it is noteworthy that we find a stronger reaction with MCA 144 in ACIS than in normal endocervical columnar cells indicating that it might be used as a neoplastic marker in endocervix. As reserve cells do not appear universally in normal cervical glands, their absence in the present study in relation to ACIS can hardly be used as a marker of malignant transformation. In accordance with Hurlimann and Gloor (1984) we have found a larger amount of high m.w.k. in invasive adenocarcinomas than in ACIS.

There are very few studies on keratin expression in normal and neoplastic endometrium (Dabbs et al. 1986), and to our knowledge none on endometrial hyperplasias. The keratin expression in normal endometrium does not differ markedly from normal endocervix, in accordance with Moll et al. (1983). It is noteworthy that despite the general view of their different biological behavior, simple hyperplasia cannot be distinguished from complex hyperplasia; both show a more variable keratin expression than normal endometrium. The heterogeneity becomes even more evident in endometrial adenocarcinomas, consistent with the general view that carcinomas of the female genital tract often are very heterogeneous (Moll et al. 1983).

A common diagnostic problem in routine pathology is distinguishing between cervical and endometrial adenocarcinomas. We find that CAM 5.2 may aid in this respect, as it stained all 8 cervical adenocarcinomas uniformly and 10 of 11 endometrial adenocarcinomas variably.

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