Changing Patterns of Keratin Expression During Progression of Cervical Intraepithelial Neoplasia

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The expression of keratins in normal cervical epithelia, metaplastic epithelium, and cervical intraepithelial neoplasia (CIN) grades I, II, and III is investigated with a panel of keratin polypeptide-specific monoclonal antibodies. This approach allowed the detection of individual keratins 4, 7, 8, 10, 13, 14, 18, and 19 at the single-cell level. By using an antibody recognizing keratins 5 and 8 (RCK 102) and two antibodies specific for keratin 8 (CAM 5.2 and M 20), it was also possible to derive information on the distribution of keratin 5. Our results show that during immature squamous metaplasia there is an acquisition of keratins typical of squamous epithelium, i.e., keratins 4, 5, 13, and 14. This process continues during further differentiation to mature squamous metaplasia. In premalignant lesions the expression pattern of the progenitor reserve cells and immature squamous metaplastic epithelium is partly conserved. However, in most cases an induction in the expression of the keratins 4, 13, and 14 was observed. Furthermore, CIN III shows a more extensive expression of keratins typical of simple epithelia, i.e., keratins 8 and 18, as compared to CIN I and CIN II. (Am J Pathol 1990, 136:657–668)

In recent years there have been a number of studies of keratin expression in normal and neoplastic cervical epithelium.1–7 These studies have been based on immunohistochemistry with anti-keratin monoclonal antibodies and/or on gel electrophoretic analysis of tissue samples. In this way it has been possible to construct a picture of the keratin phenotype of ecto- and endocervical epithelium, squamous metaplasia, reserve cell hyperplasia and, from a few cases, severe dysplasia and carcinoma of the cervix. These studies indicate that endocervical epithelum contains type II keratins 1, 4, 5, and 6 and type I keratins 13, 14, 15, and 19, with some variability in the expression of keratins 2, 8, 10, 11, 16, and 17;3,4 endocervical columnar cells contain keratins 7, 8, 18, and 19, with variable presence of keratin 4.5 Reserve cells have been shown to contain keratins 5, 17, 19, and varying amounts of keratin 8;6 Levy et al.7 also suggested that keratin 13 was present in a subpopulation of these cells. Opinions are still divided regarding the presence of keratin 18 in reserve cells.

The pattern of keratin expression in immature squamous metaplasia has been shown to differ from that of normal squamous epithelium; keratin 19 is found in the full thickness of metaplastic epithelium, as opposed to only basal cells in normal epithelia.8 Some keratin 13 and 16 expression has also been reported, but keratin 18 was not found.3,4 CIN II has not been extensively studied to date. Bobrow et al.9 showed that the antibody CAM 5.2 (to keratin 8) stained some cases of severe dysplasia and early invasive carcinoma, and keratin 18 expression has also been reported in cases of dysplasia.3

With the increasing availability of polypeptide-specific monoclonal antibodies that each recognize only a single keratin isotype, it has now been possible to extend these investigations to a systematic study of many immature and mature squamous metaplasias, as well as CIN I, II, and III lesions, and to compare the keratin expression at the single-cell level with that observed in normal endo- and ectocervical epithelium. Such detailed information may lead to greater insight into the etiologic aspects of cervical intraepithelial neoplasia.

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Materials and Methods

Tissues

The tissue specimens used in this study were derived from cervical cones removed in 50 women aged between 25 and 65 years, in whom cytologic screening had shown features consistent with dysplasia.

Before exconization the lesions were visualized by application of 4% acetic acid solution in water to the whole ectocervix.

Immediately after exconization each cone was divided into quadrants, which in turn were subdivided into three equal parts perpendicular to the cervical canal. The central specimen from each quadrant was fixed in a 4% formaldehyde solution and processed through paraffin for hematoxylin and eosin stained sections and routine light microscopic diagnosis performed independently by three qualified pathologists. The two other specimens were snap frozen in liquid nitrogen or liquid nitrogen cooled isopentane and stored in liquid nitrogen. One fragment from each quadrant was used for the immunohistochemical assays.

A total of 150 specimens representing normal or metaplastic epithelia through three grades of CIN were used. Furthermore, in many specimens normal and/or metaplastic epithelia and intraepithelial neoplasia were found along side of each other so that the following could be diagnosed: normal ectocervical epithelium in 50 specimens, normal endocervical cylindrical epithelium in 120 specimens, immature squamous metaplasia in 16 specimens, subcolumnar reserve cells in 20 specimens, mature squamous metaplasia in 45 specimens, CIN I in 15 specimens, CIN II in 14 specimens, and CIN III in 15 specimens. Furthermore, the subepithelial connective tissue with smooth muscle cells was also meticulously examined in all 150 fragments.

Antibodies

Fourteen monoclonal keratin antibodies, one monoclonal vimentin antibody, and one monoclonal desmin antibody were used in this study. The specificity of these antibodies is briefly described below.

1. **RCK 102** is a broadly reacting keratin antibody of the IgG1 subclass that recognizes keratins 5 and 8, and as a result stains virtually all epithelial tissues.

2. **RCK 103**, a broadly cross-reacting antibody of the IgG1 class, stains epithelial as well as neural tissues. Because in the prostate it decorated only basal cells in several studies, this antibody was included to examine its reactivity pattern with reserve cells.

3. **RCK 105** (IgG1) reacts only with keratin 7 in immunoblotting and stains a subgroup of glandular epithelia and their tumors, as well as transitional epithelium.

4. **RGE 53** (IgG1) and **RCK 106** (IgG1) are monospecific for keratin 18 in immunoblotting. In general these antibodies recognize columnar epithelial cells of the digestive, respiratory, and urogenital tracts, endocrine, exocrine, and mesothelial cells, as well as tumors originating from them. Generally no reaction is found in squamous epithelia.

5. **LP2K** stains most simple epithelia and basal cells in stratified squamous epithelia that are not keratinizing. This antibody only recognizes keratin 19 in immunoblotting assays.

6. **LLOO2** (IgG3) stains full-thickness epidermis (but not stratum corneum), basal, and some higher layers of noncornified stratified epithelium and basal cells in complex, mixed epithelia of glands. It is monospecific for keratin 14 in immunoblotting.

7. **RKSE 60** (IgG3) reactivity only with keratinizing epithelial cells and recognizes keratin 10 in immunoblotting.

8. **BB10** (IgG1) reacts with noncornifying squamous epithelium and with certain ciliated pseudostratified epithelia, such as the cylindrical epithelium of bronchi, and recognizes only keratin 4 in immunoblotting.

9. Antibodies 1C7 (IgG2a) and 2D7 (IgG2b) both react with noncornifying squamous epithelia. These antibodies recognize only keratin 13 in immunoblotting studies.

10. **M20** (IgG1) recognizes only keratin 8 in immunoblotting assays.

11. **CAM5.2** (IgG2a) was purchased from Becton & Dickinson (Etten-Leur, The Netherlands) and stains simple epithelia, some basal cells of squamous epithelia, and sporadically smooth muscle cells. The immunoblotting assays shown in Figure 1 demonstrate the primary specificity of this antibody for keratin 8, and not keratin 18 or keratin 19. Although earlier studies reported reactivity of this antibody with all three keratins, one-dimensional immunoblotting reactivity with the lower molecular weight bands is due to breakdown products of keratin 8. The specificity has also been confirmed in assays using bacterially synthesized individual recombinant keratins, in which we have observed strong reactivity with keratin 8, weak but distinct cross-reactivity with the closely-related keratin 7, and no reactivity with either keratin 18 or keratin 19 (CM Alexander, PC Staslik & EB Lane, unpublished observations). The immunoblotting studies on cytoskeleton preparations of RT4 cells were performed as previously described.

12. The monoclonal antibody RV 202 (IgG1) reacts with vimentin and therefore stains mesenchymal cells.
**Results**

In Figure 2 schematic representations of the keratin phenotype of normal ecto- and endocervical epithelium, immature squamous metaplasia, and mature squamous metaplasia through three grades of dysplasia, as recognized by monospecific monoclonal antibodies, are given. There were distinct variations in the expression of individual keratins in immature metaplasia and in the dysplasias, as compared to the expression in normal endo- and ectocervical epithelium. These will be described in detail.

Throughout all the squamous epithelia scattered vimentin (RV202) positive cells could be detected, representing lymphocytes or Langerhans cells. Many endocervical columnar cells showed vimentin expression of varying intensity in cervixes with immature metaplasia, those with reserve cells and also cervixes without lesions. The monoclonal desmin antibody stained all smooth muscle cells in the subepithelial connective tissue. In a number of cervixes some smooth muscle cells were also decorated by the antibodies specific for keratin 8.

**Ecto- and Endocervical Epithelium (Figures 2A, B, and 3)**

The ectocervical epithelium shows a reproducible pattern of keratin expression in all 50 specimens, with only minor variations in the staining patterns with a few antibodies between some specimens. RCK 102 (keratins 5 and 8) decorated all cells of the basal and parabasal layers, part of the cells in the intermediate layer, and only sporadic staining of some superficial cells in a few tissue fragments was observed (Figure 3a). RCK 103 stained all layers of the epithelium, with the basal layer showing a slightly more intense expression than the other layers (Figure 3c). 1C7 and 2D7 (to keratin 13) showed a similar expression pattern with strong staining of the parabasal, intermediate, and superficial cells. The basal cells were negative (Figure 3c). All cells of the superficial layer and the majority of the cells in the intermediate layer were positive for 6B10 (keratin 4) (Figure 3d). LCO02 (keratin 14) stained the basal and parabasal cells with partial staining of the intermediate cells and sporadic staining of superficial cells in a few cervical fragments (Figure 3e). Staining with LP2K (keratin 19) was uniformly positive in the basal cell compartment of the epithelium (Figure 3f) and in 14 of 50 cases a scattered reaction of individual cells was also noted in the other layers.

CAM 5.2 (keratin 8) stained a few basal cells in 15 cases and RKSE 60 (keratin 10) (Figure 3g) showed scattered decoration of cells in the intermediate and superficial cell layers in six cervical tissue fragments. RGE 53, RCK 106, CK 19-2, M 20, and RCK 106 were all negative in the ectocervical squamous epithelium.

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**Immunohistochemistry**

Cryostat sections were fixed in cold methanol (−20 C) for 5 minutes, rinsed in acetone, and air dried. The slides were incubated with the appropriately diluted or undiluted antibody for 45 minutes at room temperature.

After three subsequent 10-minute washings with PBS the peroxidase-conjugated rabbit-anti-mouse serum was applied to the sections for 30 minutes at room temperature. After a second series of washing steps with PBS, the peroxidase was detected with 3-amino-9-ethylcarbazole (AEC; Aldrich Chemical Comp., St. Louis, MO). For this purpose 40 mg of AEC was dissolved in 10 ml N,N-dimethylformamide (Merck, Darmstadt, FRG) and added to 190 ml of sodium acetate buffer (0.05 M, pH 4.95). Hydrogen peroxide was added to a final concentration of 0.01% (volume/volume). Sections were incubated for 8 minutes with this mixture, rinsed with tap water, counterstained with hematoxylin, and mounted with Kaisers glycerin-gelatin (Boon B. V. Meppel, The Netherlands).
One hundred twenty samples of endocervical epithelium were examined.

All endocervical columnar cells strongly reacted with RCK 102 (Figure 3h), RCK 103, RGE 53 (keratin 18), RCK 106 (keratin 18) (Figure 3i), CK 18-2 (keratin 18), M20 (keratin 8) (Figure 3j), CAM 5.2 (keratin 8), and LP2K (keratin 19) (Figure 3k). RCK 105 (keratin 7) (Figure 3i) stained most but not all columnar cells. 6B10 (keratin 4) displayed heterogeneous staining, with some columnar cells positive in 32 of 106 cervical fragments (Figure 3m). 1C7, 2D7 (keratin 13), LLOO2 (keratin 14), and RKSE 60 (keratin 10) were negative.

Small groups of reserve cells were found in 20 of the 150 cervical fragments. All reserve cells were uniformly decorated by RCK 102 (Figure 3h), RCK 103, and LP2K (Figure 3k).
Figure 3. Immunoperoxidase staining patterns of frozen sections from normal ectocervical squamous epithelium (a–g) and endocervical columnar epithelium with reserve cells (h–m) after incubation with (a, h) RCK 106; (b) RCK 103; (c) TCZ; (d, m) 61H; (e) L1182; (f, k) L12JK; (g) RKS8; (j) RCK 106; (l) M20; and (i) RCK 105.
Of the markers for keratin 18, RCK 106 showed the most intense reaction (Figure 5i), CK 18-2 was moderately positive, but the RGE 53 epitope was hardly detectable. M 20 (Figure 5i) and CAM 5.2 reacted with moderate intensity in most cases, some being negative. RCK 105 (Figure 5i), 6B10 (Figure 5m), and LLOO2 did not react in these cells.

**Immature Squamous Metaplasia (Figures 2C and 4)**

In 16 fragments immature squamous metaplasia was diagnosed (Figure 4a). This epithelium showed an expression pattern that only partly resembled normal ectocervical epithelium and mature squamous metaplastic epithelium. Immunoreactivity of all epithelial layers was noted with RCK 102 (Figure 4b) and RCK 103, and with LP2K (keratin 19) (Figure 4h), which all showed staining of varying intensity. RCK 106 (Figure 4d) decorated the superficial cells. The underlying epithelial cells were mostly positive with various intensities. Two cervixes were completely negative with RCK 106. CK 18-2 (Figure 4c), RGE 53, and 6B10 (Figure 4f) reacted in 7 different cases of 16 and were negative in the rest.

M20 (Figure 4a) and CAM 5.2 (Figure 4f), both to keratin 8, showed strong reactivity with the superficial columnar cells when present. The underlying cells showed some dispersed reactivity in 7 of 16 cases. 1C7 and 2D7 (to keratin 13) reacted with a few dispersed cells in 7 of 16 cases (Figure 4j). LLOO2 (keratin 14) (Figure 4k) decorated with varying intensity all epithelial layers, with exception of the superficial layer, in 8 of 16 cases. The other cases were negative.

RCK 105 (keratin 7) (Figure 4g) and RKSE 60 (keratin 10) (Figure 4l) were negative.

**Mature Squamous Metaplasia (Figure 2D)**

This was diagnosed in 45 cervical fragments. The pattern of keratin expression was almost equal to that observed in ectocervical epithelium. There were, however, a few minor differences, ie, LP2K showing expression in dispersed cells above the basal layer in 12 fragments and 1C7 and 2D7 staining basal cells in four cases. In two cases RCK 106 decorated some basal and parabasal cells.

**CIN I (Figures 2E and 5a to h)**

In 15 tissue fragments CIN I was diagnosed (Figure 5a). In these the keratin expression showed differences as compared to the epithelia described above characterized by irregularity in the pattern of keratin expression and that keratins normally occurring in simple epithelia could sometimes be detected.

RCK 103 and RCK 102 (Figure 5b) stained throughout the full thickness of the dysplastic epithelium. 1C7 (Figure 5c) and 2D7 showed expression of varying intensity in the whole epithelium in 13 cases with basally lying cells often showing positivity. Two fragments were negative. 6B10 (Figure 5d) displayed immunoreactivity in the full thickness of the epithelium with varying intensity with most basally lying cells staining rather weakly. In one fragment there was only some dispersed positivity for 6B10.

LP2K (Figure 5e) showed homogeneous staining through the whole epithelium in 9 cases, while 4 cases were negative and 1 case showed some dispersed positivity. LLOO2 (Figure 5f) reacted in all cases with varying intensity, leaving the superficial epithelial layers sometimes completely negative.

With RGE 53, RCK 106 (Figure 5g), CK 18-2, M 20, CAM 5.2, RCK 105, and RKSE 60 (Figure 5h) the major parts of CIN I lesions were nonreactive, although there were isolated positive cells detectable in a few cases. These mostly basally located cells (except RKSE 60) showed staining of variable intensity.

**CIN II (Figures 2F and 5i to p)**

CIN II was diagnosed in 14 fragments (Figure 5i). RCK 102 (Figure 5j), RCK 103, 2D7, and 1C7 (Figure 5k) showed expression through the whole thickness of the epithelium. With 2D7 and 1C7 the basal staining was less pronounced than in the other layers and with RCK 102 the intensity of immunoreactivity varied. 6B10 (Figure 5i) showed slight staining with varying intensity in the basal part of the epithelium, with more intense decoration of the superficial parts in all cases. LP2K (Figure 5m) was moderately positive through the whole thickness of the epithelium in all but one case, which was completely negative. In six fragments the superficial cells did not react. LLOO2 showed expression with variable intensity in all layers (Figure 5n); seven fragments remained negative.

RCK 106 (Figure 5o), CK 18-2, M 20, and CAM 5.2 (Figure 5p) decorated a few dispersed cells in only two cases, and staining was usually confined to the basal parts of the epithelium. Staining with RGE 53 and RKSE 60 was negative.

**CIN III (Figures 2G and 6)**

CIN III was diagnosed in 15 fragments. RCK 102 (Figure 6a) and RCK 103 (Figure 6b) decorated the full thickness of the epithelium, with the basal half of the epithelium...
staining more intensely than the upper half. Antibodies 1C7 (Figure 6c), 2D7, 6B10 (Figure 6d), LP2K, LLO02 (Figure 6e), CAM 5.2 (Figure 6f), and M 20 (Figure 6i) showed partial positivity in all epithelial layers, alternating with negative layers in most cases, while some were negative. RCK 106 (Figure 6i) stained most basal parts of the epithelium in each cervical fragment with one negative case. RGE 53 was always negative. With RCK 105, CK 18-2, and RKSE 60 (Figure 6g) only a few immunoreactive cells were seen in some cervical fragments.

**Discussion**

In this study the keratin polypeptide distribution in a large series of cervicis with normal epithelium, squamous metaplasia, and the three grades of CIN were examined immunohistochemically using a panel of 14 MAbs. By using several MAbs directed against different epitopes of the same keratin polypeptide (for example, the expression of keratin 18 and keratin 8 were monitored with 3 and 2 antisera, respectively), it is possible to detect structural
(epitope) alterations resulting from biologic activity or neoplastic transformation (see, for example, 19). An illustration of this so-called epitope masking phenomenon is the variability in the detection of keratin 18 with different MAbs. In the cervixes in which CIN III was diagnosed, RCK 106 stained the basal part of most epithelia, while CK 18-2 stained fewer cases and the reaction was less intense. Staining with RGE 53 was negative in all cases. Reserve cells and epithelia with immature squamous metaplasia were stained by all three keratin 18 antibodies, although the immunoreaction for RGE 53 was less intense when compared to CK 18-2 and RCK 106.

The pattern of keratin expression in ectocervical epithelium was reproducible in all cases with the exception of keratin 10, which is a marker of keratinization. In several cases this component occurred in some cells above the basal layer, although no morphologic signs of keratinization could be detected in these epithelia. In accord with
earlier studies, we also detected keratin 4, 13, and 19 in ectocervical epithelium. The fact, however, that we found a reaction with 6910 in all cells of the superficial layer and the majority of cells in the intermediate layer contrasts with observations by Weikel et al., who found a heterogeneous reaction with this antibody in the ectocervix. The availability of a MAB directed against keratin 14 enabled us to identify this cytoskeletal component as a major constituent in ectocervical cells.

In the endocervix the keratin expression in columnar cells was consistent with reports in the literature. Reserve cells were not often found, but in a number of cases they were distinct beneath the cylindrical cells. They showed an unequivocal, albeit moderate, expression of keratin 18 with the stronger two of the three antibodies used. Previously it was reported that the antibody RGE 53, which in this study very weakly stains the subcolumnar reserve cells using the peroxidase technique, gave negative results when the indirect immunofluorescence technique was applied. This demonstrates the greater sensitivity of the peroxidase method. Weikel et al. also found no reaction in subcolumnar reserve cells with their keratin 18 antibody CK-2. In contrast, Gigi-Leitner et al. showed a positive keratin 18 reaction in reserve cells with antibody KS 18.18. These discrepancies can be explained by presuming that these different keratin 18 antibodies recognize different epitopes or exhibit varying affinities for their respective antigens. Apparently, the epitopes recognized by RGE 53 and CK-2 are (partially) masked in subcolumnar reserve cells of the endocervix. Reserve cells were not immunoreactive with the keratin 7 antibody.

Furthermore, on the basis of the intense staining with RCK 102 (detecting keratins 5 and 8) and the weaker and variable reaction with M20 and CAM 5.2, we support the suggestion made by Weikel et al. that reserve cells contain keratin 5, and conclude that reserve cells contain, among others, keratins 5, 8, 18, and 19.

In contrast to the report of Levy et al., we could not detect keratin 13 in reserve cells with both antibodies used, ie, 1C7 and 2D7. We also could not reproduce their findings concerning the staining of keratin 13 in immature squamous metaplasia, in which they report strong staining of all cells in the vicinity of the transitional zone using antibody KS-1A3. In immature squamous metaplasia we found only some dispersed reactivity with 1C7 and 2D7 of cells in the epithelium, and the whole mature epithelium, with exception of the basal layer, stained intensely. Epitope masking or recognition of other keratins by the antibody KS-1A3 of Levy et al. should be considered explanations for these discrepancies.

However, different affinities of the antibodies for their respective epitopes may play a major role in the staining variability observed. For example, the different antibodies to keratin 18 may be ranked in order of decreasing tritrability as follows: RCK 106 >> CK 18.2 >> RGE 53. Therefore, if the amount of keratin 18 is low in a given cell type, a negative reaction with RGE 53 may be a result of its high threshold of sensitivity. This may also hold true for keratins 8 and 13. The keratin 8-specific antibody CAM 5.2, for example, which is a strong antibody, shows a somewhat more intense and extensive staining pattern than M 20. The keratin 13 antibody used by Levy et al. might show a lower threshold of sensitivity than 1C7 and 2D7, which are relatively weak antibodies as compared to other keratin 13 antibodies available (e.g., AE 8).

Immature squamous metaplasia can be considered a proliferation of reserve cells in which they acquire squamous characteristics. This state of differentiation is, however, far removed from normal ectocervical epithelium. No significant staining with keratin 13 antibodies was detected, which means the protein level must be at least very low, and substantial amounts of simple epithelial keratins 8 and 18 were present. The irregular distribution of both keratins 14 and 18 throughout most of the epithelial thickness suggests that these two usually nonoverlapping keratins may be expressed reciprocally. Therefore, our results indicate that this type of squamous epithelium has a keratin expression pattern that is, on the one hand, characteristic of simple epithelia and, on the other hand, characteristic of squamous differentiation. This fact is emphasized when we compare the expression in immature squamous metaplasia to mature squamous metaplasia and ectocervical epithelium. The latter two show a highly comparable keratin expression pattern. In mature squamous metaplasia keratin 8 and 18 are virtually absent with the exception of two cases, while keratin 19 was restricted to the basal cell layer. Furthermore, as a result of this progressive stabilization, the expression of keratins 4, 10, 13, and 14 increases. As compared to the study of Gigi-Leitner et al. who used antibody KS 18.18, we found keratin 18 in immature squamous metaplasia.

Next to these changes in keratin expression during progressive metaplastic lesions, we also identified profound changes in keratin expression during progression of CIN. Through the three grades of CIN we found that keratins characteristic of simple epithelia were also present in dysplastic lesions. In CIN I approximately one half of the cases and in CIN II one third of the cases showed some dispersed reactivity for antibodies against keratins 8 and 19, while keratin 18, which only stains in the basal compartment in ectocervical epithelium, showed a loss of polarity, staining the full thickness of the epithelium, often in an irregular fashion. CIN I and CIN II would appear to be somewhere in between the immature metaplastic and the mature metaplastic epithelium in terms of their keratin expression. They share with immature metaplasia the homogeneous full-thickness staining with anti-keratin 19 antibody LP2K. Keratin 19 has been associated with variable or transitional areas of differentiation or with premalig-
nancy,26 in this case the former would seem to fit better. In contrast to the sporadic staining for keratins 8 and 18 in some CIN I and CIN II lesions, CIN III showed reactivity for these keratins in most of the cases, with areas of intense, moderate, or slight staining alternating with negative areas. Throughout this study, the CIN III samples stand out as the most disorganized condition. Simple epithelial keratins are synthesized by cells in the basal compartment as opposed to appearing preferentially in groups of cells higher up in the epithelium. All the keratinocyte keratins tested (keratins 19, 13, 14, and 10) were highly irregular in their expression. This suggests that the proliferating cells are incompletely programmed, leading to a degree of randomness in differentiation, possibly reflecting an origin from a more pluripotent progenitor cell. The degree of disorganization seen in CIN III samples is thus in keeping with their poorer prognosis.

The role of other keratins, ie, keratins 5, 6, 15, and 17, which may constitute an important part of the cytoskeleton of basal (reserve) cells and squamous epithelium, must still be investigated.

Antibody RCK 102, recognizing both keratins 5 and 8, could only be used to detect keratin 5 expression when the keratin 8 antibodies M20 and CAM 5.2 were negative. Based on this consideration, we conclude that keratin 5 is a major constituent of not only normal ectocervical epithelium but also of reserve cells, squamous metaplasia, and CIN lesions.

In contrast to a recent study by Raju,27 who suggested that the keratin(s) recognized by CAM 5.2 seldom occur in CIN III, our findings with CAM 5.2 in frozen sections show a positive reaction in a varying number of cells in 13 of 15 cases of CIN III. The study of Angus et al28 largely confirms our data, although we wish to stress in our study that almost 80% of all CIN III lesions reacted with CAM 5.2, while Angus et al report reactivity in only 30% of their cases. This difference can probably be explained by the fact that they used formalin-fixed, paraffin-embedded cervical excisions. Also Whitaker et al29 showed a profound difference in keratin expression when CIN I and II are compared to CIN III, resulting in a loss of stratification. In their study, however, only three antibodies were used (K 8.60, K 8.12, and PKK1), each recognizing more than one keratin, and thus not allowing the exact determination of which keratins are expressed. Furthermore, all material studied was routinely processed, which could be partly responsible for the unexpectedly restricted staining pattern of the keratin antibody PKK1 and for their observation that both mature and immature squamous metaplasia showed comparable staining patterns.

It is widely accepted that CIN I, II, and III can progress to cervical carcinoma.24-26 The probability of malignant transformation depends first on the severity of the dysplasia. Most cases of CIN I and CIN II regress, while a small percentage show progression to CIN III and carcinoma of the cervix. Future studies will have to reveal whether those cases of dysplasia in which we have observed fundamental changes in the keratin phenotype are the ones that will show progression to carcinoma. On the other hand, those cervical excisions with microscopic characteristics consistent with CIN I and II that show no substantial alterations in keratin phenotype could be cases that will revert to a normal epithelial architecture when the factors inducing the dysplasia are removed.

We have observed also keratins 8, 18, and 19, among others, in squamous cell carcinoma and adenocarcinoma of the cervix (unpublished observations). The progenitor cells for the adenocarcinoma are considered to be the columnar cells of the endocervical canal. The observation of keratins 8, 18, and large amounts of 19 in CIN III indicate that these cells may be the common progenitor that can show dual differentiation, on one hand, to a squamous cell carcinoma and, on the other hand, to an adenocarcinoma. This supposition is further supported by the occasional finding of dysplasia in the vicinity of adenocarcinoma.

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