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Cytokeratin expression in squamous metaplasia of the human uterine cervix

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Abstract. The expression of cytokeratin polypeptides in squamous metaplasia of the human uterine cervix was investigated by immunocytochemical labeling with polypeptide-specific antibodies against cytokeratins. Immunofluorescence microscopic examination of cervical tissues using various monoclonal antibodies indicated that squamous cervical metaplasia expresses a unique set of cytokeratin polypeptides, this being distinctively different from that expressed by all of the normal epithelial elements of the exo- and endocervix. The development of metaplastic foci was accompanied by the expression of cytokeratin polypeptide no. 13, which is commonly detected in stratified epithelia, and by a reduction in the level of polypeptide no. 18, which is typical of simple epithelia. The 40-kilodalton cytokeratin (no. 19) described by Moll et al., which is abundant in the columnar and reserve cells of the endocervix, was found throughout the metaplastic lesions. Only in 'well-differentiated' metaplasias did we detect polarity of cytokeratin expression reminiscent of the staining patterns in the exocervix. This was manifested by the exclusive labeling of the basal cell layer(s) with antibodies K_B 8.37 and K_M 4.62, which stain the basal cells of the exocervix. Furthermore, a comparison of cervical metaplasia with squamous areas occurring within endometrial adenocarcinomas pointed to a close similarity in the cytokeratin expression of the two. We discuss the use of cytokeratins as specific markers of squamous differentiation, the relationships between squamous metaplasia and cervical neoplasia, and the involvement of reserve cells in the metaplastic process.

Introduction

The process of squamous metaplasia involves the transformation of differentiated nonsquamous epithelium into squamous epithelium [31, 46]. This process is often detected in various human tissues such as the bronchi [3, 49], stomach [9, 32], urinary bladder [25], and salivary glands [11], and is especially common in the uterine cervix [12, 13]. The human cervix consists of two major areas that are anatomically and histologically distinct: the exocervix and endocervix. The former is characteristically composed of nonkeratinizing stratified squamous epithelium, while the latter contains a simple epithelial monolayer of columnar cells which line the mucosal surface and invaginate into the stroma. The sharp squamocolumnar junction detected between the two areas is normally located at the cervical portio [8, 12].

This boundary area has been found to be the most common site for the development of squamous metaplasia [6, 12, 43]. This metaplasia is preceded by the outward extension of the endocervical mucosa into the exocervical portion, a process denoting erosion or ectopy [12]. Subsequently, and usually throughout the reproductive period of the individual, changes may occur in the ectopic endocervical mucosa, leading to various degrees of stratification of the epithelium and the formation of 'transformation-zone metaplasia' (TZM). The metaplastic squamous epithelium thus formed is usually less ordered than that of the neighboring exocervix and can usually be identified by conventional light microscopy [12]. Nevertheless, is often retains an apparent continuity with the exocervical epithelial layers, and the exact point of transformation is difficult to determine (see Fig. 1c).

Less frequent, yet still quite common, is the appearance of 'metaplastic plaques' (MPs) in the endocervical canal, which are completely disconnected from any normal squamous epithelium. These MPs may exhibit variable dimensions and may differ with regard to the extent of stratification. Similar metaplastic changes may be found not only in the normal mucosa but also in malignancies of glandular tissues of the female genital tract, including the endocervix, forming 'adenocarcinomas with squamous differentiation' [26].

The factors which induce metaplastic transformation are still poorly understood, yet several possibilities have been suggested. These include alterations in environmental conditions, mechanical irritation, chronic inflammation, changes in pH and in hormonal balance, etc. [12, 43]. Another debatable issue concerns the cellular basis of metaplastic transformation: is there a direct ingrowth from the native portio epithelium into the transformation zone ([43]; or even beyond it), or does the process involve cells of purely endocervical origin? Attempts to identify cellular precursors for squamous metaplasia exhibiting the latter mechanism have focused on two endocervical cell types, i.e., 'basal' or 'reserve' cells and columnar cells. As will be discussed in detail later, the former are commonly scattered in between the columnar cells of the endocervix and confined to the area close to the basement membrane (for

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further details, see Discussion). For a number of years, these reserve cells have been accepted as being the source of cervical squamous metaplasia, a theory originally proposed by Fluhmann, who called this process "prosoplasia" [14]. Until recently, the major, if not only, means of studying metaplastic transdifferentiation was morphological observations using light and electron microscopy.

Recently, immunocytochemical techniques employing cell-type-specific antibodies have been widely used for the identification of the histogenetic origins as well as the state of differentiation of cells. Particularly useful in this respect are specific antibodies reactive with intermediate filament (IF) subunits (for reviews, see [17, 36, 37, 41, 51]). It has been extensively documented that there are five major, biochemically and antigenically distinct families of IF subunits which are expressed in a cell-type-restricted fashion [2, 28, 29]. Among those, the cytokeratin family, which is characteristic of epithelial cells, is further diversified [16, 33, 40]: about 20 different cytokeratin polypeptides from various human epithelia have been isolated and biochemically, immunochemically, and genetically characterized [18, 23, 33, 40, 45]. It has further been shown that each type of epithelial cell contains a characteristic combination of cytokeratin polypeptides which may be used to identify that particular cell type either in the normal state or after malignant transformation (for reviews, see [33, 40, 47]). This approach has been extensively employed in recent years for the diagnosis of anaplastic tumors and the determination of their histogenetic origins [4, 19, 34, 42].

In the present study, we investigated the expression of specific cytokeratin polypeptides in different forms of squamous metaplasia of the human cervix. Using both biochemical and immunohistochemical approaches, we showed that cells undergoing metaplastic changes express a unique combination of cytokeratin polypeptides including the stratification-related cytokeratin polypeptide no. 13 (which is otherwise absent from the normal mucosa of the endocervix), polypeptides nos. 8 and 19, and minute and variable amounts of polypeptides nos. 18 and 10/11 (numbers according to the classification of Moll et al. [33]). These results suggest that the metaplastic process involves a unique step of squamous differentiation of an endocervical cell (probably a reserve cell) which is molecularly distinct from the process of stratification of the exocervix. The significance of these results and their relevance to cervical neoplasia are discussed.

Methods

Tissues

The cervical tissues studied were obtained from 31 patients aged 36–82 years (mean, 54 years) at the Kaplan Hospital, whose uteri were removed due to leiomyomas and prolapse. The cervix was opened through the external os within 15 min of hysterectomy, and several sections were obtained through the exocervix and endocervix, including the squamocolumnar junction in a plane parallel to the long axis of the cervical canal. The tissues used for immunocytochemical studies were snap frozen in isopentane that had been precooled in liquid nitrogen, and then stored at −70° C. For routine histologic examinations, the tissues were fixed in 4% buffered formaldehyde, embedded in paraffin [1], and stained with hematoxylin and eosin (HE). In 8 out of the 31 cases examined, various degrees of cervical squamous metaplasia were observed, either in continuity with the exocervix or in isolated foci within the cervical canal.

Immunochemical reagents

The murine monoclonal antibodies used included:

1. K_G 8.13, a broad-spectrum cytokeratin antibody which reacts with the cytokeratin filaments present in all human epithelial cells tested, i.e., both normal and malignant cells. This antibody, raised against bovine muzzle keratin, reacts with a relatively broad range of polypeptides, including cytokeratins nos. 1, 5, 6, 7, 8, and 18, as well as reacting weakly with cytokeratins nos. 10 and 11 [22].

2. K_K 8.60, an antibody reactive with human cytokeratin polypeptides nos. 10 and 11. As previously suggested, this antibody might be a specific marker of keratinization [24].

3. K_S 8.12, an antibody that reacts with polypeptides nos. 13 and 16, which are present in stratified nonkeratinizing epithelia as well as in squamous carcinomas [24].

4. K_B 8.37, an antibody which reacts with IFs of cultured keratinocytes of murine and bovine origin (data not shown), as well as with cytokeratin filaments in the basal layer of stratified squamous epithelium (skin, exocervix, etc.; see insert in Fig. 2e). This antibody does not react with simple, pseudostratified or transitional epithelia in humans. The exact polypeptide specificity of this antibody has not been defined, since it does not react with electrophoretically separated polypeptides of the exocervix in Western-blot analysis. The epitope specifically recognized by this antibody may be conformation dependent and thus be irreversibly destroyed by electrophoretic separation. Regardless of its fine molecular specificity, we used antibody K_B 8.37 as a marker of the basal layer of the squamous epithelium.

5. K_M 4.62, a monoclonal antibody prepared against cytoskeletal polypeptides of cultured human adenocarcinoma line MCF-7. This antibody reacts with only one human polypeptide, i.e., no. 19 [21].

6. K_S 18.18, a murine monoclonal antibody which reacts with human cytokeratin polypeptide no. 18 and stains simple and pseudostratified epithelia as well as the basal layer of several noncornifying stratified squamous epithelia (W.W. Franke, unpublished data). This antibody was kindly supplied by Prof. W.W. Franke (German Cancer Research Center, Heidelberg, FRG).

The different monoclonal antibodies used were usually applied as undiluted hybridoma culture supernatants.

The secondary antibodies were affinity-purified goat antibodies raised against mouse $F(ab')_2$, and conjugated to lissamine rhodamine sulfonyl chloride as previously described [5, 20].

Immunohistochemistry

Frozen sections of tissue blocks were cut at about −20° C in a Frigocut 2700 cryostat (Jung-Reichert, FRG). The sections (4–5 μm thick) were placed on clean glass slides, air dried, acetone fixed, and immunolabeled as previously described [15]. Antibody-stained sections were dehydrated in absolute ethanol, mounted in Entelan (Merck, FRG) and examined using a Zeiss Photomicroscope III equipped for epifluorescence observations with oil-immersion Plan Neofluar objectives (×25/0.8 or ×16/0.5).

Gel electrophoresis and immunoblotting analyses

Relevant regions of 20-μm-thick frozen sections were dissected out under microscopic control and extracted with high-salt buffer and detergent [33]. Analysis of the cytokeratin composition in the sections was carried out using one-dimensional gel electrophoresis [27] and immunoblotting [48].

Electron microscopy

Freshly obtained surgical samples were dissected into small blocks (2–3 mm) and immediately fixed in 2% glutaraldehyde in 0.1 *M* cacodylate buffer, pH 7.2. The samples were postfixed in 1% OsO_4, embedded in Polybed 8.12 (Polyscience, USA), cut at the desired orientation, and examined using a Phillips 410 electron microscope at an accelerating voltage of 80 kV.

Results

The histological appearance of the various normal and metaplastic epithelial elements of the human cervix is shown in Fig. 1.

Cytokeratin expression in the epithelial elements of normal human cervix

To establish the pattern of cytokeratin expression as revealed by immunofluorescence labeling, frozen sections of various regions along the cervix were stained with the six cytokeratin-specific monoclonal antibodies.

The normal exocervix, throughout its entire length, had an appearance typical of stratified-squamous epithelium, with a distinct layer of basal cells and well-ordered suprabasal squamous cells (Fig. 1a). All epithelial layers of the exocercix were intensely labeled with the broadly cross-reacting K_G 8.13 cytokeratin antibody (Fig. 2a) as well as with the 'stratification-specific' antibody, K_S 8.12 (Fig. 2b). Two of the antibodies used, K_M 4.62 and K_B 8.37, exclusively labeled the basal cell layer (Fig. 2c and e, respectively). It should be pointed out, however, that in other squamous epithelial tissues, there are marked differences between the staining patterns produced by these last two antibodies; antibody K_M 4.62 stains most simple epithelia but does not label keratinizing squamous epithelia (e.g., epidermis), while K_B 8.37 labels the basel cell layer of keratinizing and nonkeratinizing squamous epithelia but is negative in all nonsquamous epithelia. Staining of the exocervix with K_K 8.60 produced sporadic labeling of individual cells or groups of cells within the suprabasal layers of the exocervix (Fig. 2f); the extent of this labeling varied somewhat from region to region and from sample to sample. Antibody K_s 18.18 (reactive with only polypeptide no. 18 did not significantly or reproducibly stain any component of the squamous epithelia of the exocervix (Fig. 2d). Occasionally, faint staining of the basal cells of the exocervix was observed (see Fig. 2d insert).

The normal endocervix was uniformly positive for antibodies K_G 8.13, K_M 4.62, and K_s 18.18 (Fig. 3a, c, and d, respectively). No labeling of the endocervix was obtained with the other three antibodies testes, i.e., K_S 8.12, K_B 8.37 and K_K 8.60 (Fig. 3b, e, and f, respectively). Histological examination of HE-stained sections of the endocervix often revealed the presence of cuboidal cells within the columnar epithelium. These cells were situated near the basal portion of the columnar cells and were not exposed at the surface of the mucosa (Figs. 1b and 4a). These cells, identified as being reserve cells, exhibited the same labeling pattern as columnar cells with all of the cytokeratin antibodies testes (Fig. 4a–f).

A high-resolution view of these reserve cells of the endocervix was obtained using transmission electron microscopy. Examination of the endocervical mucosa indicated that these cells were cuboidal cells with electron-lucent cytoplasm and a large, round nucleus (Fig. 5a). These cells did not reach the luminal surface of the endocervix, nor were they directed attached to the basement membrane (Fig. 5b, arrowheads). Examination of a large number of samples indicated that the reserve cells were 'trapped' between the columnar cells, and were attached at their basal aspects to membrane projections and lamellae of the columnar cells (Fig. 5b; see Discussion).

Reserve cells were usually sparsely distributed along major parts of the endocervix, and the unequivocal identification of individual cells was often difficult. However, we occasionally detected endocervical regions in which various degrees of reserve-cell hyperplasia were apparent (Fig. 4f, arrowheads). This manifested itself by a local accumulation of cuboidal cells in one or a few layers, in which the 'normal' columnar cells could be detected at their mucosal aspect (Fig. 4a). Staining of hyperplastic reserve cells with the cytokeratin antibodies revealed positive reactivity with K_G 8.13, K_s 18.18, and K_M 4.62 (Fig. 4f, arrowheads) as in the endocervical mucosa (Fig. 3). No labeling of hyperplastic reserve cells was observed with antibody K_S 8.12 (Fig. 4c) or with antibodies K_B 8.37 and K_K 8.60 (data not shown).

Cytokeratin expression in squamous metaplasia of the human cervix

We distinguished four types of squamous metaplasia. In the first two metaplasia of the TZM in continuity with the normal exocervix (type a; Figs. 1c, e and 6a–e) and metaplasia situated within the endocervical canal and its invaginations at a distance from the MP (type b; Figs. 1f and 6f), the metaplasia exhibited diminished maturation and lacked a definite basal layer when compared to normal exocervical squamous epithelium (Fig. 1c). We also found a more mature type of metaplasia of the transformation zone, with distinct, hyperplastic basal cells occupying more than the usual one layer of cells (type c; Fig. 7). Finally, we identified metaplasia occurring in glandular elements of an endometrial carcinosarcoma (type d; Fig. 8).

Immunofluorescence labeling of metaplasia of the first category (type a) with the cytokeratin antibodies resulted in the metaplastic cells being extensively labeled with K_G 8.13 (Fig. 6a), K_S 8.12 (Fig. 6c), and K_M 4.62 (Fig. 6e, f). No labeling was obtained with the basal-cell-specific antibody, K_B 8.37 (data not shown), and individual positive cells were detected throughout the sections after labeling with antibody K_K 8.60 (Fig. 6d). Staining with antibody K_s 18.18 produced essentially no labeling of most of the metaplastic cells (Fig. 6b), although in some cases, faint staining of the basal cell layer of the squamous metaplasia was noticed. Occasionally, strongly labeled residual endocervical epithelial cells were detected at the luminal aspect

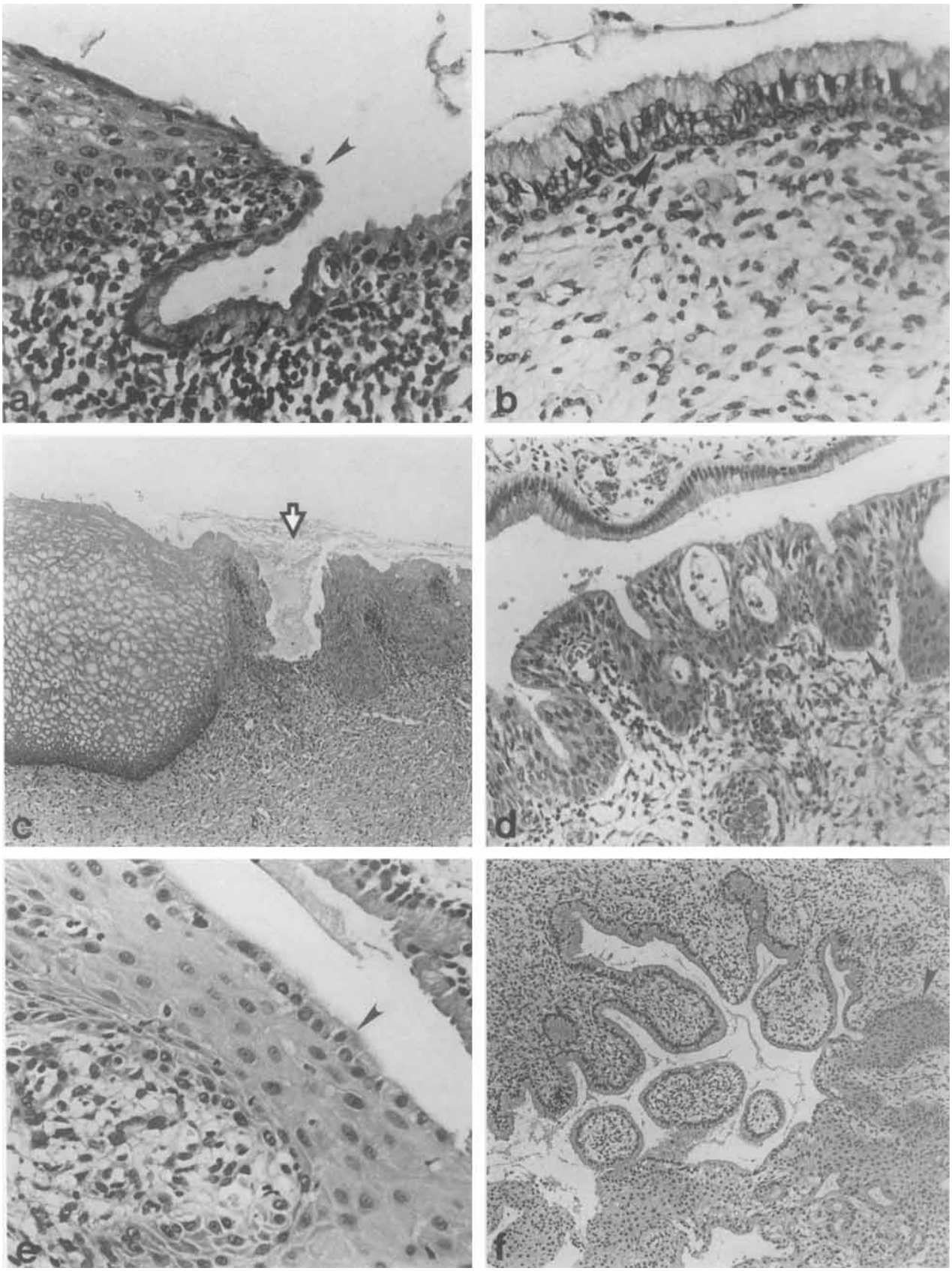

Fig. 1 a–f. Light-microscopic appearance of HE-stained sections of normal hyperplastic and metaplastic human cervix. The regions examined were near to the squamocolumnar junctions (**a, c, e**) and in the endocervical canal (**b, d, f**). **a** The junction between the squamous epithelium of the exocervix (*left*) and the simple epithelium of the endocervix (*right*). The *arrowhead* marks the junction area (×225). **b** Endocervix displaying both simple columnar epithelium and a layer of reserve cells (*arrowhead;* ×225). **c** Squamous metaplasia at the transformation zone (TZM). The *arrow* indicates a site of previous biopsy (×60). **d** Reserve cell hyperplasia (*arrowhead*) in the endocervical canal; notice the apparently normal epithelium at the top (×150). **e** Squamous metaplasia. The *arrowhead* indicates the residual columnar cells (×240). **f** Metaplastic plaques (MP) in the endocervical glands (*arrowhead;* ×150)

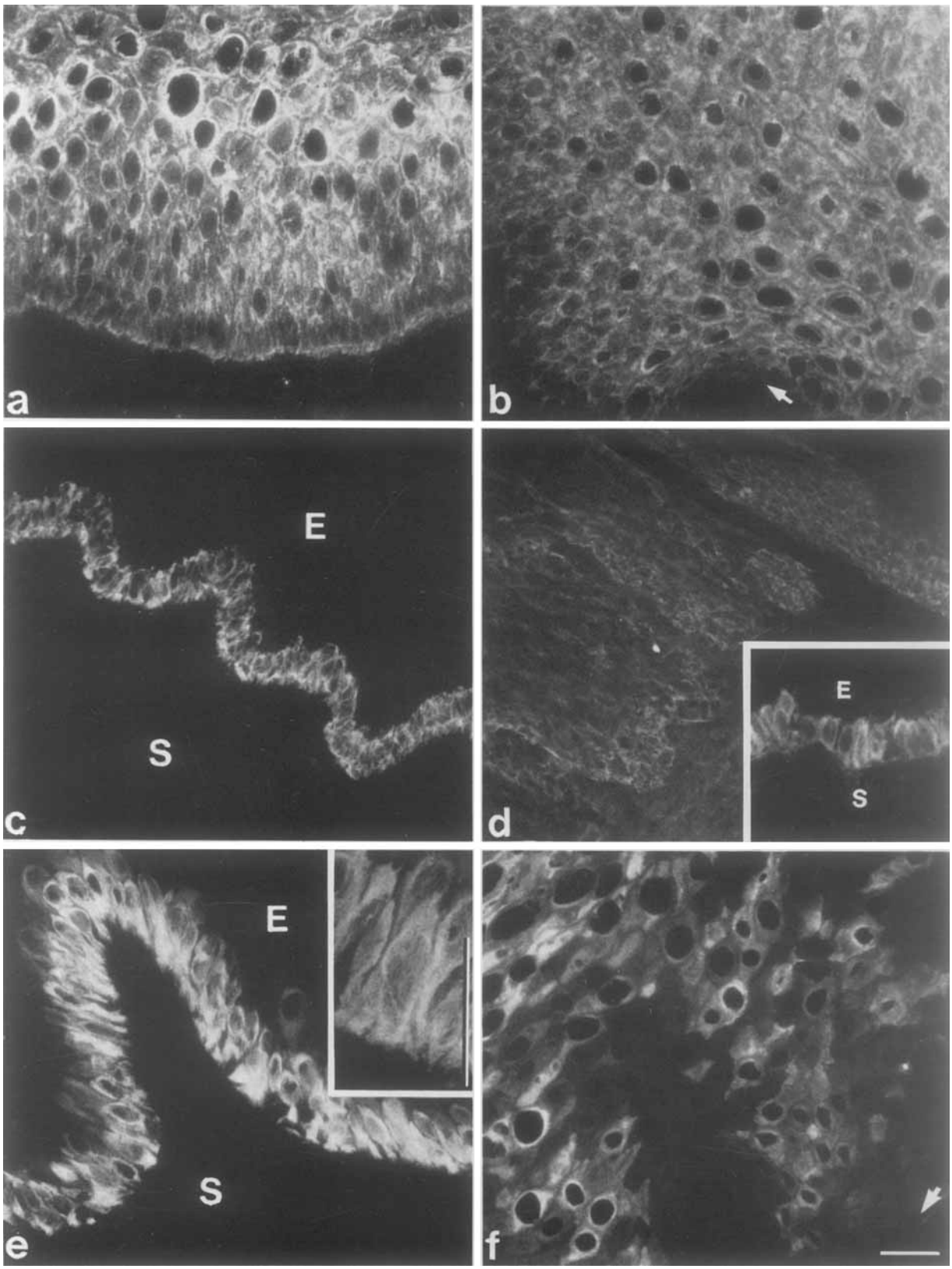

Fig. 2a–f. Immunofluorescence microscopic labeling of the exocervix with monoclonal antibodies. **a** K_G 8.13; **b** K_S 8.12; **c** K_M 4.62; **d** K_s 18.18; **e** K_B 8.37 (insert in **e** shows staining of filaments in the basal cell layer at a higher magnification); **f** K_K 8.60. Note that antibody K_G 8.13 uniformly labeled all of the epithelium, while K_S 8.12 stained the suprabasal layers more intensely. K_M 4.62 and K_B 8.37 stained only the basal layer, while antibody K_K 8.60 stained individual cells or groups of cells. Antibody K_s 18.18 was essentially negative, except for occasional faint labeling of the basal cells (insert in **d**). *E*, epithelium; *S*, stroma. The *arrows* in **b** and **f** point to the basal lamina. *Bars*, 25 μm

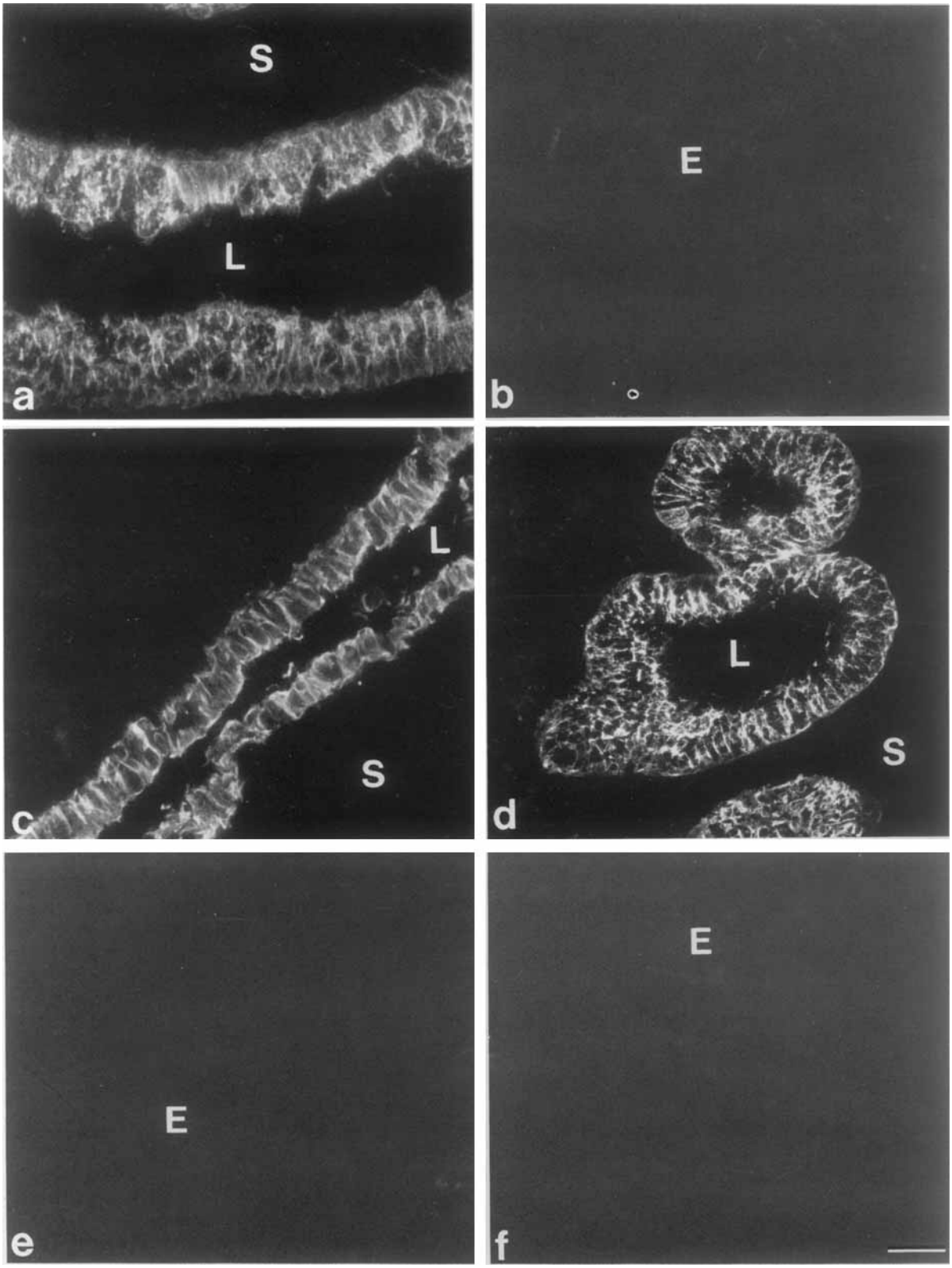

Fig. 3a–f. Immunofluorescence microscopic labeling of frozen sections of endocervical simple epithelium with monoclonal antibodies. **a** K_G 8.13; **b** K_S 8.12; **c** K_M 4.62; **d** K_s 18.18; **e** K_B 8.37; **f** K_K 8.60. Note the positive reaction of K_G 8.13, K_M 4.62, and K_s 18.18 with epithelial cells, this being in contrast to antibodies K_S 8.12, K_B 8.37, and K_K 8.60 which were negative. *S*, stroma; *E*, epithelium; *L*, lumen. *Bar*, 25 μm

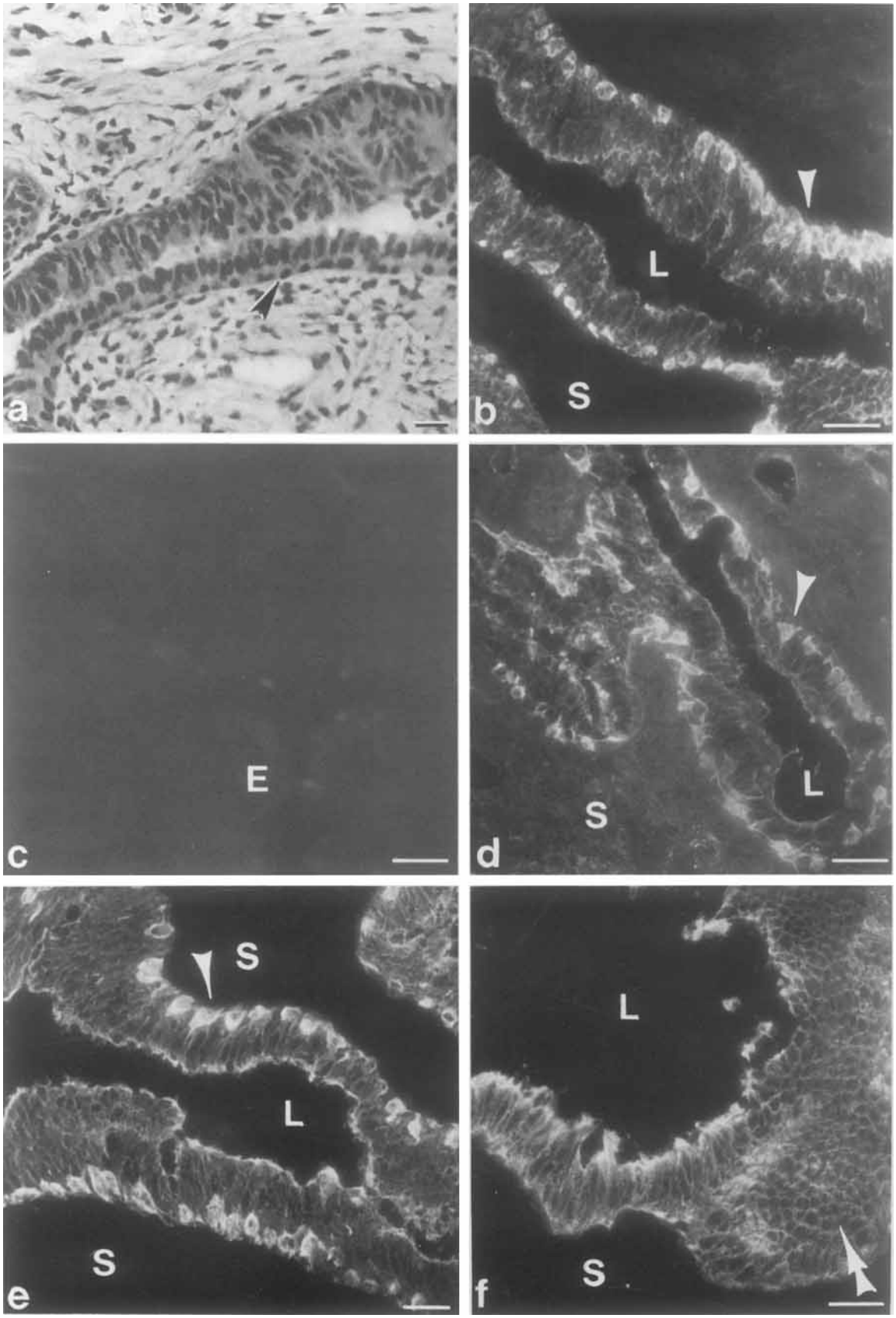

Fig. 4a–f. Hematoxylin-eosin staining (**a**) and immunofluorescence labeling (**b–f**) with monoclonal antibodies of the endocervix revealing either normal or hyperplastic reserve cells. **b** K_G 8.13; **c** K_S 8.12; **d** K_s 18.18; **e, f** K_M 4.62. Note the extensive staining of reserve cells (*arrowheads*) by antibodies K_G 8.13, K_s 18.18, and K_M 4.62. The *double-arrowhead* in **f** indicates reserve cell hyperplasia. Antibody K_S 8.12 did not label epithelial cells in the endocervix. *E*, epithelium; *S*, stroma; *L*, lumen. *Bar*, 25 μm

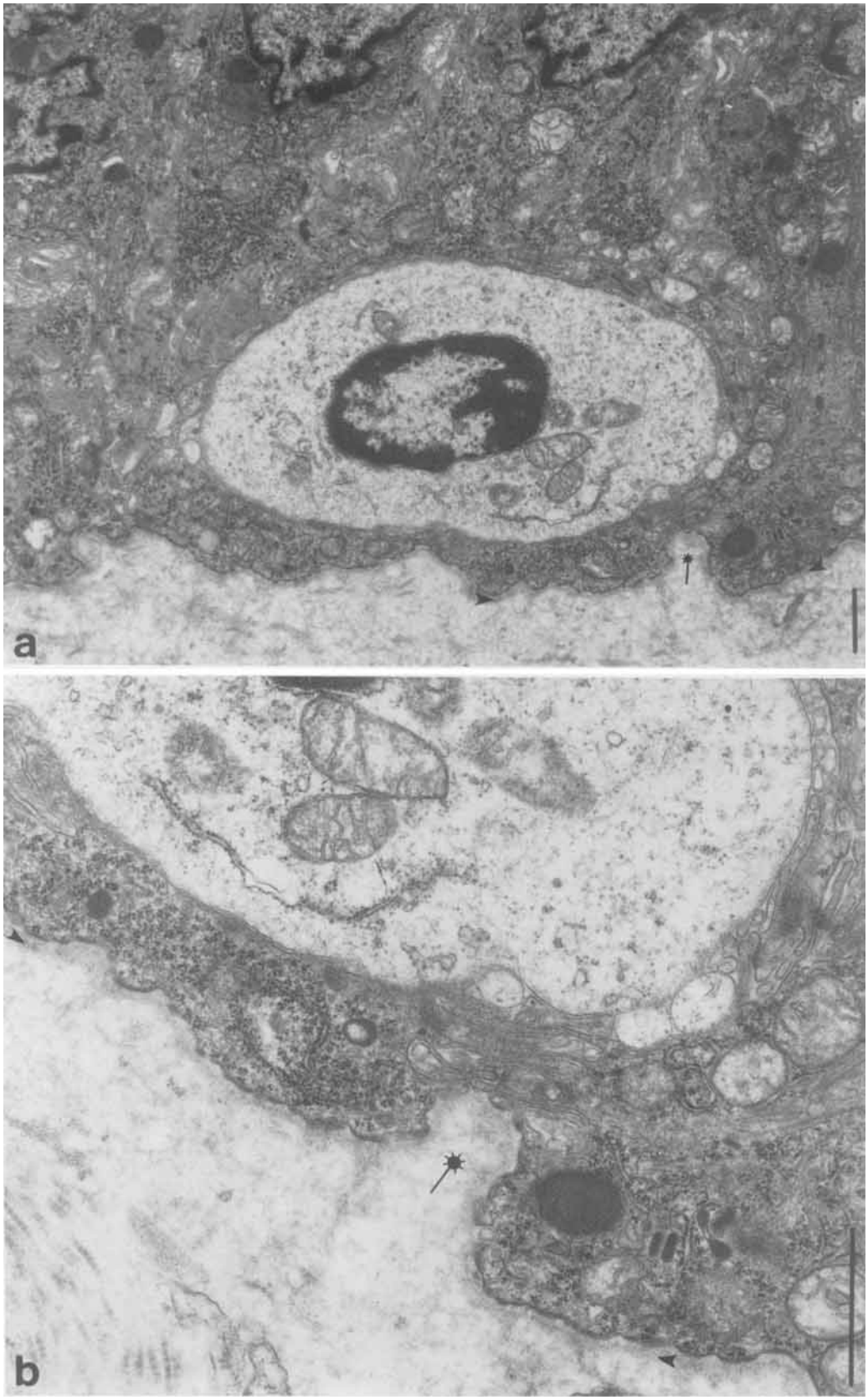

Fig. 5a, b. Transmission electron micrographs of the endocervix showing a reserve cell at low (**a**) and high (**b**) magnification. (The *asterisks* and *arrowheads* in **a** and **b** mark the same positions). Note that the reserve cell has a clear cytoplasm and does not reach the lumen, nor is it directly attached to the basement membrane (*arrowheads*). The reserve cell appears to be permanently associated with the neighboring columnar cells and their process. *Bars*, 0.2 μm

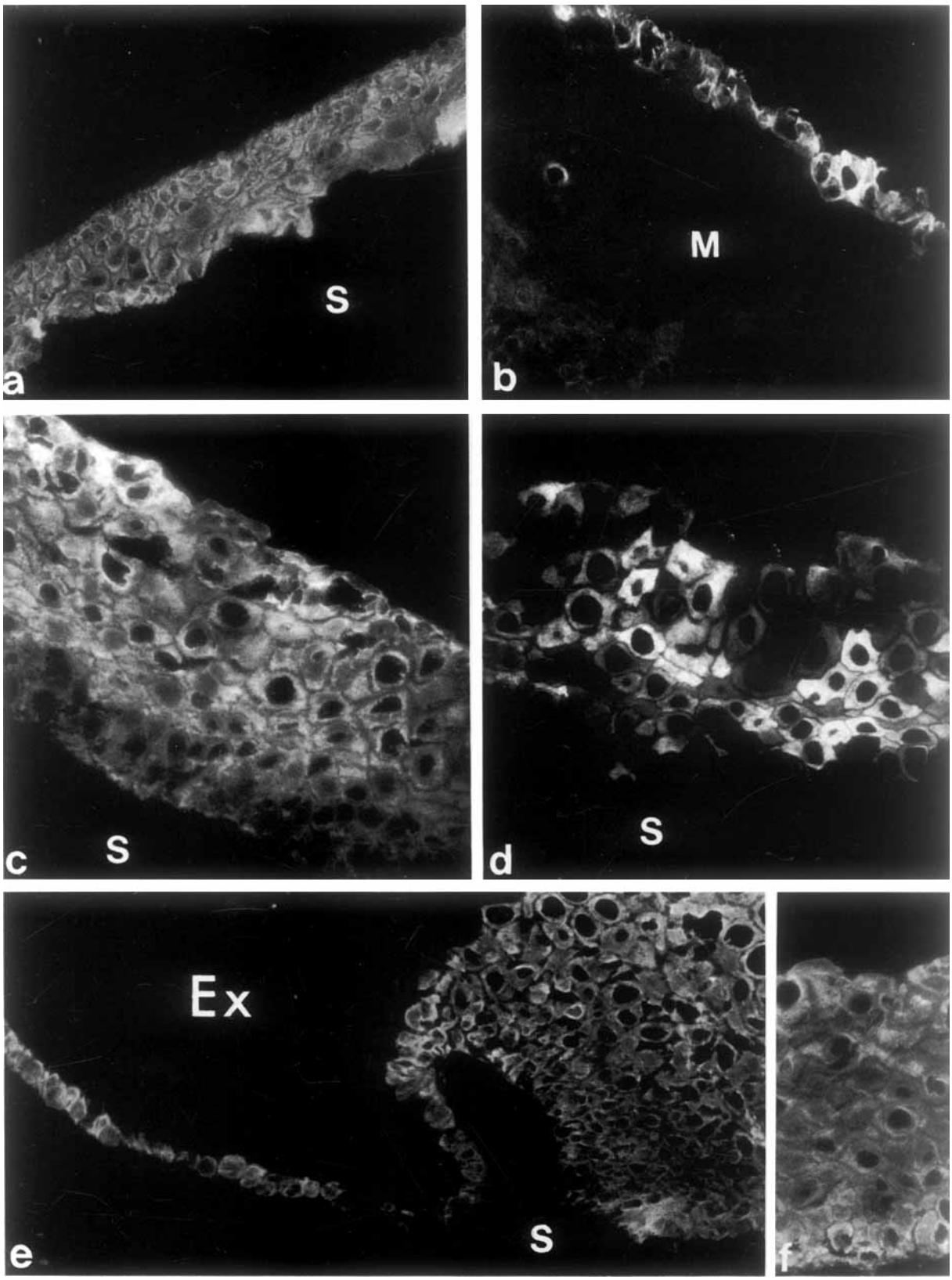

Fig. 6a–f. Immunofluorescence microscopic labeling of cervical squamous metaplasia using different monoclonal antibodies. **a** K_G 8.13; **b** K_s 18.18; **c** K_S 8.12; **d** K_K 8.60; **e, f** K_M 4.62. Note that antibodies K_G 8.13 and K_s 8.12 stained the squamous metaplasia uniformly, while K_s 18.18 stained only the residual columnar cells of the simple epithelium of the endocervix. Antibody K_K 8.60 labeled individual cells or groups of cells in the suprabasal layers of the metaplasia, and K_M 4.62 uniformly stained all cells of the squamous metaplasia, both at the transformation zone (**e**) and in the endocervical canal (**f**). Note the sharp boundary between the negative suprabasal cells of the exocervix and the metaplastic cells in **e**. *Ex*, exocervix; *S*, stroma; *M*, metaplasia. *Bars*, 25 μm

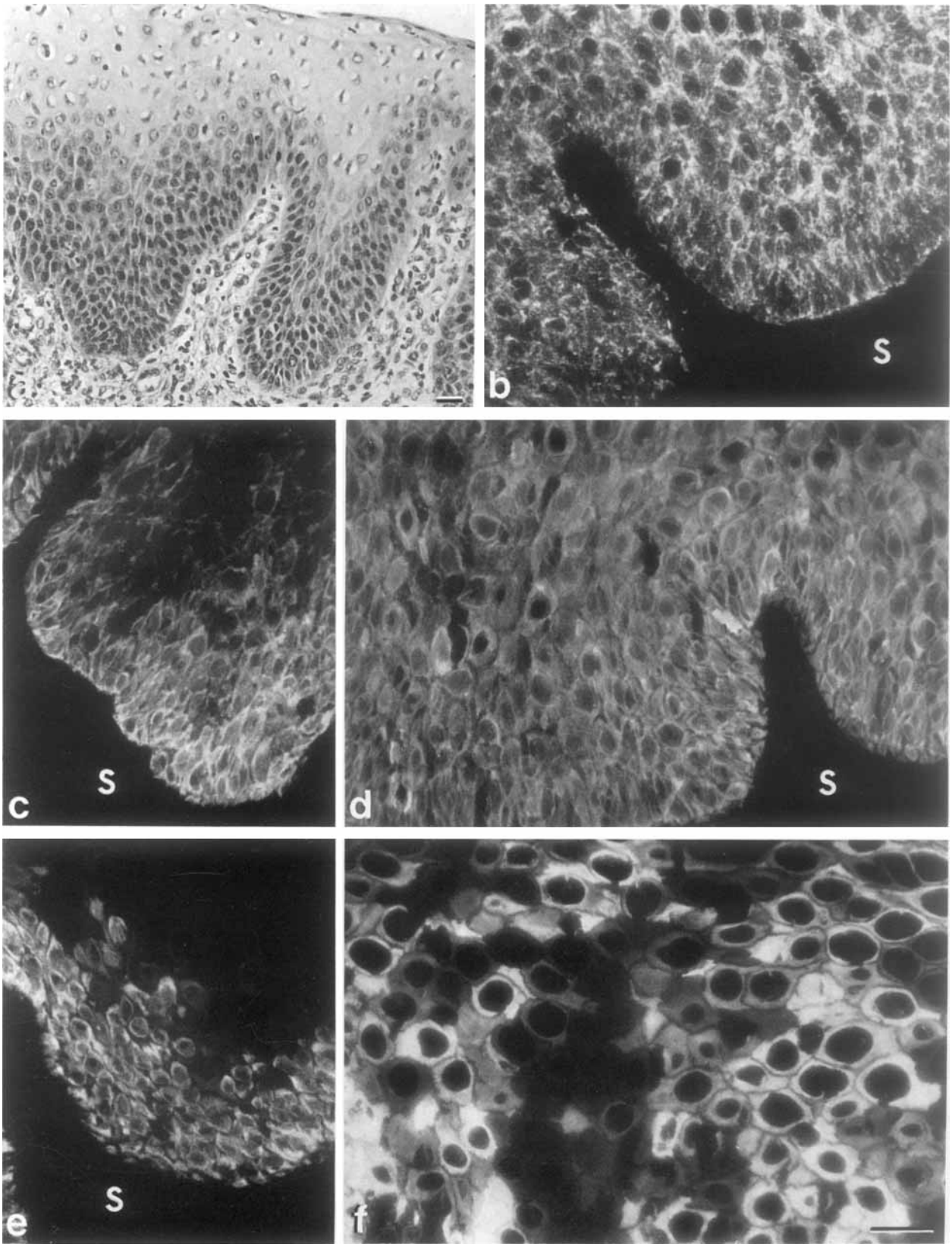

Fig. 7a–f. Hematoxylin-eosin staining (**a**) and immunofluorescence microscopy (**b–f**) of a transformation zone metaplasia exhibiting a high degree of squamous differentiation. **b** K_G 8.13; **c** K_M 4.62; **d** K_S 8.12; **e** K_B 8.37; **f** K_K 8.60. Note that K_G 8.13 and K_S 8.12 uniformly stained the squamous metaplasia, while K_M 4.62 and K_B 8.37 stained predominantly the basal layers. Antibody K_K 8.60 stained individual cells or groups of cells within the suprabasal region. *S*, stroma. *Bars*, 25 μm

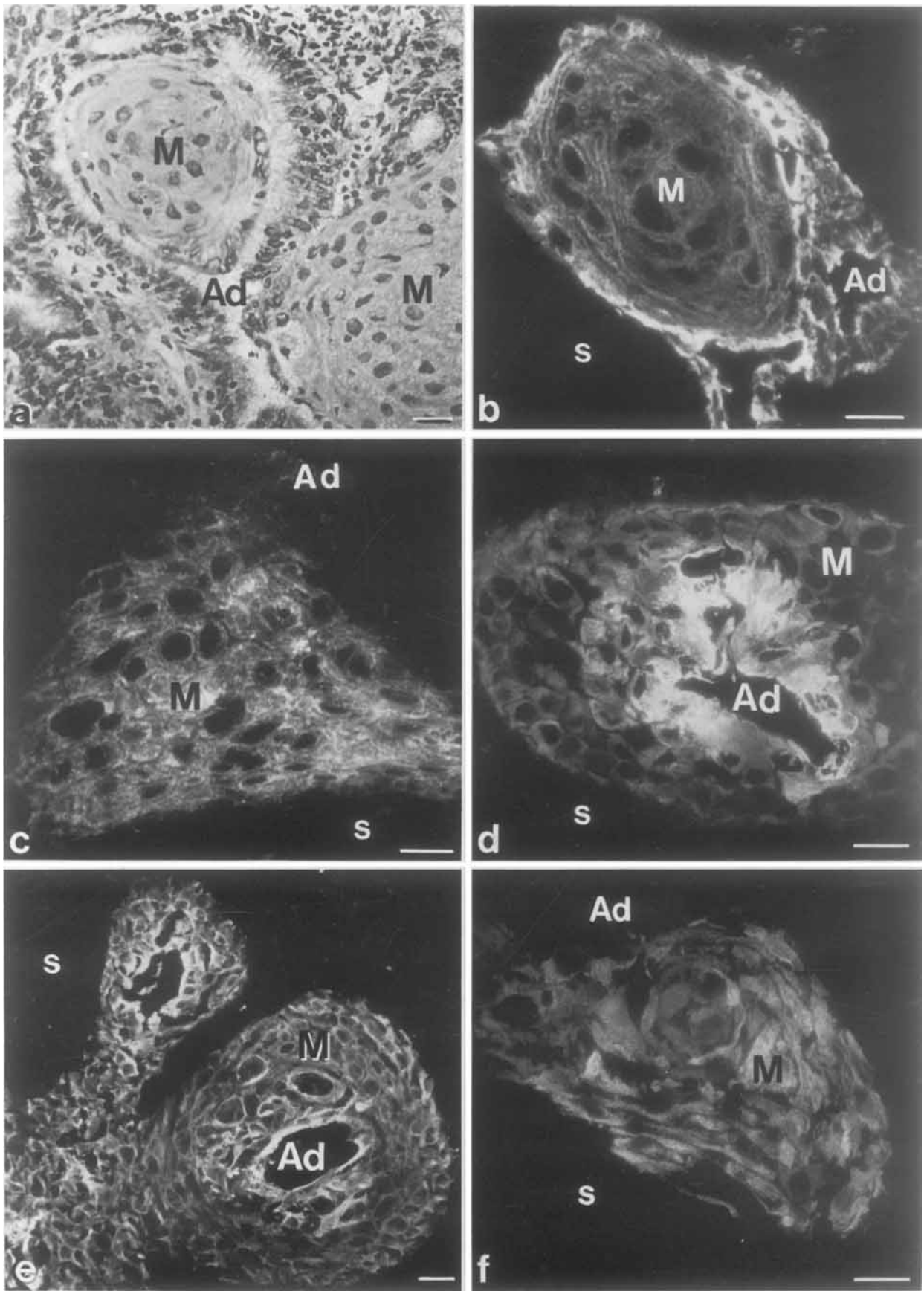

Fig. 8a–f. Hematoxylin-eosin (**a**) and immunofluorescence microscopy (**b–f**) of a squamous area within an endometrical adenocarcinoma. **b** K_G 8.13; **c** K_S 8.12; **d** K_s 18.18; **e** K_M 4.62; **f** K_K 8.60. Note that antibodies K_G 8.13 and K_M 4.62 stained the metaplasia as well as the surrounding adenocarcinoma, while K_S 8.12 stained only the metaplasia. K_K 8.60 stained individual cells in keratinizing foci only in the metaplasia. K_s 18.18 stained the metaplasia faintly as compared to its intense labeling of adenocarcinoma cells. *M*, metaplasia; *S*, stroma; *Ad*, adenocarcinoma. *Bars*, 25 μm

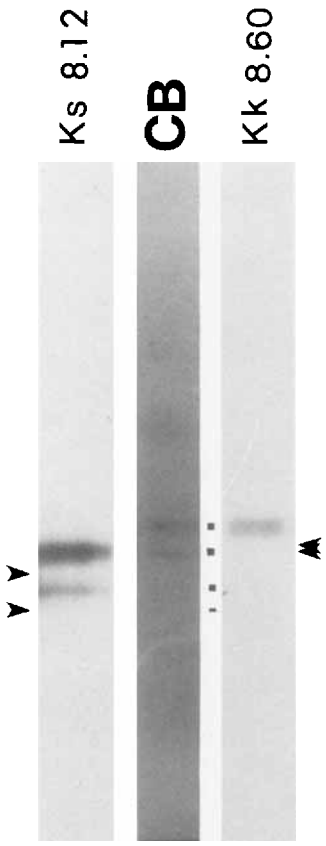

Fig. 9. Immunoblotting analysis of human cervical metaplasia cytokeratins using antibodies K_S 8.12 and K_K 8.60. Metaplastic regions were microdissected and examined by one-dimensional gel electrophoresis. The Coomassie-blue (*CB*)-stained gel contained polypeptides in the 57- to 59-kilodalton range (corresponding to polypeptides 5 [35] and 10/11; *upper dot*) as well as in the ~54-kilodalton area (comigrating with polypeptide no. 13; *second dot from top*). The two *lower dots* mark the position of polypeptides nos. 16 (48-kilodaltons) and 19 (40-kilodaltons) which are barely detectable by Coomassie-blue staining. The *double-arrowhead* on the *right* indicates the presence of polypeptides nos. 10 and 11 in comparable amounts. The reaction with antibody K_S 8.12 shows the major reactivity of the antibodies with polypeptide no. 13 (*upper arrowhead*). The lower band (*lower arrowhead*) comigrated with polypeptide no. 16

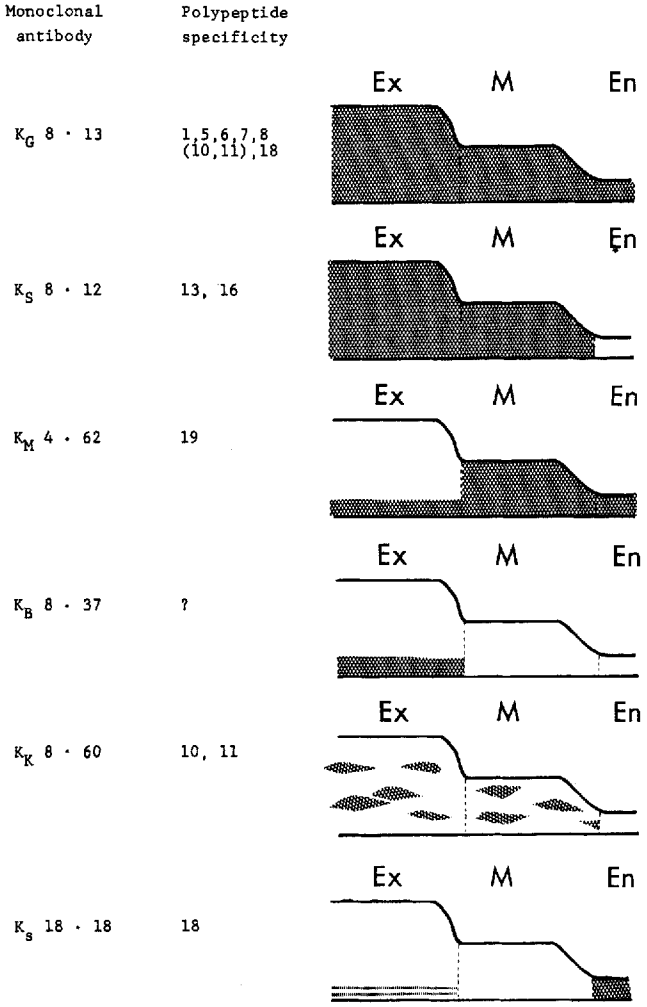

Fig. 10. Schematic diagram showing the different patterns of staining produced by the monoclonal antibodies used in this study. The different regions of the cervix, exocervix (*Ex*), metaplasia (*M*), and endocervix (*En*) are marked, and positive reactivity is indicated by the *shaded areas*. The *partial shading* of basal cells with antibody K_s 18.18 represents the faint occasional labeling obtained with this antibody. The polypeptide specificities refer to the nomenclature of Moll et al. [33, 40]

of the metaplasia (Fig. 6b). Results identical to those obtained with TZM metaplasia were also obtained with cases of MP metaplasia (type b), in which the squamous metaplasia was situated at different sites along the cervical canal and its invaginations (data not shown). The information obtained from these observations indicated that metaplastic cells do not express the same combination of cytokeratin polypeptides as any particular cell type of the normal cervix. Metaplastic cells were positively labeled with antibody K_M 4.62 but were negative or nearly negative for K_s 18.18, unlike the normal mucosa which was positive for both antibodies. In contrast to the basal layer of the exocervix, the metaplastic cells were not labeled with antibody K_B 8.37. In being uniformly positively labeled with antibody K_S 8.12, metaplastic cells differed from the columnar and reserve cells of the endocervix, both of which were not labeled with this antibody (see Discussion).

Another form of metaplasia (type c) involved a more mature type of TZM. This metaplasia retained some order of layers, contained prominent basal cells, and was generally similar to the neighboring normal exocervix. It could, however, be distinguished from the normal exocervix both by its anatomical location and by its less ordered stratification (Fig. 7a). The staining patterns of this metaplasia with the battery of cytokeratin antibodies used was different from that of the 'common' forms of metaplasia described above (types a and b); while all were positively labeled with antibodies K_G 8.13 and K_S 8.12 (Fig. 7b, d), type c was only partly positive with K_M 4.62 (Fig. 6c), and its basal cells were positively stained by antibodies K_B 8.37 (Fig. 6e) and K_s 18.18 (not shown). This is in contrast with the common forms of metaplasia which were uniformly positive with antibody K_M 4.62, and negative with K_B 8.37. Antibody K_K 8.60 labeled individual cells throughout the metaplasia in all three forms (Fig. 7f).

Finally, we examined the transformation of malignant

epithelia, rather than normal simple epithelia, into stratified squamous epithelium. Figure 8a shows a glandular region within a carcinosarcoma of the endometrium exhibiting distinct foci of squamous metaplasia. Immunofluorescence labeling of this tissue with the cytokeratin-specific antibodies (Fig. 8b–f) produced exactly the same staining pattern obtained in the TZM and MP of the endocervix described above.

Immunoblotting analysis of cytokeratin polypeptides present in metaplastic cervical tissues

Immunoblotting analysis of microdissected, high-salt-extracted tissue sampes revealed the major groups of cytokeratin polypeptides present in this metaplastic tissue. The major groups of polypeptides detached were bands corresponding to polypeptide no. 5, which has previously been detected in this tissue [35], as well as to polypeptide no. 10/11. A second group was found near polypeptide no. 13, and a few smaller polypeptides with molecular masses of 40–46 kilodaltons were barely detectable. Immunoblotting analysis of this sample was carried out in order to determine which of the polypeptides recognized by antibodies K_S 8.12 and K_K 8.60 was actually present in the metaplasia. The results (Fig. 9) showed that antibody K_S 8.12 predominantly recognized polypeptide no. 13 and only small amounts of no. 16 (both of which react with this particular antibody). Antibody K_K 8.60, on the other hand, was bound to a polypeptide doublet corresponding to cytokeratins nos. 10 and 11 (present in essentially equal amounts).

Discussion

The present study focused on a relatively common type of 'transdifferentiation' event which occurs in the human cervix, i.e., the development of squamous metaplasia. The major tool used for studying the nature of the metaplastic process was the immunocytochemical and biochemical identification of the cytokeratin polypeptides expressed by normal cells of the cervix and their metaplastic derivatives. As pointed out in the Introduction, the expression of cytokeratins in different epithelia has proved to be a most useful marker both of the histogenetic origin of cells as well as of their state of differentiation [47, 50]. In previous studies carried out in several laboratories, the various cytokeratins expressed in normal and pathological specimens of the human female genital tract have been identified [10, 30, 35, 39]. In accordance with the present findings, these studies have indicated the widespread occurrence of the 40-kilodalton cytokeratin (no. 19) in the endocervix, metaplastic cells, and the basal layer of the exocervix, as well as the presence of cornifying foci in normal exocervix and in squamous metaplasia.

In the present study, we applied a battery of monoclonal antibodies with restricted and defined polypeptide specificities. Staining of normal and metaplastic cervical tissue with these antibodies revealed several interesting features relating to the process of squamous differentiation in general and to the formation of squamous cervical metaplasia in particular.

A striking property of squamous metaplasia was revealed by the occurrence of a cytokeratin-polypeptide combination which is markedly different from that found in the epithelial components of the normal cervix. This is schematically illustrated in Fig. 10, which shows the labeling patterns obtained in the exocervix, in metaplasia, and in the endocervix using our six monoclonal antibodies. The marked differences between the metaplastic cells and the normal cervical components indicate that, regardless of the nature of the cellular precursor of the metaplasia, the pattern of cytokeratin expression in cells changes during metaplastic transformation. Thus, the metaplastic lesions exhibit a largely nonpolar expression of cytokeratins, this being in contrast to the exocervix; the basal cell layer of the exocervix was positively labeled with antibodies K_B 8.37 and K_M 4.62, whereas the metaplasia was uniformly negative with the former and uniformly positive with the latter (Fig. 6e). The only suggestion of a limited degree of differential expression of certain keratins in distinct regions of the metaplasia was the faint, often barely discernible labeling of its basal cell layer with antibody K_s 18.18 (Fig. 6b), and the sporadic labeling with K_K 8.60 (Fig. 6d). Comparison of the metaplasia with cells of the normal endocervix revealed remarkable differences, the most conspicuous of which was the expression in the metaplasia of cytokeratin no. 13 and its apparently diminished expression of cytokeratin no. 18, which is abundant in normal endocervical mucosa. From these findings, we propose that the formation of squamous metaplasia represents a new route of differentiation which differs from those detected in the various epithelial elements of the normal cervix. The cells which are induced to undergo metaplastic squamous differentiation probably reside in the endocervix. This hypothesis is based on anatomical considerations and was corroborated by the results of antibody labeling. Our study of a large number of cases indicated that metaplastic lesions with similar morphologies and identical cytokeratin patterns may develop at a distance from the squamocolumnar junction and may even be detected within adenocarcinomas, thus excluding the possibility that squamous metaplasia may be formed by a lateral migration of the exocervix. However, we cannot at present exclude the possibility that the latter process is responsible for the formation of the 'mature' squamous metaplasia of the transformation zone. This mature form of metaplasia may develop either by further differentiation of the 'common' form of metaplasia or by displacement of the exocervical epithelium.

Findings for human cervical metaplasia in combination with the results presented here suggest that reserve cells may be at least bipotent. They may normally terminally differentiate into columnar, mucous-secreting cells, but under certain circumstances, they may adopt a stratification pattern of differentiation and form metaplasia [12, 38]. This view is corroborated by the finding of apparently intermediate stages in the development of metaplasia, i.e., reserve cell hyperplasia (Figs. 1d, 4f). On the basis of the results of antibody labeling and immunoblotting following gel-electrophoresis analyses, it is further proposed that, throughout the stratification process, new cytokeratins, including polypeptides nos. 5 and 13, small amounts of nos. 16 and 17, and finally, nos. 10 and 11, are gradually co-expressed. These polypeptides appear in metaplasia along with three (nos. 7, 8, and 19) of the four cytokeratins initially present in the endocervical mucosa. The expression of cytokeratin polypeptide no. 18 decreases during the course of the metaplastic process. Interestingly, the paired polypeptides, nos. 5 and 13, are co-expressed in metaplastic cells; studies in several laboratories have indicated that

there are cytokeratin polypeptides pairs that are commonly co-expressed in a differentiation-restricted fashion [50]. The positive staining of groups of metaplastic cells by antibody K_K 8.60 suggests that, following stratification, another step towards keratinization may occur, this being manifested by the appearance of cytokeratins nos. 10 and 11, which are commonly found in keratinizing squamous epithelia (see [24, 47, 50]). At least some of these polypeptides (i.e., nos. 5, 7, 8, 17, 18, and 19) have also been detected using two-dimensional gel electrophoresis [35]. However, the use of various monoclonal antibodies, particularly K_S 8.12, K_K 8.60, and K_M 4.62, revealed unequivocally the presence of the stratification-specific polypeptides nos. 13 and 16, polypeptides nos. 10/11, and polypeptide no. 19, respectively.

Another aspect highlighted by our findings involves the relationships between squamous cervical metaplasia and neoplastic lesions of the cervix. These will be discussed at two levels: first, the capacity of squamous metaplasia to transform into a neoplastic lesion, i.e., squamous cell carcinoma, and second, the capacity of malignant simple epithelia (i.e., adenocarcinoma) rather than normal simple epithelia to undergo squamous differentiation.

The possibility that squamous metaplasia may constitute a preneoplastic site which is prone to malignant transformation is supported by clinical and histopathological data [7, 44]. The suggestion of a common origin for squamous metaplasia and neoplasia is corroborated by the present findings as well as by the results of recent studies concerning the expression of cytokeratins in squamous cell carcinomas and adenocarcinomas (unpublished results). Most prominent in this respect is the positive labeling of both lesions with antibody K_S 8.12 and their common expression of the 40-kilodalton (no. 19) polypeptide [33] which is stained by antibody K_M 4.62 [21]. This is also in line with a previous report of diminished levels of cytokeratin no. 18 in nonkeratinizing squamous cell carcinoma of the cervix [35]. It is thus concluded that metaplasia and neoplasia may exhibit a similar pattern of differentiation despite the marked difference in their proliferative and invasive properties.

The other aspect, which can only be briefly dealt with here, concerns the development of squamous metaplasia within adenocarcinoma. The existence of adenocarcinomas with regions exhibiting squamous differentiation has been well established by conventional histopathology [26]. The data presented in our study support the view that such metaplastic cells express polypeptides largely similar to those of 'conventional' squamous metaplasias derived from normal, nonneoplastic epithelium. This is mainly shown by the persistent expression of cytokeratin no. 19 and the positive labeling with antibody K_S 8.12. We observed variable degrees of labeling with antibodies to polypeptide no. 18, although we still do not know whether this should be attributed to the fact that the tissue of origin studied here was derived from the endometrium rather than the endocervix or to the fact that is was malignant. This aspect is now under investigation. It might, however, be concluded that virtually the same changes in cytokeratin expression occur during metaplastic transformation regardless of whether the epithelium of origin is normal or neoplastic.

In conclusion, the present study shed light on basic processes of squamous differentiation from its early stages, characterized by the hyperplastic growth of reserve cells through stratification, to the development of focal keratinizing centers, as well as on the molecular relationships between squamous metaplasia and neoplastic transformation of the cervix.

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