LITERATURE CITED

1. N. G. Blokhina, V. A. Agafonov, S. N. Bykovskaya, et al., Ter. Arkh., No. 12, 125 (1984).
2. S. N. Bykovskaya, N. G. Blokhina, E. R. Vasileva, et al., Byull. Éksp. Biol. Med., No. 3, 38 (1983).
3. S. N. Bykovskaya, M. S. Iobadze, T. A. Kupriyanova, et al., Byull. Éksp. Biol. Med., No. 1, 86 (1987).
4. M. O. Raushenbakh, G. V. Kruglova, A. V. Sergeev, et al., Abstracts of Proceedings of the 9th International Symposium on the Comparative Study of Leukemia and Related Diseases [in Russian], Sukhumi (1979), p. 150.
5. B. Bonavida, J. Exp. Med., 145, 293 (1977).
6. A. Boyum, Scand. J. Clin. Lab. Invest., 21, 77 (1968).
7. V. Diehl, H. H. Kirchner, H. Burrichter, et al., Cancer Treat. Rep., 66, 615 (1982).
8. F. Healy and R. C. Rees, Cancer (Philadelphia), 21, 775 (1985).
9. S. Levy and F. Reins, Nouv. Rev. Francs. Hematol., 28, 323 (1986).
10. M. Julius and E. Simpson, Eur. J. Immunol., 3, 645 (1973).

IMMUNOHISTOCHEMICAL IDENTIFICATION OF RESERVE CELLS OF THE ENDOCERVICAL CANAL BY MONOCLONAL ANTIBODIES EE_{21-06d}

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UDC 612.627.017.1.083.3

KEY WORDS: immunohistochemistry; monoclonal antibodies; reserve cells; cervix uteri

The mucous membrane of the cervical canal is lined with cylindrical epithelium, beneath which, on the basement membrane, may lie reserve (or cambial) cells, capable of differentiating into either glandular or stratified squamous epithelium [1, 2]. Although the epithelial nature of the reserve cells can be taken as proven, the precise mechanism of their formation is not known [8]. In recent years research into proteins of the cytoskeleton of epithelial cells (cytokeratins, prekeratins) has been conducted on an extensive scale in biology and medicine, with the aid of monoclonal antibodies (McAb). After the compiling of a catalogue of cytokeratins, in which 19 polypeptides have been distinguished [7], it was shown that cells of different epithelia differ from one another in the set of expressed cytokeratins [3, 7]. Previously prekeratins Nos. 7, 8, 18, and 19, characteristic of simple epithelium, and also cytokeratins of squamous-cell type Nos. 5 and 17 were found in the reserve cells. Cytokeratins Nos. 7, 8, 18, and 19 are present in the cylindrical cells of the cervical mucous membrane, but prekeratins Nos. 1, 2, 4, 10, 13, 14, 15 of squamous-cell type are not presented [3, 7, 8].

The aim of this investigation was to detect reserve cells with the aid of new McAb against a complex of cytokeratin polypeptides, which are markers of squamous-cell differentiation. For this purpose we used original McAb EE_{21-06d} (IgG_1k), obtained by G. Serre by the hybridoma method after immunization of mice with extract of human callus. These antibodies react with five cytokeratin polypeptides Nos. 1, 2, 9, 10, and 11, characteristic of stratified squamous epithelium [7].

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(Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 108, No. 11, pp. 603-606, November, 1989. Original article submitted January 18, 1989.

0007-4888/89/0011-1627$12.50

TABLE 1. Binding of Cytokeratin McAb with Endocervical Cells (after Weikel [8] with additions)

McAb	Endocervix	
	Cylindrical cells	Reserve cells
KG 8.13 (wide spectrum)	+	+
A53-B (to cytokeratin No. 19)	+	+
KA 1 (in prekeratins of squamous epithelium)	+—	+
Kg 8.1 (to cytokeratin No. 8)	+	+—
Monospecific guinea pig antibodies (after [4])	+—	+
McAb EE_{21-06d}	—	+

Legend. +) Weak reaction in not all cases.

Table 1 shows that only McAb EE_{21-06d} stain the reserve cells without staining cylindrical epithelial cells. McAb KA1 [8] and polyclonal guines pig antibodies against bovine callus [4] are evidently the closest in similarity to our own antibodies with respect to specificity of action, but they possess the property of staining cylindrical cells of the mucosa of the cervical canal weakly. Incidentally, in hyperplasia of the reserve cells their persistent staining is preserved, so that McAb EE_{21-06d} can be used to investigate the reserve cells of the cervical canal and also for the diagnosis of squamous-cell metaplasia and metaplastic carcinoma of the cervical mucous membrane.

EXPERIMENTAL METHOD

Tissue was taken from the mucous membrane of the endocervical canal of eight women aged from 32 to 65 years during biopsy or during hysterectomy, and frozen with liquid nitrogen. Frozen sections were incubated with McAb, after which FITC-labeled commercial goat serum against mouse immunoglobulins, in a dilution of 1:100 (from "Sigma"), was used as the second antibodies. Sections were mounted in buffered glycerol solution.

EXPERIMENTAL RESULTS

Single reserve cells, difficult to identify, were sometimes found in preparations of the cervical mucous membrane (Fig. 1a).

In all observations McAb EE_{21-06d} reacted immunohistochemically in the endocervix only with reserve cells, and were found in their cytoplasm. Stromal cells did not react with these antibodies in any single case. The intensity of luminescence of the cytoplasm of the reserve cells was always sufficiently high, regardless of their number in the section (Fig. 1b, c, d), whereas in the cylindrical cells the reaction was negative (Fig. 1b, c, d). Brightly luminescent reserve cells were most frequently arranged in a single row, in the form of a continuous chain (Fig. 1c, d), but sometimes a definite distance could be seen between them (Fig. 2a). In areas of reserve-cell hyperplasia, the staining properties of McAb EE_{21-06d} were the same as in places where the reserve cells were arranged in a single line (Fig. 2a, b). Sometimes reserve cells or foci of their proliferation were located deep in the endocervical canal, a long way from the transitional zone of the exoendocervix.

We studied expression of cytokeratin polypeptides in the reserve cells of the cervical canal with the aid of antibodies against five prekeratins, markers of squamous-cell differentiation. We showed that McAb EE_{21-06d} detect both single reserve cells and foci of their proliferation highly constantly, without staining cylindrical cells. Our results were com-

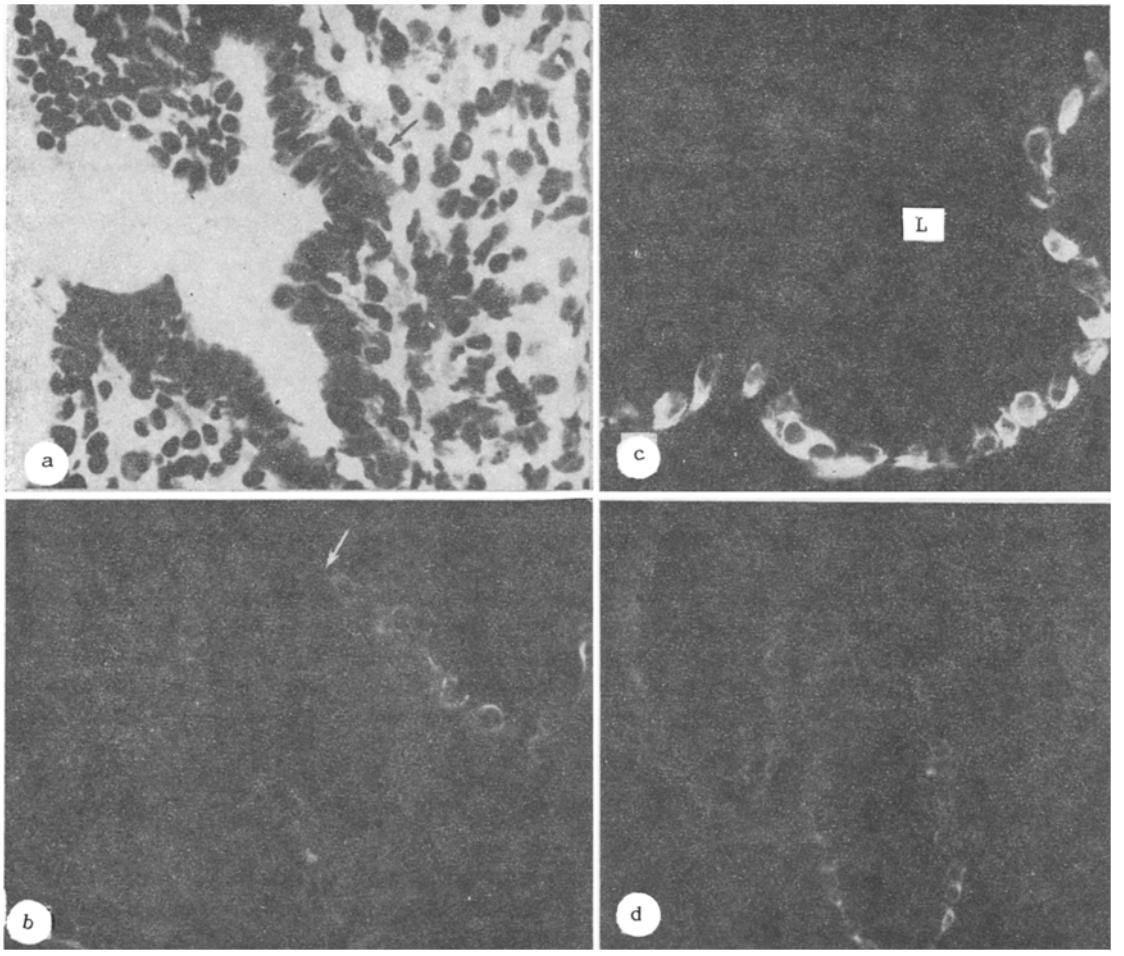

Fig. 1. Endocervical crypts with reserve cells. a) Reserve cells (arrows) beneath cylindrical epithelium can be identified with difficulty; b, c) reserve cells arranged in one row; d) fluorescence of reserve cells around crypts of endocervix, outlines of cylindrical cells can be seen. a) Stained with hematoxylin and eosin; b, c, d) immunofluorescence study with McAb EE_{21-06d}. L) Lumen of endocervical crypt; magnification: a) 330; b, d) 300; c) 400.

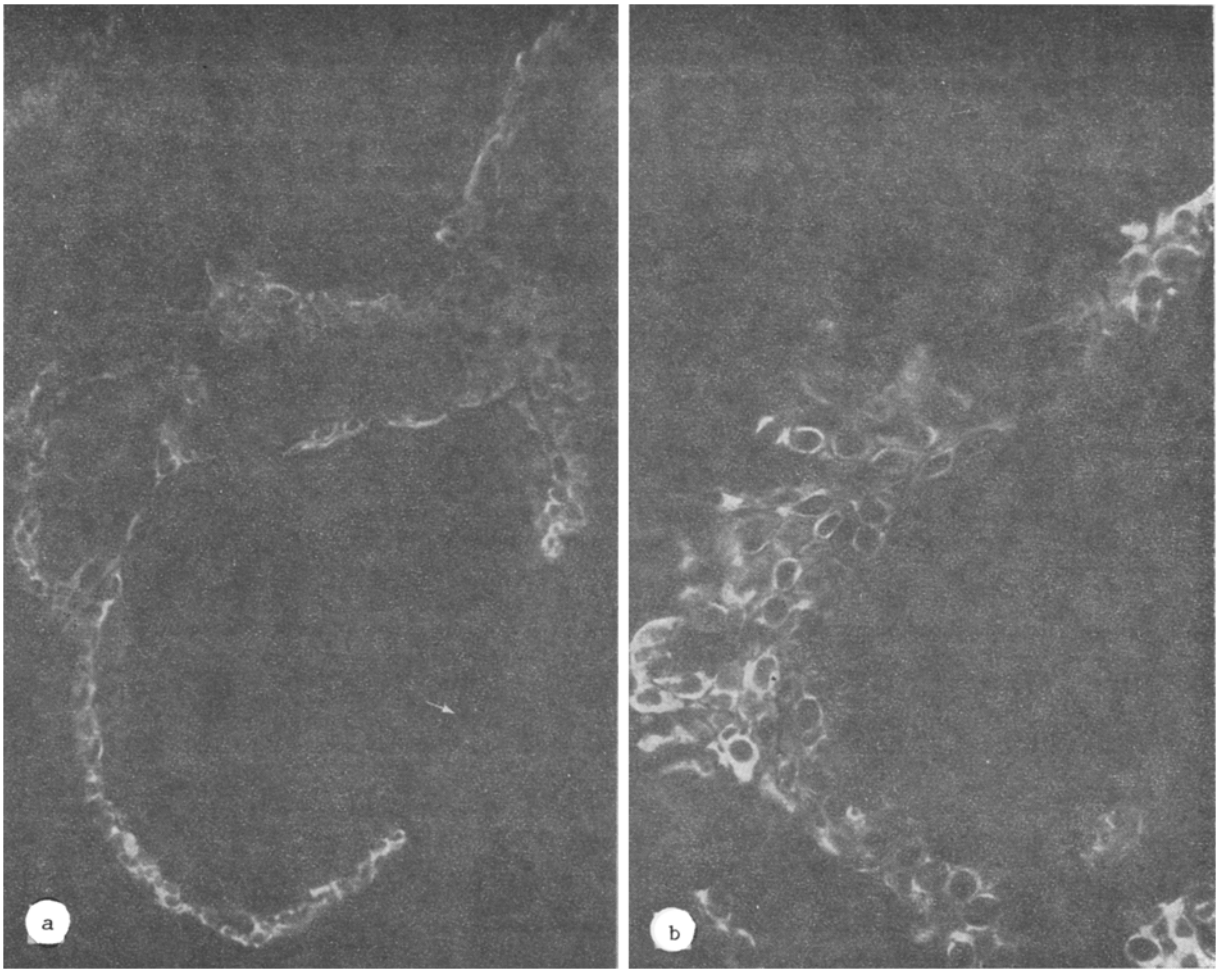

Fig. 2. Immunofluorescence detection of areas of hyperplasia of reserve cells. a) Reserve cells in crypt form a broken line (arrow). Focal concentration of reserve cells seen below; b) focus of hyperplasia of reserve cells in region of a cervical crypt. Magnification: a) 300; b) 450.

parable with those of investigations [5, 8] in which McAb with established specificity for particular types of cytokeratins were used to identify reserve cells (Table 1).

LITERATURE CITED

1. J. A. de Brux and J. Dupré-Froment, Acta Cytol., 5, No. 4, 265 (1961).
2. R. Carmichael and B. L. Jeafferson, J. Path. Bact., 49, 63 (1939).
3. D. Cooper, A. Schermer, and T.-T. Sun, Lab. Invest., 52, 243 (1985).
4. W. W. Franke, B. Appelhans, E. Schmid, et al., Differentiation, 15, 7 (1979).
5. O. Gigi-Leitner, B. Geiger, and B. Czernobilsky, Differentiation, 31, 191 (1986).
6. W. D. Lawrence and H. M. Shingleton, Am. J. Obstet. Gynecol., 137, 661 (1980).
7. R. Moll, M. Franke, D. Schiller, et al., Cell, 31, 11 (1982).
8. W. Weikel, R. Wagner, and R. Moll, Virch. Arch. Abt. B. Zellpathol., 54, No. 2, 98 (1987).