Virchows Archiv A Pathol Anat (1992) 421:315–322

Virchows Archiv A
Pathological Anatomy and Histopathology

An immunohistochemical study of the breast using antibodies to basal and luminal keratins, alpha-smooth muscle actin, vimentin, collagen IV and laminin

Part I: normal breast and benign proliferative lesions

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Received November 9, 1991 / Received after revision May 14, 1992 / Accepted May 15, 1992

Summary. The distribution of simple epithelial (K8/18/19) and basal (myoepithelial) (K5/14) keratins, α-smooth-muscle actin, vimentin, collagen IV and laminin in normal mammary glands and in benign proliferative lesions was studied using monoclonal antibodies (mAbs). These antibodies (Abs) identified myoepithelial cells and luminal cells specifically. In lesions with adenosis and papillomas, the two-layered formation resembled that of normal glands with a purely myoepithelial-epithelial differentiation. In scleradenotic lesions, the main cell was of myoepithelial immunophenotype with intermixed trabecular-tubular proliferations of simple-type epithelium. The sclerosis seems to be the result of an irregular basal lamina synthesis by the myoepithelial cells. In contrast to these lesions, epitheliosis represents a purely intraluminal cell proliferation of clearly simple epithelial immunophenotype and of cells with a basal keratin phenotype, lacking myoepithelial differentiation antigen actin. The basal keratin type epithelium may represent post-stem or intermediate cells developing into luminal epithelium. Epitheliosis appears to be a purely epithelial hyperplasia with striking similarity to the regeneration of normal breast epithelium. The different proliferative patterns may give an explanation for differences in potential cancer risks of patients with these lesions.

Key words: Hyperplastic breast lesions – Anti-keratin antibody – Anti-smooth muscle actin antibody – Anti-vimentin antibody – Anti-collagen IV antibody – Immunohistology

Dedicated to Prof. Dr. G. Seifert on the occasion of his 70th birthday

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Introduction

Benign proliferative mammary lesions show a wide range of reactions with an unusual morphological diversity (Azzopardi 1979; Bässler 1978; Fechner and Mills 1990; Millis 1984). Basically, these may be divided into lesions with no increased cancer risk (adenosis, scleradenosis, solitary duct papilloma, adenoma of the nipple) and those with a statistically slightly increased cancer risk (epitheliosis, multiple papillomas) (Dupont et al. 1980; Haagensen et al. 1981; Page et al. 1978; Stegner 1975). In recent publications we described the immunohistochemical reaction pattern of benign proliferative breast lesions, using mAbs against simple epithelial keratins 8/14/15/16/18/19 and against basal myoepithelial keratins 5/14 (Böcker et al. 1986; Jarasch et al. 1988; Nagle et al. 1986). From these studies we concluded that benign proliferative lesions are essentially epithelial-myoepithelial in nature, including epitheliosis as defined by Azzopardi (1979).

Recently, several immunohistochemical investigations have been performed on normal breast and on benign and malignant lesions, focusing on further keratins (Altmannsberger et al. 1981; Ash et al. 1981; Franke et al. 1980; Krepler et al. 1981; Moll et al. 1982; Nathrath et al. 1982; Ramaekers et al. 1983; Van Muijen et al. 1984; Wetzels et al. 1991). Furthermore, antigens such as the glial fibrillary acidic protein (GFAP), vimentin, alpha-smooth muscle specific actin (α-sm actin), S-100, common acute lymphoblastic leukaemia antigen (CALLA), laminin and collagen IV have been studied (Bussolati 1980; Bussolati et al. 1980; Charpin et al. 1986; Gould et al. 1990; Gusterson et al. 1982, 1986; Lissitsky et al. 1988; Siegal et al. 1981; Walther et al. 1986; Willebrand et al. 1986). Generally, these studies confirm differences in antigen expression of keratins in proliferating cells of epitheliosis and intraductal carcinoma. These dif-

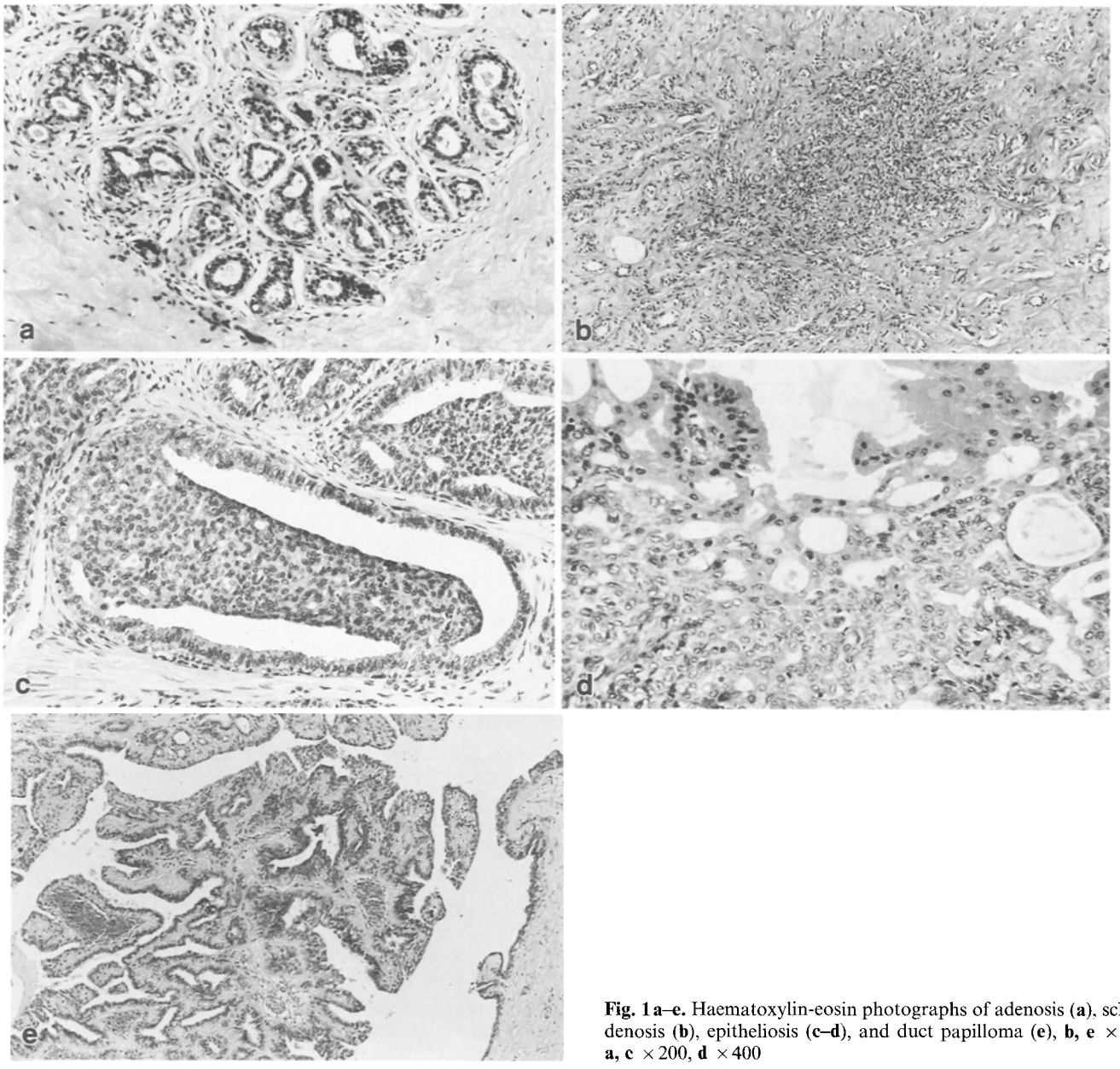


Fig. 1 a–e. Haematoxylin-eosin photographs of adenosis (**a**), scleradenosis (**b**), epitheliosis (**c–d**), and duct papilloma (**e**), **b, e** ×100, **a, c** ×200, **d** ×400

ferent immunophenotypes may reflect biologically distinct forms of proliferation (Gould et al. 1990; Jarasch et al. 1988; Raju et al. 1990). In the present study we have examined the normal mammary gland and diverse benign proliferative lesions with an extended panel of antibodies. Our findings suggest two different immunophenotypes of benign proliferative lesions; one with a distinct myoepithelial-epithelial immunophenotype including adenosis, papillomas, scleradenosis and adenoma of the nipple, and the other with a heterogeneous epithelial immunophenotype including epitheliosis. Immunohistologically, these lesions consisted of two different subsets of epithelial cells. Thus, these results may explain the distinct clinical differences in relation to cancer risk in the two groups.

Materials and methods

Tissue was obtained from biopsies and lumpectomies of 102 patients. Upon surgical removal, samples were frozen in liquid nitrogen and stored at −70° C. In all instances, histological slides from paraffin-embedded material were critically reviewed and classified according to the criteria of Azzopardi (1979), Carter (1990), Haagensen et al. (1981) and Page and Anderson (1987) (Table 1; see also Fig. 1).

Primary Abs used are listed in Table 2. The secondary Ab used were fluorescein-isothyocyanate (FITC)-coupled or rhodamine (tetramethylrhodamine-isothiocyanate)-coupled Abs (Dianova, Hamburg, FRG) raised to either mouse or rabbit IgG (Table 2).

Immunofluorescence microscopy was performed on 5 μm thick cryostat sections that had been air-dried and fixed in acetone at −20° C for 10 min as described (Moll et al. 1982). For double-label immunofluorescence microscopy, both primary Abs were applied

Table 1. Samples of normal breast tissues and of various benign proliferative lesions (185 samples from 102 patients)

Diagnosis	*n*
Normal breast	40
Adenosis	50
Scleradenosis	42
Solitary duct papilloma	7
Adenoma of the nipple	3
Epitheliosis	37
Radial scar	6
Total	185

simultaneously, as were the secondary Abs. In addition, occasional sections were stained using the alkaline phosphatase anti-alkaline phosphatase method (Cordell et al. 1984).

Results

On frozen sections of normal breast tissue the luminal epithelium of ducts and lobules was stained distinctly and consistently with K8/18 TPA-Abs and K19 mAb KA 4. K5/14 mAb KA1 reacted strongly with the basal myoepithelial cell (Fig. 2a). In addition to the K5/14 mAb KA1-positive myoepithelial cells, there were also cells in luminal portions in both ducts and in lobules revealing a bright K5/14 mAb KA1-positive reaction (Fig. 2b, see also Figs. 3a, b and 6a, b). These cells did not reacted with actin and K8/18 TPA. In contrast, α-sm actin was found only in basal myoepithelial cells (Fig. 2c). Vimentin was found in myoepithelial cells, although these cells displayed a much weaker reaction than the stromal cells of the adjacent lobule with variable interlobular staining intensity (Fig. 2d). The whole parenchymal tree was enmeshed by a collagen IV (laminin)-positive continuous basal membrane (Fig. 2e).

Biopsy specimens of more than 50 patients have been studied, displaying the typical histological appearance of adenosis, scleradenosis and duct papilloma as described by Azzopardi (1979). Adenosis and duct papilloma disclosed the same immunophenotypic appearance as the normal gland.

In adenotic lesions, nearly all of the luminal cells reacted with the keratin 8/18 Abs TPA and mAb KA4 (Fig. 3a). The basal myoepithelial cell layer was decorated with K5/14 mAb KA1. There were also intensively stained K5/14 KA1-positive cells in the luminal layer which did not react with K8/18/19 mAb KA4 (Fig. 3b). Occasionally even all acinar cells in luminal portions were stained with K5/14 mAb KA1. Myoepithelial cells showed an extensive staining pattern with α-sm actin Abs (Fig. 3c). The vimentin reaction in myoepithelial cells was variable and much weaker than in the surrounding stromal cells (Fig. 3d). Collagen IV mAb showed a broad basal lamina around the glands (not shown in Figs). Radial scars exhibited a similar immunophenotypical reaction pattern as adenosis.

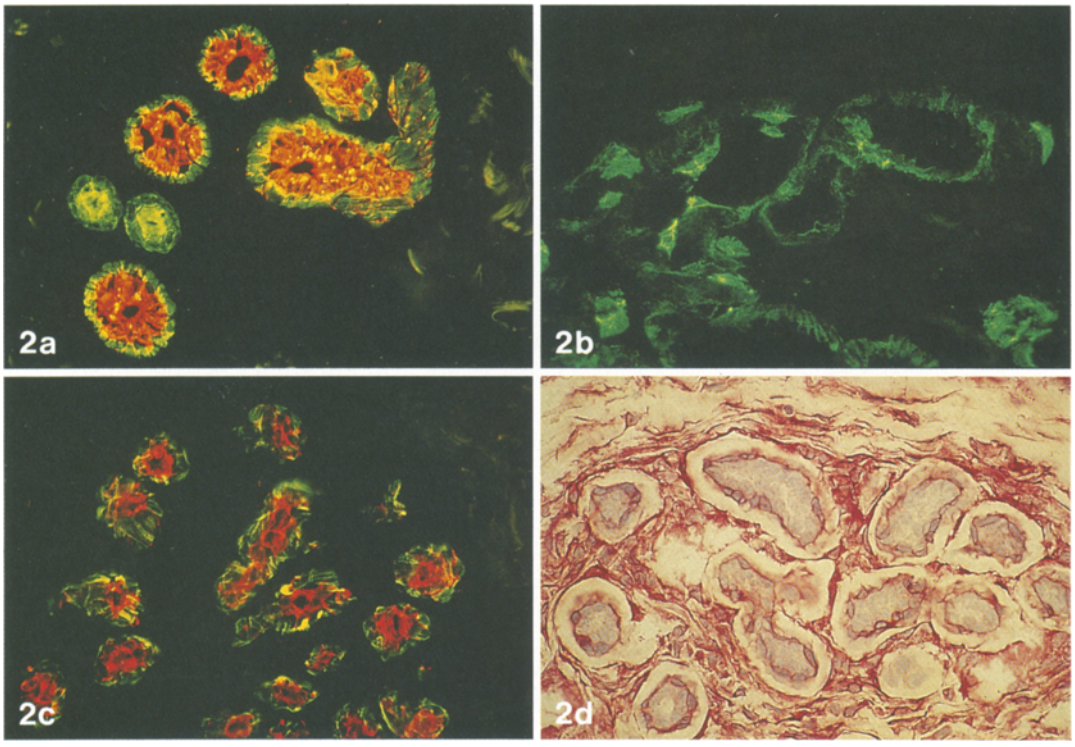
Papillomas differed from adenosis only by showing an infolding into "ductal" lumina. At its base a collagen IV positive basal membrane was developed, as was demonstrated with collagen IV mAb (Fig. 4a). With K5/14 mAb KA1 occasional luminal cells were also be found to react in addition to a very intensive myoepithelial staining pattern (Fig. 4b). A similar intense staining reaction of only these basal cells was obtained with α-sm actin mAb (not shown in Figs). Most luminal cells stained intensively with K14, 15, 16, 19 mAb KA4 and the polyclonal K8/18 TPA (Fig. 4b).

Scleradenotic lesions were characterized by a pronounced and rather disorderly myoepithelial proliferation, expressing the myoepithelial markers actin (HHF-35), K5/14 (KA1) and vimentin (V9) as shown in Fig. 5a. Within the myoepithelial proliferations there were irregular tubular or trabecular ducts, originating from larger ducts, showing a staining with simple epithelial keratin 8/18/19 Abs (TPA, KA4) (Fig. 5a). The myoepithelial components were found to be enmeshed in convoluted collagen IV/laminin-positive basal membranes (Fig. 5a, b). In one case an almost purely myoepithelial proliferation was found (not shown in Figs).

Table 2. Primary immunohistochemical reagents

Antigens recognized	Antibody	Source	Dilution	Reference
K5/14	KA1	Dr. R.B. Nagle	1:2000	Nagle et al. 1986)
K14, 15, 16, 19[a]	KA4	Dr. R.B. Nagle	1:2000	Nagle et al. 1986)
K8, 18	TPA	AB Sangtec Medical Co., Bromma, Sweden (rabbit polyclonal antibody)	1:10	Björklund (1984)
Vimentin	V9	Boehringer, Mannheim, FRG	1:80	Osborn et al. (1984)
α-sm Actin	HHF-35	Boehringer, Mannheim, FRG	1:4	Tsukada et al. (1987)
Collagen IV		Dianova, Hamburg, FRG	1:80	Zuk et al. (1989)
Laminin		Dianova, Hamburg, FRG	1:100	Lissitsky et al. (1988)

[a] KA4 antibody reacted preferentially with keratin 19



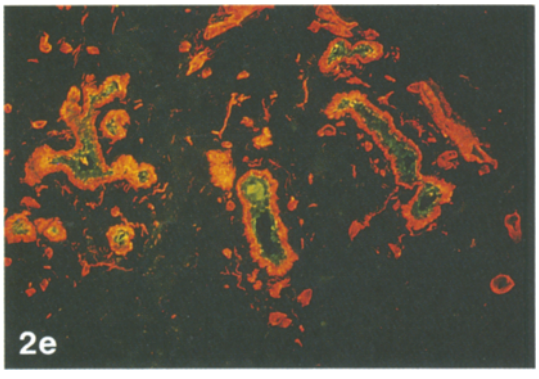


Fig. 2a–e. *Normal breast lobules:* **a** Lobule, double immunostained with keratin mAb KA1 (FITC) and keratin TPA (rhodamine). Myoepithelial cells are clearly and extensively stained with "basal keratin" mAb KA1 and the luminal cells with TPA Ab, (×639). **b** Lobule, immunostained with keratin mAb KA1. Note the strong reaction of occasional luminal cells in addition to myoepithelial cells. The luminal cells show a somewhat brighter reaction than the myoepithelial cells, (×630). **c** Lobule, double immunostained with α-sm actin mAb (FITC) and keratin TPA Ab (rhodamine). There is a similar reaction pattern as in **Fig. 1a**, (×200). **d** Lobule, immunostained with vimentin mAb V9. Stromal cells in the lobular tissue react very strongly. There is however a clear decoration of myoepithelial cells, (×400). **e** Lobule, immunostained with collagen IV mAb, showing a decoration of the basal lamina of acini and of capillaries, (×200)

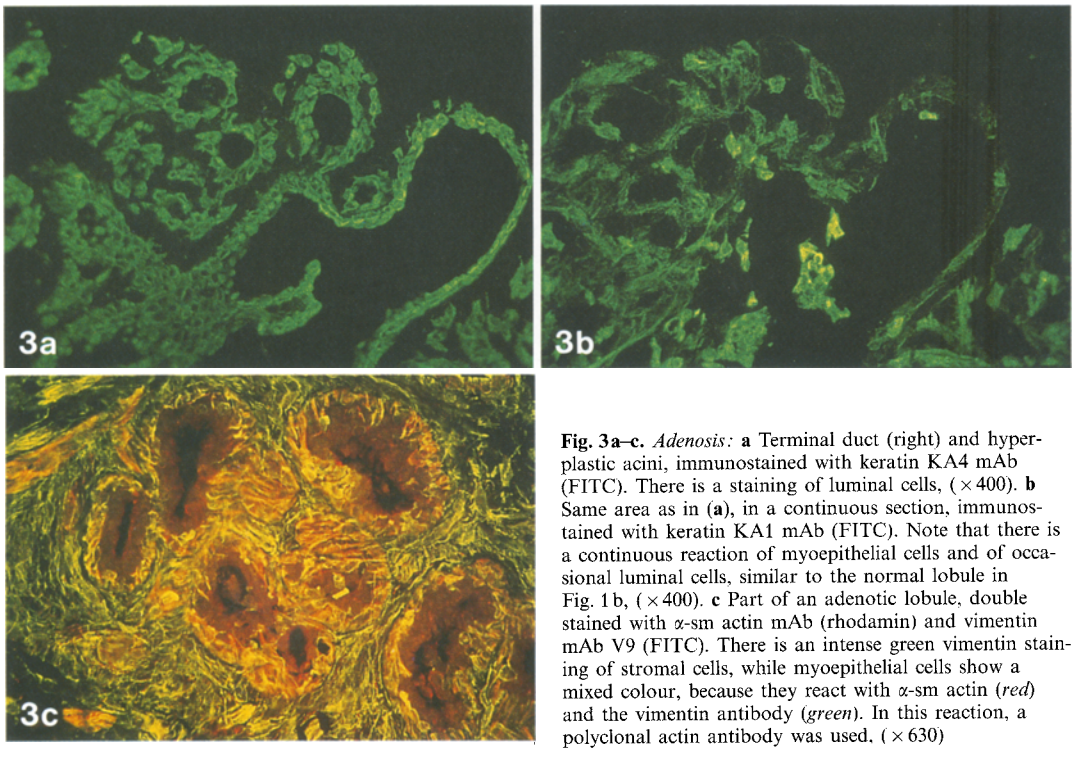


Fig. 3a–c. *Adenosis:* **a** Terminal duct (right) and hyperplastic acini, immunostained with keratin KA4 mAb (FITC). There is a staining of luminal cells, (×400). **b** Same area as in (**a**), in a continuous section, immunostained with keratin KA1 mAb (FITC). Note that there is a continuous reaction of myoepithelial cells and of occasional luminal cells, similar to the normal lobule in Fig. 1b, (×400). **c** Part of an adenotic lobule, double stained with α-sm actin mAb (rhodamin) and vimentin mAb V9 (FITC). There is an intense green vimentin staining of stromal cells, while myoepithelial cells show a mixed colour, because they react with α-sm actin (*red*) and the vimentin antibody (*green*). In this reaction, a polyclonal actin antibody was used, (×630)

In addition to the expression of actin, most intertubular spindle cells showed a reaction with K5/14 mAb KA1.

The epitheliotic lesions showed some basic qualitative differences when compared with the lesions described above. Epitheliotic intraluminal proliferation displayed an intense K5/14 mAb KA1 and K19 mAb KA4 positivity in the same lesions with a preference of one group of keratins in each cell (Fig. 6a, b). In addition, there was occasional staining of intraluminal cells with vimentin mAb (Fig. 6c). The intraluminal K5/14 KA1-positive cells did not react with α-sm actin mAb HHF-35 as did the myoepithelial cells at the periphery of these lesions (Fig. 6d). There was a continuous peripheral myoepithelial reaction with mAb HHF-35 which was similar to that seen in ductal carcinoma in situ (not shown in Figs). These myoepithelial cells reacted also with K5/14 mAb KA1, but weaker than the K5/14 KA1-positive intraluminal cells (Fig. 6b).

All three cases of nipple adenoma showed a complex immunophenotype consisting of elements of papillomas, adenosis scleradenosis and epitheliosis.

Discussion

The normal mammary gland shows a dual differentiation of ducts and lobular acini. There are luminal epithelial cells expressing simple cytokeratins 8, 18, 19 shown in this study by K19 mAb KA4 and K8/18 TPA decoration and a myoepithelial cell type showing a triple expression of keratins 5/14 (mAb KA1), α-sm-actin (HHF 35) and vimentin (V9) (Böcker et al. 1986; Bussolati et al. 1980; Jarasch et al. 1988; Nagle et al. 1986; Russo and Russo 1988). Vimentin, GFAP, CALLA, myosin and S-100 have so far rarely been described in myoepithelial cells (Gould et al. 1990; Gusterson 1986; Raju et al. 1990; Viale et al. 1991). Of all these myoepithelial antigens α-sm-actin seems to be the most consistent and diagnostically reliable market, whereas vimentin, GFAP and even K5/14 are variably present in a subset of myoepithelial cells (Gould et al. 1990; Nagle et al. 1986; Purkis et al. 1990; Viale et al. 1991). Purkis et al. (1990) recently found that the antigenic determinants of keratin 5 (MW 56,000) and keratin 14 (MW 50,000) of myoepithelial cells are shared with basal keratinocytes of the skin.

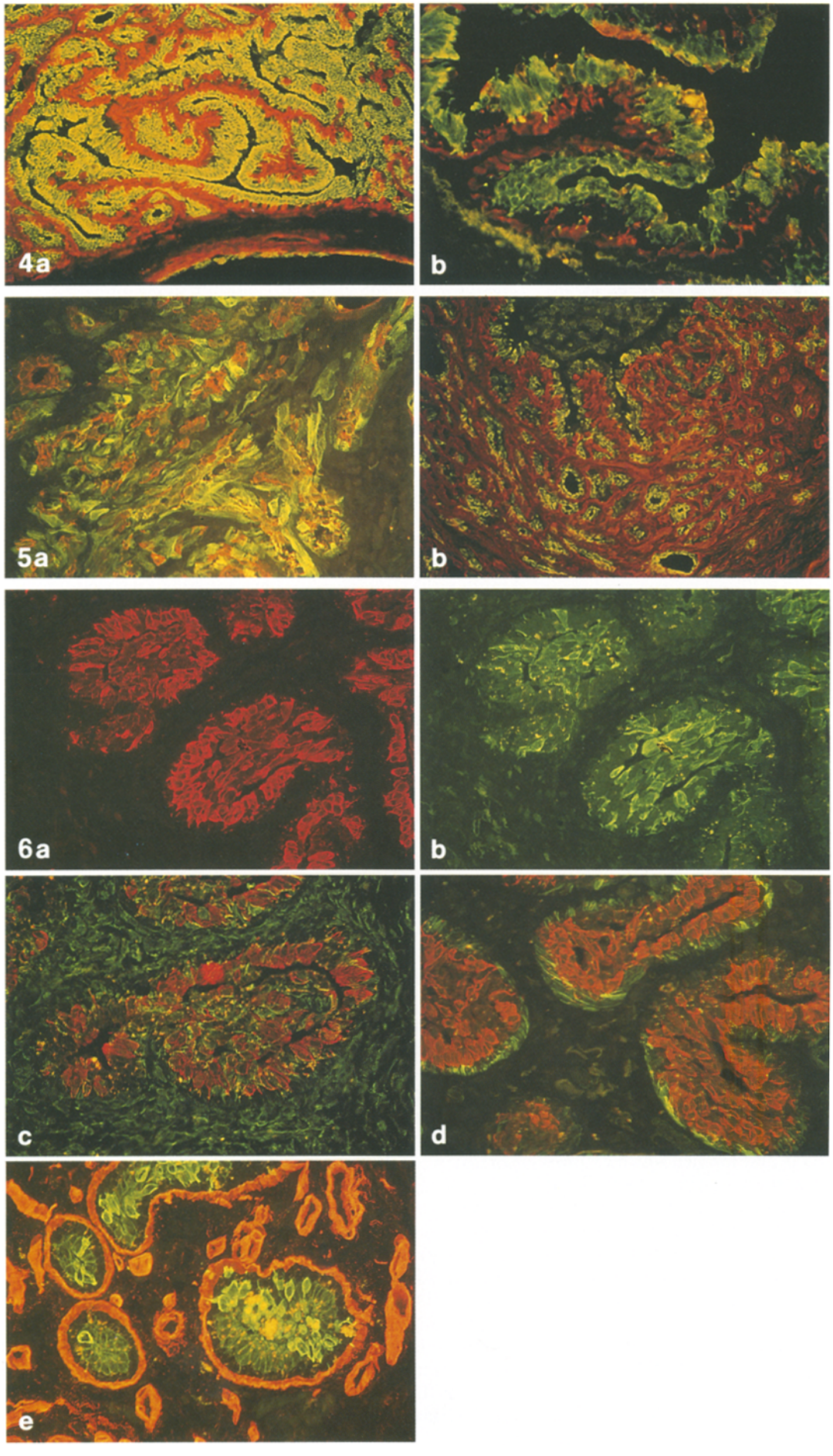
Of particular interest is the fact that there are some luminal cells in terminal ducts and lobules which express basal keratins 5/14, without showing the myoepithelial differentiation marker α-sm actin or the luminal keratins 8, 18, and 19. Interestingly, cells of this immunophenotype are one essential component of epitheliotic lesions (see below). It may be speculated whether cells with this immunophenotype (K5/14-positive, α-sm-actin negative) represent post-stem cells.

Our results suggest that the different proliferative lesions of the breast have two qualitatively different immunophenotypes, summarized schematically in Fig. 7.

In adenosis and duct papilloma a similar immunophenotypic reaction pattern was observed with an epithelial-myoepithelial differentiation, showing close resemblence to the normal breast. These lesions reveal an epithelial layer with a typical luminal keratin pattern (8, 18 and 19), a basal myoepithelial layer with basal keratins 5/14, actin and vimentin positivity and finally, a basal lamina with type IV collagen and laminin positivity forming a continuous lining around the myoepithelial cells. In this regard, duct papilloma may be regarded as the "ductal" (cyst) counterpart of lobular adenosis. We speculate that the intraluminal growth of papillomas is due to fibrous tissue surrounding ducts and cysts.

The proliferative pattern of scleradenotic lesions is characterized by an overwhelming myoepithelial proliferation (Hamperl 1970) usually with thick layers of collagen IV-laminin-positive basal lamina surrounding the myoepithelial cells (Fig. 7). We believe that the basal lamina is synthezised by the myoepithelial cells. The epithelial component consists of typical or closed tubules and is usually scanty. As these lesions have few or no capillary structures, this may offer an explanation for a secondary atrophy of the cellular components. Thus, papillomas and scleradenotic lesions represent a myoepithelial-epithelial hyperplasia of the breast tissue. The immunohistochemical pattern explains very well the difficulties these lesions may present in distinguishing them from small invasive tubular carcinomas. The differential diagnostic importance of membrane deposition and/or myoepithelial cells in scleradenosis versus tubular carcinoma has been underlined by several authors (Walther et al. 1986; Willebrand et al. 1986). All adenomas of the nipple analysed in this study showed a mixture of papillomatous, scleradenotic and epitheliotic phenotypes.

The second immunophenotypic pattern comprises epitheliotic lesions. In a previous analysis of these lesions, we studied the keratin patterns with mAb KA1 (k5/14) and mAb KA4 (K14/15/16/19). We observed intraluminal proliferation with luminal keratin and a basal (myoepithelial) keratin pattern and concluded that these lesions are of epithelial-myoepithelial nature (Jarasch et al. 1988). The results of the current report, which are in accordance with Raju et al. (1990), with the additional use of α-sm actin mAb forced us to modify our concept. The immunostain for α-sm actin yielded constant negative results of the intraluminal cell proliferations, thus the most important myoepithelial differentiation marker is negative. However, actin-positive myoepithelial cells can be demonstrated at the periphery of these lesions. On considering the immunophenotype of the normal breast we believe that these cells with a basal keratin 5/14 phenotype possibly represent post-stem cells or intermediate cells in transition to luminal cells, as already suggested by Azzopardi (1979). If this holds true, epitheliosis represents an epithelial hyperplasia of post-stem (intermediate) cells and of cells with a luminal keratin pattern. At least we can exclude the assumption that epitheliotic lesions are an epithelial-myoepithelial proliferation (Jarasch et al. 1988). It may be theorized that a common (K5/14-positive, actin-negative) stem cell of both epithelial and myoepithelial regeneration is situated in the basal (myoepithelial) layer. This is under investigation.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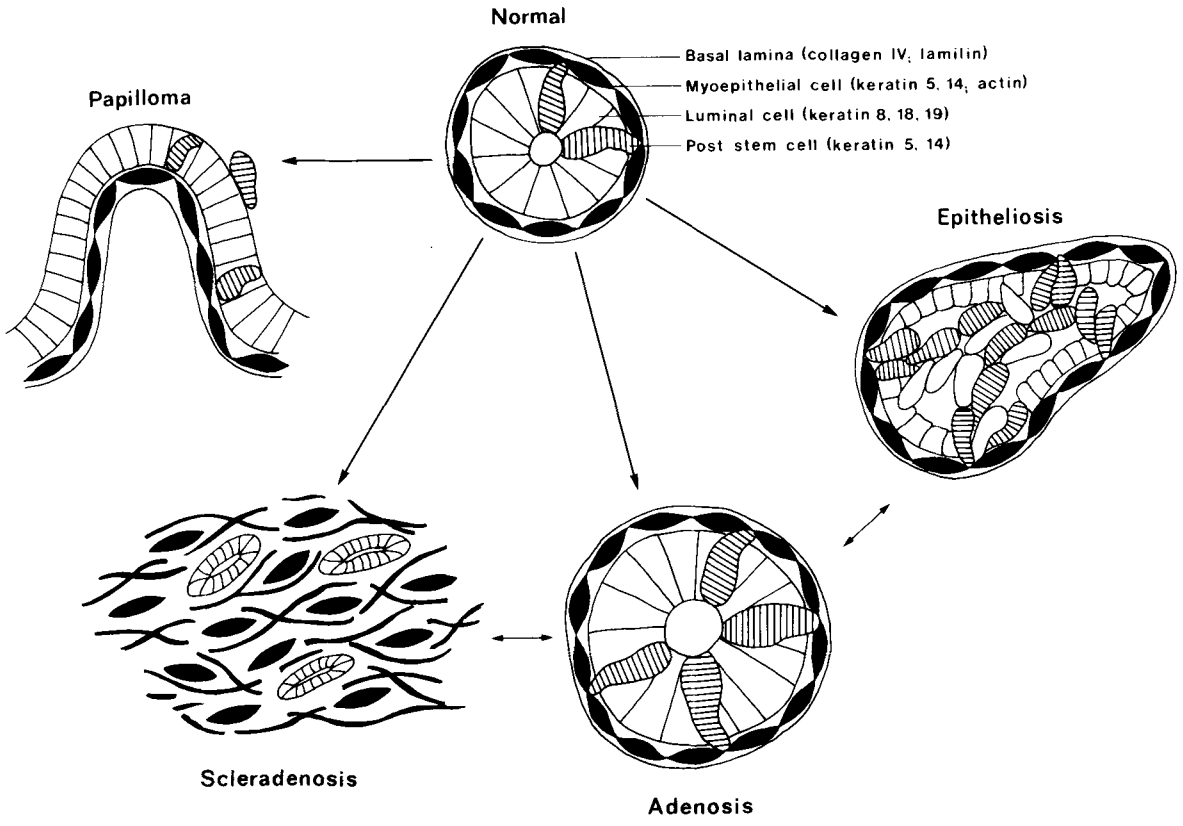

Fig. 7. Schematic presentation of our immunohistochemical findings of benign proliferative breast lesions (for details see text)

The two different patterns of benign epithelial lesions of the breast might explain the differences in biological significances of these lesions in terms of carcinogenesis. Patients with scleradenosis, ductal papilloma and adenoma of the nipple have a normal cancer risk, whereas the risk of those patients with epitheliosis is slightly increased (Dupont et al. 1980; Page et al. 1978). In an experimental breast cancer study in the rat Russo and Russo (1980) were able to show that the incidence of breast cancer is conversely related to the maturity of the normal breast tissue. These results may explain why the cancer risk of epitheliotic lesion is somewhat higher than that of adenotic lesions. Further studies are in preparation, investigating the relationship of eptiheliosis to ductal carcinoma in situ.

Fig. 4a, b. *Duct papilloma:* **a** Portion of papilloma immunostained with keratin TPA Ab (FITC) and collagen IV mAb (rhodamine). The papillary infoldings of both basal lamina and cells can clearly be seen, (× 400). **b** Papillary projection of a duct papilloma, double immunostained with keratin KA1 mAb (FITC) and keratin TPA Ab (rhodamine). Note that in addition to the KA1 reaction of myoepithelial cells there are occasional flattened luminal cells also stained, (× 630)

Fig. 5a, b. *Scleradenosis:* **a** A scleradenotic lobule, double immunostained with keratin KA1 mAb (FITC) and keratin TPA Ab (rhodamine). Note strong reaction of myoepithelial cells. (× 200). **b** Scleradenotic lobule, double immunostained with keratin TPA Ab (FITC) and collagen IV mAb (rhodamine). Note intensive staining of basal lamina material, (× 200)

Fig. 6a–e. *Epitheliosis:* **a, b** Double immunostaining of a portion of epitheliosis with keratin TPA Ab (rhodamine) and keratin KA1 mAb (FITC). Note the clearly exclusive reaction of cells with either TPA Ab (**a**) or KA1 mAb (**b**), (× 400). **c** Epitheliotic area, double immunostained with keratin TPA Ab (rhodamine) and vimentin mAb V9 (FITC). Note that quite a number of intraluminal cells stain positive for vimentin, (× 630). **d** Epitheliotic area, double immunostained for α-sm-actin mAb (FITC) and TPA Ab (rhodamin). Contrary to keratin KA1, there are only peripheral myoepithelial cells decorated by α-sm-actin mAb, (× 400). **e** Epitheliotic area, double immunostained with collagen IV mAb (rhodamin) and keratin TPA Ab (FITC). The basal lamina of glands and cpaillaries are brightly stained, (× 400)

Acknowledgements. The authors wish to thank Prof. Stegner and Prof. Schneider for providing the material, Dr. Nagle for providing the two mAbs KA1 and KA4, Dr. Fahrenkamp for preparing the schematic drawings, Ms. Gerdes for her photographic work and Mrs. Giffiths for typing the manuscript.

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