Virchows Archiv A Pathol Anat (1991) 418:339–348

Virchows Archiv A
Pathological Anatomy and Histopathology

Glial fibrillary acidic protein immunoreactivity in normal and diseased human breast

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Received October 31, 1990 / Accepted November 1, 1990

Summary. Immunostaining for glial fibrillary acidic protein (GFAP) identifies a minor subpopulation of immunoreactive myoepithelial cells in the normal resting human breast. The GFAP-immunoreactive cells also express a panel of myoepithelial cell markers, including cytokeratin 14 (CK 14), vimentin, smooth-muscle-specific actin isoforms, nerve growth factor receptor (NGFR) and common acute lymphoblastic leukaemia antigen (CALLA). The percentage of GFAP-immunoreactive myoepithelial cells is greatly increased in various neoplastic and non-neoplastic diseases of the breast, being highest in adenomyoepitheliomas. Furthermore, in all the instances of fibroadenoma, phyllodes tumour, epitheliosis and gynaecomastia, a variable number of epithelial cells also acquires immunoreactivity for GFAP, vimentin, CK 14, NGFR and, to a lesser extent, for CALLA. Conversely, GFAP immunoreactivity has never been encountered in the malignant cells of the different types of breast carcinoma. These findings suggest that the expression of GFAP might be a (possibly transient) feature of proliferating epithelial and myoepithelial cells in breast diseases other than carcinomas.

Key words: Glial fibrillary acidic protein – Intermediate filament co-expression – Myoepithelial cells – Breast

Introduction

The localization of specific cell markers has revealed a remarkable immunophenotypic heterogeneity in normal and diseased human cells belonging to the same cell type. In particular, subsets of different epithelial and non-epithelial cells can be defined, according to their expression of individual or multiple classes of intermediate filament (IF) proteins. Among these cytoskeletal proteins, glial fibrillary acidic protein (GFAP) has been consistently detected in a subpopulation of myoepithelial cells of the parotid gland (Achstätter et al. 1986), in individual nerve sheath cells (Fields and Yen 1985; Achstätter et al. 1986; Budka 1986), in fetal and adult chondrocytes of the respiratory tract cartilages (Kepes et al. 1984; Budka 1986; Viale et al. 1988) and in some neoplastic cells of mixed tumours of the salivary glands (Nakazato et al. 1982, 1985; Rozell et al. 1985; Achstätter et al. 1986; Stead et al. 1988) and of the skin (Argenyi et al. 1988), in nerve sheath tumours (Tascos et al. 1982; Memoli et al. 1984; Gould et al. 1986; Reifenberger et al. 1987), in chondromatous hamartomas of the lung (Viale et al. 1988) and in mixed müllerian tumours (Liao and Choi 1986).

In the course of a study aimed to ascertain a possible expression of GFAP in myoepithelial cells of glands other than the parotid, we noticed that a subset of myoepithelial cells of the normal resting human breast was consistently immunoreactive for GFAP, in addition to other myoepithelial cell markers, such as cytokeratin 14 (CK 14) (Dairkee et al. 1985; Nagle et al. 1986; Caselitz et al. 1986; Sorenson et al. 1987; Wetzels et al. 1989; Rudland and Hughes 1989), vimentin (Guelstein et al. 1988), smooth-muscle-specific actin isoforms (Gugliotta et al. 1988), nerve growth factor receptor (NGFR) (Garin Chesa et al. 1988; Thompson et al. 1989) and common acute lymphoblastic leukaemia antigen (CALLA) (Gusterson et al. 1986; Mahedran et al. 1989). Because GFAP-immunoreactive cells in the human breast have not been previously documented, we extended our preliminary investigation to a large series of normal and diseased breast specimens to evaluate the distribution of these cells in diverse pathological conditions. We wished to determine whether GFAP immunoreactivity could be a useful adjunct in elucidating the pathway of myoepithelial cell proliferation and differentiation in different pathological conditions of the human breast.

Because of the reported discrepancies on GFAP immunolocalization using different polyclonal and monoclonal antibodies (mAbs) (Achstätter et al. 1986; Viale et al. 1988) and to the adverse effects of fixatives on the immunoreactivity of the GFAP molecule (Viale et al.

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1988), the current investigation included use of one polyclonal and six different well-characterized mAbs to GFAP and the staining of frozen and methacarn- or formalin-fixed tissue specimens.

Our findings document that, at variance with the normal resting condition, in diseases of the breast other than carcinoma, immunoreactivity for GFAP and for the myoepithelial cell markers under study is not restricted to the myoepithelial cell component. It is shared by a subpopulation of epithelial (luminal) and stromal cells.

Table 1. Samples of benign and malignant breast tissues (451 samples from 313 patients)

Category[a]	No. of specimens
Normal tissue	50
Cystic disease	43
Adenosis (blunt duct)	38
Sclerosing adenosis	28
Sclerosing adenosis with pseudo-infiltration (infiltrating epitheliosis, radial scar)	9
Epitheliosis (papillomatosis)	30
Gynaecomastia	6
Hamartoma	4
Chondrolipomatous hamartoma	1
Papilloma	21
Fibroadenoma	23
Juvenile fibroadenoma	1
Phyllodes tumour	4
Duct adenoma	3
Tubular adenoma	4
Adenomyoepithelioma	3
Ductal carcinoma in situ[b]	19
Invasive ductal carcinoma (various types)[c]	138
Lobular carcinoma in situ[d]	8
Invasive lobular carcinoma	16
Mixed (ductal and lobular) carcinoma	2

[a] Lesions defined according to Azzopardi (1979) and Azzopardi and Salm (1984)
[b] Associated with invasive ductal carcinoma in 16 cases
[c] Classified as follows: 108 not otherwise specified, 8 medullary, 9 mucoid, 5 papillary, 4 tubular, 1 with prominent squamous cell metaplasia and 1 with chondroid metaplasia
[d] Associated with invasive lobular carcinoma in 6 cases

Materials and methods

A total of 451 specimens obtained from 313 (307 female, 6 male) patients undergoing lumpectomy or mastectomy for benign or malignant breast disease were included in the current study (Table 1). Normal tissue samples were obtained by sampling the specimens far away from the main lesions.

Immediately after surgery, the tissue samples were subdivided in two or three parts, one of which was snap-frozen in liquid nitrogen while the remaining part(s) were fixed in methacarn and/or in formalin and embedded in paraffin.

Adjacent tissue sections were immunostained for GFAP, vimentin, CK 14 (on frozen sections only), muscle-specific actin isoforms (m-actin), NGFR and CALLA (on frozen sections only), according to the avidin-biotin-peroxidase complex (ABC) staining technique (Hsu et al. 1981), as reported previously (Doglioni et al. 1987). The source, working dilution and specificity of the primary antibodies are detailed in Table 2; normal goat and horse sera, biotinylated antibodies and the ABC components in kit form were purchased from Vector (Burlingame, Calif. USA). Immunostaining of known positive controls for all the antigens and of negative controls, obtained replacing the specific antisera with the immunoglobulin fraction of non-immune rabbit or mouse sera, confirmed the specificity of all the immunoreactions.

Selected cases (5 cases each of normal breast tissue, fibroadenoma, epitheliosis, and papilloma and all the carcinomas) were also immunostained for oestrogen (ER) and progesterone (PgR) receptors using commercially available reagents in kit form (Abbott Laboratories, Chicago, Ill., USA).

Double immunocytochemical experiments for the simultaneous localization of ER or PgR proteins and GFAP on the same tissue section were performed using the peroxidase-antiperoxidase (PAP) complex staining method (Sternberger et al. 1970) for the localization of GFAP and the ABC technique with biotinylated alkaline phosphatase for the localization of the steroid receptors. Alkaline phosphatase activity was developed with the McGadey reagent, as reported by Unger et al. (1986) to give a blue end product. The reagents for the PAP complex reaction were purchased from Dakopatts (Copenhagen, Denmark).

Table 2. Source and working dilution of specific antisera

Reagent	Dilution	Source	Reference
Anti-GFAP (polyclonal)	1:2000	Dakopatts (Copenhagen, Denmark)	
Anti-GFAP (GA5 mAb)	1:5	Boehringer (Mannheim, FRG)	Debus et al. 1983
Anti-GFAP (1B4-2E1-4A11 mAb)	1:5	Biomedical Technologies (Stoughton, MA)	McLendon et al. 1986
Anti-GFAP (G1 mAb)	1:1000	Amersham (Amersham Bucks, England)	Gheuens et al. 1984
Anti-GFAP (GM mAb)	1:200	Labsystem (Helsinki, Finland)	Virtanen et al. 1985
Anti-GFAP (12–24 mAb)	1:25	Progen (Heidelberg, FRG)	Achstatter et al. 1986
Anti-GFAP (GFAP3 mAb)	1:25	Milab (Malmo, Sweden)	Jie et al. 1986
Anti-vimentin (V9 mAb)	1:5	Boehringer	Osborn et al. 1984
Anti-gytokeratin 14 (CKB1 mAb)	1:100	Sigma (St. Louis, MO)	Osborn et al. 1986
Anti-α smooth muscle actin (1A4 mAb)	1:3000	Sigma	Skalli et al. 1986
Anti-CALLA	1:10	Dakopatts	Newman et al. 1981
Anti-NGFR (ME20 mAb)	1:50	Amersham	Ross et al. 1984
Anti-oestrogen receptor	1:1	Abbott (Chicago, Ill.)	Greene et al. 1980
Anti-progesterone receptor	1:1	Abbott	Press and Greene 1988

CALLA, Common acute lymphoblastic leukaemia antigen; GFAP, glial fibrillary acidic protein; NGFR, nerve growth factor receptor

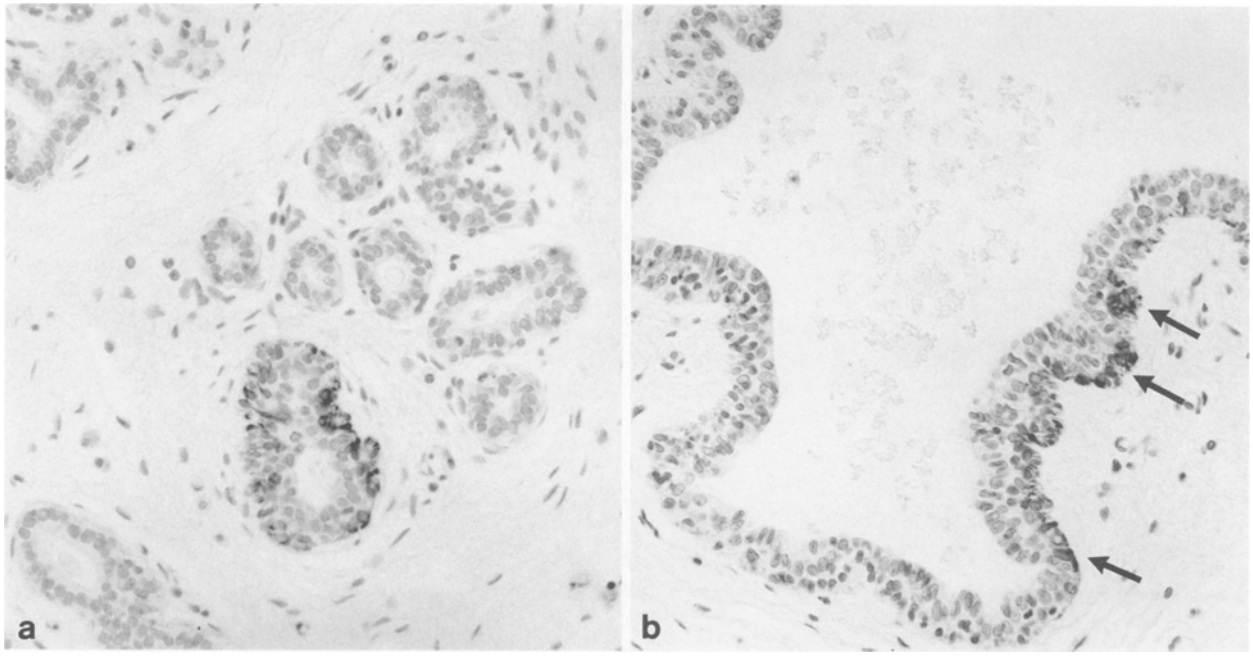

Fig. 1. Glial fibrillary acidic protein (GFAP)-immunoreactive cells in a small (**a**) and in a large duct (**b**) of normal breast samples. *Arrows* indicate immunostained cells. Methacarn fixation, GA5 mAb, ×250

Results

A preliminary experiment performed on a total of 25 cases (5 cases each of normal breast samples, fibroadenoma, adenosis, epitheliosis and papilloma) which had been partly frozen and partly fixed in methacarn and in formalin consistently demonstrated the occurrence of GFAP-immunoreactive cells in all the specimens. The number of these cells in the different samples, however, was highly variable mainly due to the diverse primary antibodies used and to the staining of frozen or fixed tissues. In particular, the immunostaining of serial sections of frozen tissue samples indicated that GA5 mAB decorated 80% of the cells which were immunostained by the polyclonal antiserum, the 1B4-2E1-4A11 cocktail and GFAP 3 mAb 75%, GM mAb 60%, G1 and 12–40 mAbs only 40% of the same cells.

Moreover, in fixed and embedded tissues the immunoreactivity for the different anti-GFAP antibodies was variably reduced. In methacarn-fixed tissues, best results were obtained with the polyclonal antiserum and the GA5 mAb, which decorated 90% and 80% respectively of the cells immunoreactive to the same antibodies in frozen sections. However, in formalin-fixed tissues the immunoreactivity for GA5 mAb was severely affected, whereas that for the polyclonal antiserum and GM mAb was only slightly decreased. Because of these findings, the polyclonal antiserum and the GA5 mAb were selected for immunostaining frozen and methacarn-fixed sections of all the remaining normal and pathological samples, whereas the formalin-fixed tissue sections were immunostained with the polyclonal antiserum and the GM mAb.

GFAP-immunoreactive cells were identified in all tissue specimens which did not show any histological abnormality (normal tissues). These cells were basally located and were distributed in a similar fashion to myoepithelial cells (Fig. 1). Moreover, the staining of adjacent serial sections allowed us to demonstrate that GFAP-immunoreactive cells invariably co-expressed m-actin, vimentin, CK14, NGFR and CALLA, so that they could be convincingly interpreted as a true subpopulation of myoepithelial cells of the human breast. Double immunolabelling experiments demonstrated that the GFAP-immunoreactive cells did not express ER and PgR.

The number of GFAP-immunoreactive cells was quite variable in the samples from different patients as well as in different fields of the same tissue specimens. In frozen sections, they ranged from 5% to 20% of the myoepithelial cells, irrespective of the patient's age, pre- or post-menopausal status, previous lactation and underlying disease.

GFAP-immunoreactive cells were unevenly distributed along the duct and lobular system of the breast, being more numerous around the nipple ducts, lactiferous sinuses and extralobular ducts than around the terminal ductal-lobuloalveolar units (TDLUs) (Fig. 1). Vimentin, m-actin and NGFR were consistently expressed in all the myoepithelial cells; CK 14 immunoreactivity was shown by all the myoepithelial cells of the ductal system, and by almost 50% of those surrounding the TDLUs, whereas CALLA immunoreactivity was detected in all the myoepithelial cells except for the vast majority of those surrounding the lactiferous sinuses.

The epithelial (luminal) cells of the ducts and TDLUs consistently failed to immunostain for any of the antigens tested. In the stroma, the polyclonal antiserum to

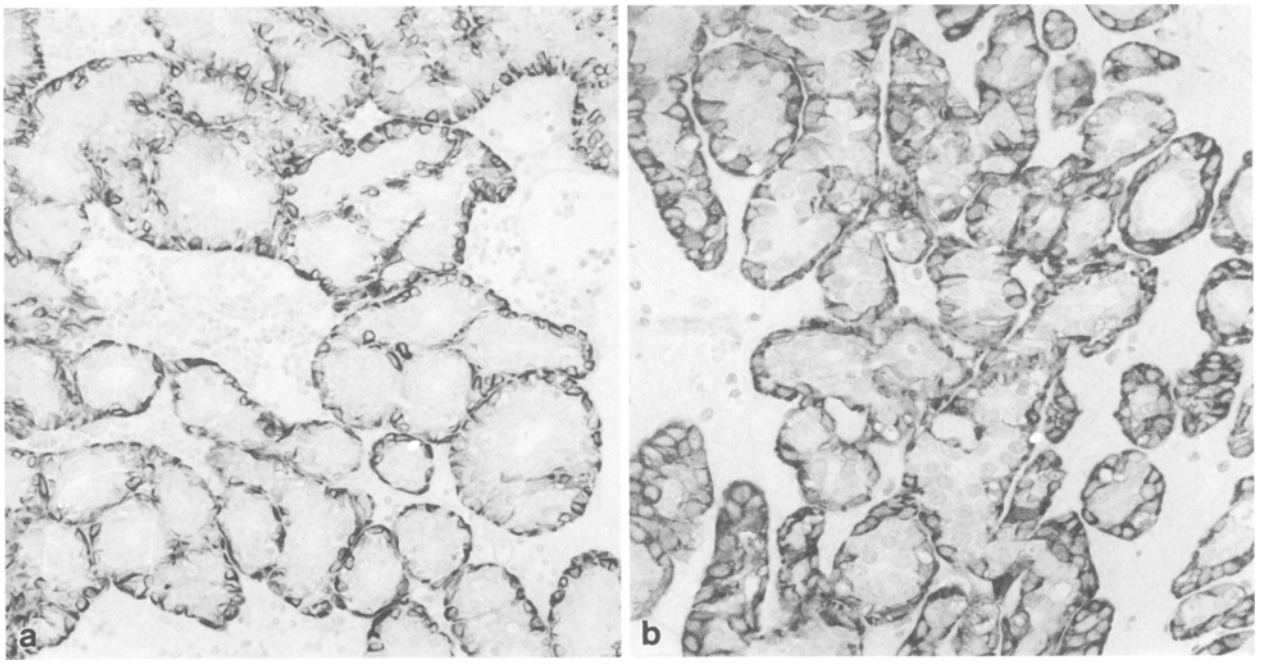

Fig. 2. The myoepithelial cell component of this formalin-fixed adenomyoepithelioma is simultaneously immunoreactive for GFAP (GM mAb, **a**) and for smooth muscle actin (**b**). **a**, ×250; **b**, ×325

GFAP decorated both nerve sheath cells and axons (possibly reflecting contaminating specificities of the antiserum, as previously suggested) (Achstätter et al. 1986), whereas the anti-GFAP mAbs immunostained only occasional nerve sheath cells. The antibody to NGFR decorated some stromal cells. As expected, the mAb to m-actin immunostained the pericytes and the smooth muscle cells of the vessel walls, whereas the anti-vimentin mAb stained all the stromal and inflammatory cells, including most smooth muscle cells of the vessel wall and the nerve sheath cells. Stromal cells immunoreactive for CK 14 and CALLA were not identified.

Variable patterns were seen in pathological tissues; however, the staining pattern of the simple and apocrine cysts for the diverse antigens did not significantly differ from that of the normal tissues.

A remarkable increase in the number of myoepithelial cells immunoreactive for GFAP was observed consistently in all the remaining pathological conditions of the breast, other than carcinomas. In the adenomyoepitheliomas most of the myoepithelial cells expressed GFAP (Fig. 2). In fibroadenomas, phyllodes tumours (Fig. 3a) and duct adenomas the immunoreactive cells accounted for 25–95% of the basally located cells. In the different types of adenosis (blunt duct, sclerosing adenosis with or without pseudoinfiltration) (Fig. 3b) and in intraductal papillomas the immunoreactive cells ranged from 50% to 95%. In all cases of duct epithelial hyperplasia (epitheliosis and gynaecomastia) the basally located positive cells ranged from 20% to 30%.

In all these instances, the GFAP-immunoreactive myoepithelial cells showed co-expression of vimentin, CK 14, m-actin, NGFR and CALLA, except for fibroadenomas, in which the co-expression of CK 14 was restricted to approximately 50% of the myoepithelial cells, in a similar manner as the normal TDLUs.

The myoepithelial cells surrounding ductal and lobular in situ carcinomas did not show any consistent GFAP immunoreactivity.

Apart from the basally located myoepithelial cells, in almost 50% of the fibroadenomas and phyllodes tumours and in all samples of duct hyperplasia a variable subpopulation of epithelial (luminal) cells displayed simultaneous immunoreactivity for GFAP, CK 14, vimentin, NGFR, and – to a lesser extent – for CALLA (Figs. 4, 5). These cells never exhibited m-actin immunoreactivity, whereas they expressed ER and PgR, as documented by double immunostaining experiments. V9 mAb to vimentin not only decorated the GFAP-immunoreactive cells but also immunostained an additional 20–30% of epithelial cells which did not co-express other markers.

The GFAP-immunoreactive cells accounted for 10–25% of the epithelial cells; they were not evenly distributed along the ducts, being especially clustered in areas where the epithelium was multilayered and showed papillary hyperplasia (Fig. 4).

At variance with the above findings, the malignant epithelial cells of in situ and infiltrating carcinomas – irrespective of the histological subtype – never displayed GFAP immunoreactivity. In the GFAP-immunostained sections, a clear-cut distinction between negative malignant cells and immunoreactive cells of intrapped hyperplastic ducts was often apparent. Especially in the case of tubular carcinomas, immunostaining for GFAP highlighted the positive hyperplastic ducts against the unstained neoplastic tubules. In a very minor percentage of cases, however, a variable number of malignant epi-

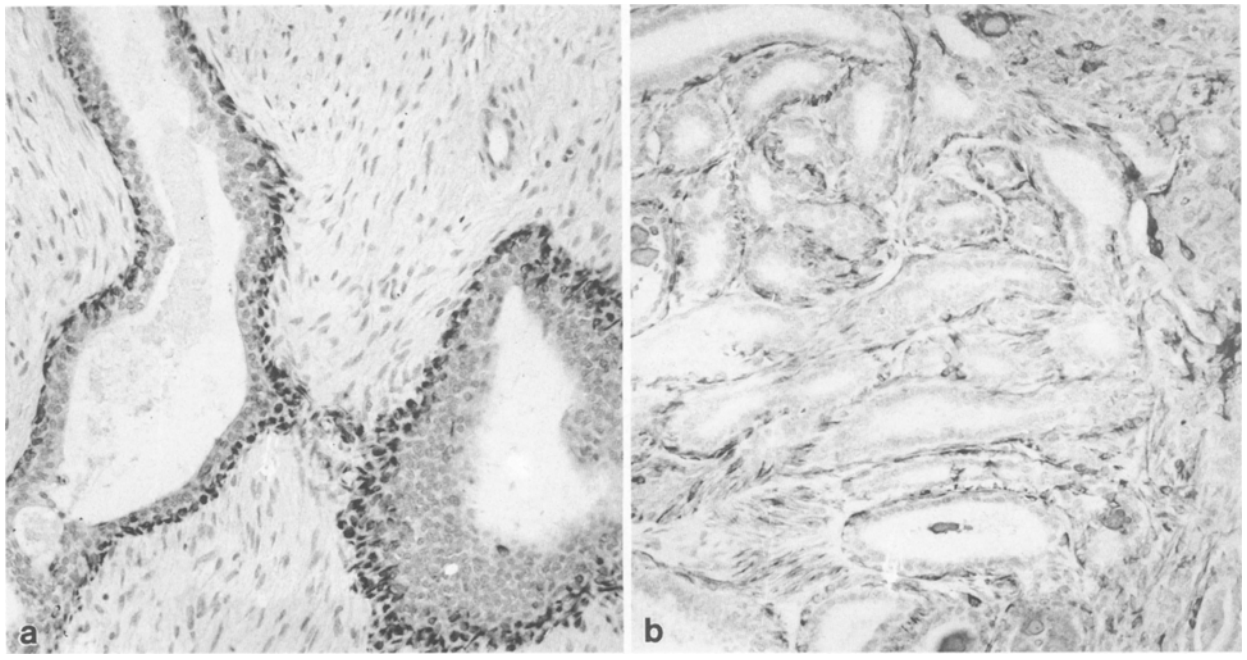

Fig. 3a, b. A phyllodes tumour (**a**) and a case of sclerosing adenosis (**b**) showing intense GFAP immunoreactivity in most of the myoepithelial cells. Methacarn fixation, polyclonal antiserum, ×250

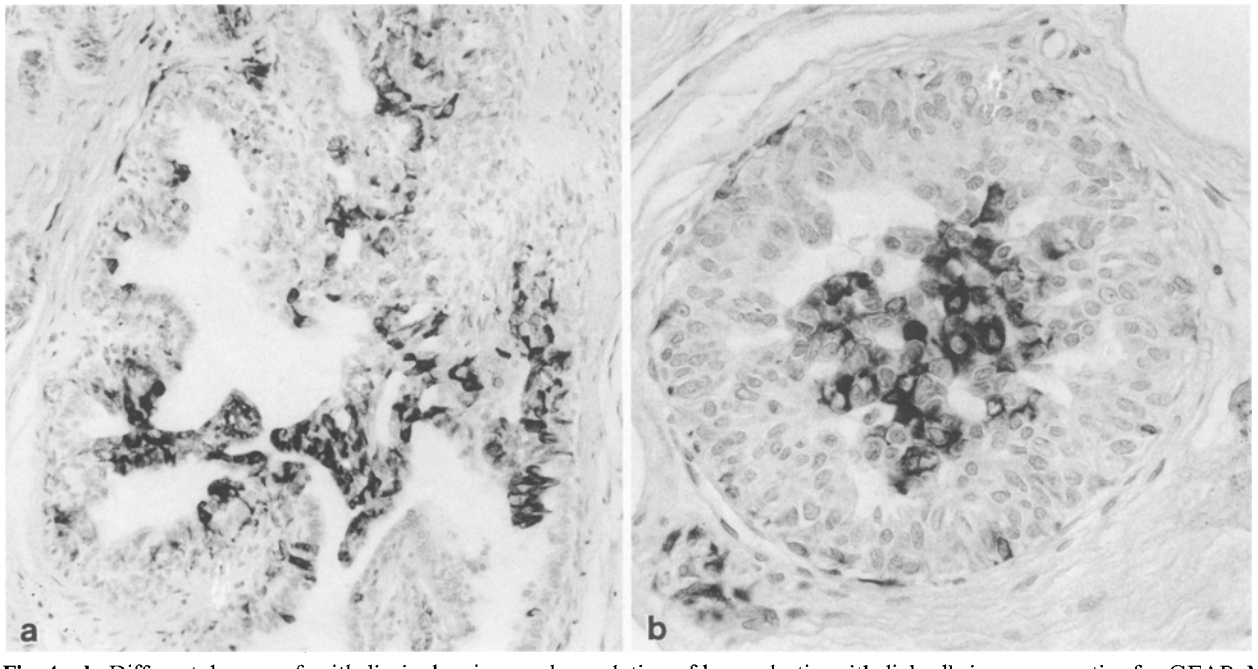

Fig. 4a, b. Different degrees of epitheliosis showing a subpopulation of hyperplastic epithelial cells immunoreactive for GFAP. Methacarn fixation. **a**, GA5 mAb, ×250; **b**, polyclonal antiserum, ×400

thelial cells displayed immunoreactivity for vimentin, CK 14 or NGFR. These findings will be dealt with in a subsequent report.

Apart from myoepithelial and epithelial cells a variable number of elongated stromal cells immunoreactive for GFAP occurred in areas of stromal reaction surrounding the proliferating ductules, in all the cases of adenosis, and occasionally, in fibrotic regions distant from the epithelial component. These stromal cells were particularly prominent in the lesions associated with a more intense sclerotic reaction, namely sclerosing adenosis and radial scars.

Though it was almost impossible to identify the very same cells in adjacent serial sections exactly, the number, morphological features and distribution of the cells immunoreactive for GFAP were strikingly parallel to those

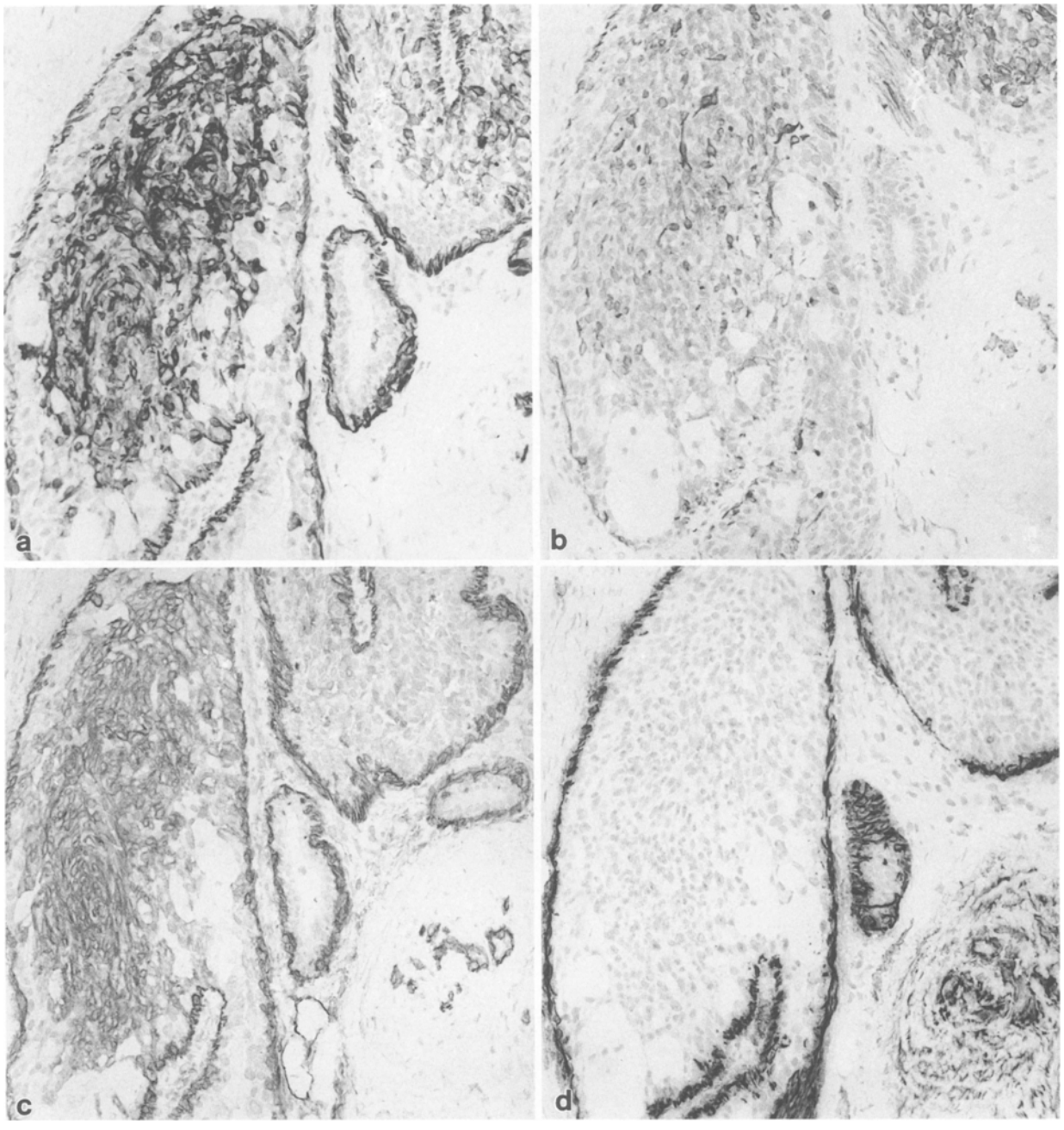

Fig. 5. Consecutive serial sections of a frozen sample showing severe epitheliosis immunostained for GFAP (polyclonal antiserum, **a**), cytokeratin 14 (**b**), nerve growth factor receptor (**c**) and smooth muscle actin (**d**). Smooth muscle actin immunoreactivity remains strictly confined to the myoepithelial cells. ×250

of cells expressing m-actin, CALLA and NGFR. Likewise, V9 mAb to vimentin also decorated these cells, among other stromal cells, whereas CKB1 mAb did not react with any of them. A variable number of elongated stromal cells with the same immunophenotypic pattern was also identified in the stalk of intraductal papillomas and in a single case of duct adenoma with prominent fibrotic areas (Fig. 6a). Finally, definite immunoreactivity for GFAP was detected in several chondrocytes and perichondrial cells of the chondrolipomatous hamartoma (Fig. 6b) and of the metaplastic chondroid foci in a duct adenoma and in a duct carcinoma.

In none of the malignant tumours, including those showing dense fibrotic reaction, were GFAP-immunoreactive stromal cells identified, although a variable number of m-actin and NGFR expressing elongated stromal cells were usually detected in the carcinomas associated with stromal fibrosis. In the single case of

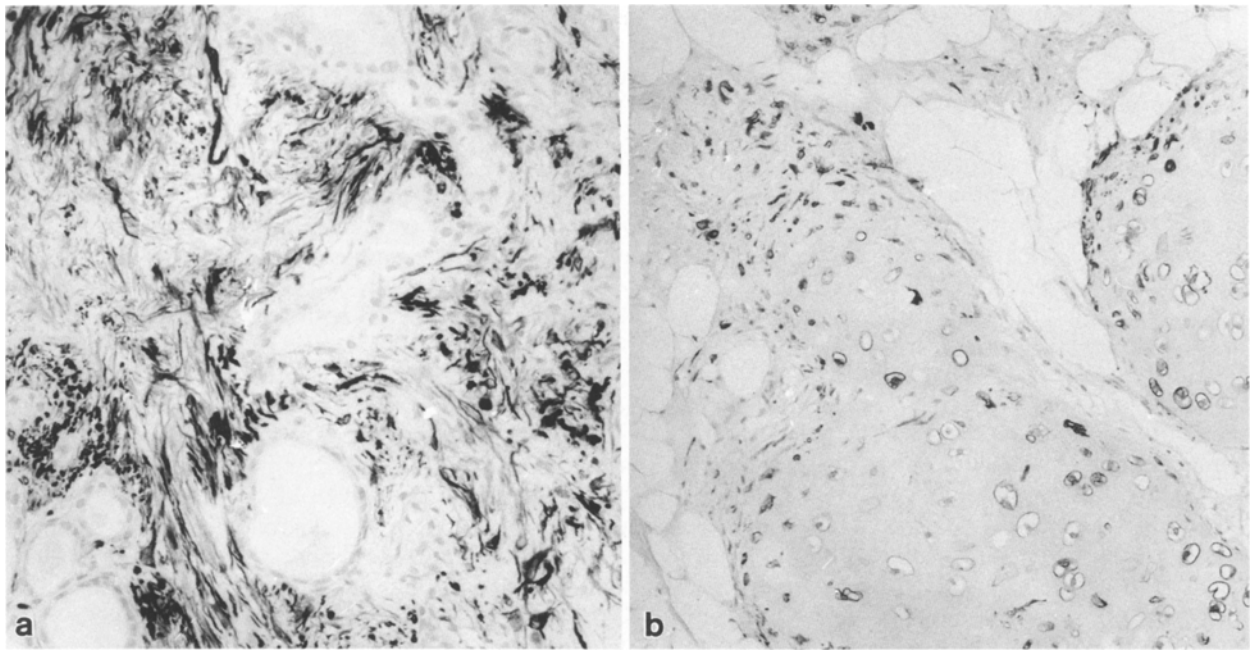

Fig. 6a, b. Immunoreactivity for GFAP in several elongated stromal cells of a duct adenoma (**a**) and in numerous chondrocytes of the chondrolipomatous hamartoma (**b**). Methacarn-fixed tissues immunostained with GA5 mAb, ×200

duct carcinoma showing chondroid metaplasia, however, the metaplastic chondrocytes exhibited intense GFAP immunoreactivity.

Discussion

The current investigation documents a previously unrecognized immunophenotypic heterogeneity of different cell types (myoepithelial, epithelial and myofibroblastic) of the human breast, with regard to the expression of GFAP and of other cell markers.

As far as the myoepithelial cells are concerned, we have identified a subset of this cell type which expresses GFAP in addition to other well-established immunocytochemical markers, namely CK 14, vimentin, smooth muscle actin, NGFR and CALLA. In the tissues without histological abnormalities, GFAP immunoreactivity is restricted to a minor subpopulation of myoepithelial cells of the ducts and TDLUs. Although we did not have access to truly normal mammary glands, we have obtained samples of histologically proven normal tissues far away from the pathologically altered areas in benign and malignant tumours and mammary hamartomas. It is therefore unlikely that the appearance of GFAP-immunoreactive myoepithelial cells could be produced by any underlying breast disease. In diverse neoplastic and non-neoplastic breast diseases, the percentage of the immunoreactive cells is greatly increased, being highest in adenomyoepitheliomas. Although, in the latter tumours, a selective clonal proliferation of the subpopulation of myoepithelial cells normally exhibiting GFAP co-expression cannot be excluded, the reported findings suggest that GFAP immunoreactivity could represent a (possibly transient) phenotypic expression during different stages of the differentiation pathway of these cells.

The occurrence and distribution of GFAP-immunoreactive myoepithelial cells in the human breast have never been documented, though Stead et al. (1988) and Gould et al. (1990) incidentally alluded to the presence of GFAP-immunoreactive mammary myoepithelial cells in the discussion of their articles on pleomorphic adenomas of salivary glands and on central neuroectodermal tumours, respectively. Immunoreactivity for GFAP in some cells of a single case of breast adenomyoepithelioma has been also reported by Erlandson (1989).

The myoepithelial cells of the breast should therefore be added to their counterparts in the parotid gland (Achstätter et al. 1986) and to some chondrocytes (Viale et al. 1988) as the only normal human cells not associated with the nervous system showing immunoreactivity for GFAP. In a similar manner as for the myoepithelial cells of the parotid, these cells are characterized by a complex pattern of IF expression, being immunoreactive for three distinct classes of IF proteins, namely GFAP, CKs and vimentin.

Besides the myoepithelial cells, we have also shown a remarkable immunophenotypic heterogeneity of the epithelial (luminal) cells in some benign proliferative conditions of the breast, both neoplastic (fibroadenomas, phyllodes tumours) and non-neoplastic (epitheliosis, gynaecomastia). Such heterogeneity has never been encountered in the normal resting gland or in the different histotypes of breast carcinomas.

Indeed, in the benign proliferative diseases, some of the epithelial cells, most often occurring in clusters, ac-

quire immunoreactivity for GFAP, vimentin, CK 14, NGFR and CALLA. Our results are in keeping with previous reports showing that in the same proliferative diseases of the breast at least some hyperplastic luminal cells acquire immunoreactivity for vimentin (Guelstein et al. 1988; Raymond and Leong 1989) and for CK polypeptides 5, 14 and 17 (Nagle et al. 1986; Guelstein et al. 1988; Jarash et al. 1988). The latter CK polypeptides are most often expressed in addition to those normally present in the resting luminal epithelium.

These data document that the benign proliferative activity of the mammary epithelial cells is consistently associated with changes in the expression of genes coding for distinct IF proteins and for other markers. The resulting immunophenotype is strikingly similar to that of (differentiating) myoepithelial cells, sharing the same complement of distinct IF proteins (CK 14, vimentin and GFAP), NGFR and CALLA. In contrast with the myoepithelial cells, however, the epithelial cells never synthesize smooth-muscle actin, while they express ER and PgR proteins.

A large body of evidence points to the occurrence, both in experimental animals and in humans, of a stem cell population able to proliferate and differentiate into both luminal and myoepithelial cells (Vorheer 1974; Rudland et al. 1980; Russo et al. 1983; Williams and Daniel 1983; Ferguson 1985; Ormerod and Rudland 1986; Walker 1988). Furthermore, cells showing morphological aspects of both progenitor (basal clear cells) and fully differentiated myoepithelial cells may be found in the normal human breast (Smith et al. 1984), while cells within areas of epitheliosis have already been shown to be of intermediate type, having ultrastructural features of both epithelial and myoepithelial differentiation (Ozzello 1971). Cells with such intermediate features have been also identified in different proliferative conditions, namely in the developing rat mammary glands (Ormerod and Rudland 1984), during regeneration of the glands from isolated ducts (Ormerod and Rudland 1986) and in cultured cell lines from normal rat and human mammary glands as well as from benign rat and human tumours (Rudland 1987). Conversely, no such intermediate cells have been identified in rat and human mammary carcinomas or in cultures thereof (Dunnington et al. 1984; Rudland et al. 1985; Sonnenberg et al. 1986; Rudland 1987). We speculate that the heterogeneous immunophenotypic patterns of luminal epithelial and myoepithelial cells in benign proliferative conditions of the breast might be strictly related to the proliferation of precursor or intermediate cells and to their differentiation into fully differentiated cells. The lack of a similar cell heterogeneity in malignant tumours suggests that in these conditions different pathways of cell proliferation and differentiation take place.

Finally, we have also documented GFAP expression in the elongated stromal cells seen in the fibrotic areas of some benign breast diseases (sclerosing adenosis, ductal adenomas and papillomas). These cells show morphological and immunocytochemical (vimentin and alpha smooth-muscle actin immunoreactivity) features similar to those of the myofibroblasts identified by Sappino and coworkers (1988). Our data demonstrate that the cytoskeletal complement of these cells in non-malignant diseases of the breast also includes expression of GFAP, and that they are simultaneously immunoreactive for NGFR and CALLA. These findings point to the existence of closer relationships between stromal myofibroblasts and myoepithelial cells. Conversely, in breast carcinomas, the stromal myofibroblasts do not show any GFAP immunoreactivity. Thus, a divergent origin and/or differentiation of these cells in benign and malignant conditions of the breast is suggested.

Besides the myofibroblasts, GFAP immunoreactivity is an invariable feature of the chondroid cells and of the perichondrial elongated cells of the metaplastic cartilaginous foci in both benign and malignant tumours of the breast as well as in chondrolipomatous hamartomas. The immunoreactivity for GFAP of these cells is in keeping with the recent data documenting expression of GFAP in normal human chondrocytes of the respiratory tract and chondromatous hamartomas of the lung (Kepes et al. 1984; Budka 1986; Viale et al. 1988).

In conclusion, the current investigation re-emphasizes the plasticity of IF gene expression, not only documenting a new example of unexpected expression of GFAP in a subpopulation of myoepithelial cells of the resting human breast and in some myofibroblasts of the fibrotic stroma of various benign diseases, but also showing that a complex immunophenotypic pattern (including the simultaneous expression of CKs, vimentin, and GFAP and the immunoreactivity for NGFR and CALLA) is a consistent feature of a subset of epithelial cells in some benign proliferative conditions of the human breast. The findings indicate that the typing of the distinct IF proteins (including the identification of different CK polypeptides) and of muscle actin isoforms, together with the localization of the newly recognized myoepithelial cell markers NGFR and CALLA, may be useful adjuncts for a better understanding of the mechanism(s) and the pathway(s) of epithelial cells proliferation and differentiation in various benign and malignant diseases, and for ascertaining the actual precancerous potential of different proliferative lesions of the breast, with particular reference to those showing atypical features.

Acknowledgements. This work was partly supported by the Ministero della Universita' e della Ricerca Scientifica e Tecnologica, Roma, Italy.

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Note added in proof. The expression of GFAP in myoepithelial cells of the human breast has been recently confirmed (Gould VE, Koukoulis GK, Jansson DS, Nagle RB, Franke WW, Moll R (1990) Coexpression patterns of vimentin and glial filament protein with cytokeratins in the normal, hyperplastic, and neoplastic breast. Am J Pathol 137:1143–1155)

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