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Mammary foam cells

Characterization by immunohistochemistry and in situ hybridization

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Abstract Cells showing abundant, finely vacuolized cytoplasm (foam cells) are found frequently in most benign lesions of the breast and in certain malignant breast tumours. The origin of mammary foam cells (FCs) has not been clarified, and we therefore studied the morphological features of mammary FCs in a series of 50 benign lesions. The FCs were subdivided, on the basis of their distribution into FCs lining the glandular lumina, intraluminal FCs, intraepithelial-pagetoid FCs, and stromal FCs. The lesions were tested with a panel of antibodies against macrophage (MAC 387, CD68) and epithelial (epithelial membrane antigen [EMA], gross cystic disease fluid protein 15 [GCDFP15] and cytokeratin) markers. The lesions were examined for the presence of PIP/GCDFP15-specific mRNA by an in situ hybridization technique. Three different types of FCs were identified. Type A FCs are epithelial cells (positivity with EMA and cytokeratin) and show apocrine differentiation (positivity with GCDFP15 antiserum and expression of PIP/GCDFP15 mRNA). Type B FCs are of macrophage origin, as they are positive with the macrophage markers and lack cytokeratin and PIP/GCDFP15 mRNA. Finally, type C FCs show an intermediate profile between an epithelial cell and a macrophage: they are both CD68 and GCDFP15 positive and show a thin peripheral rim of positivity with anti-cytokeratin antibody. They lack PIP/GCDFP15 mRNA. Our results indicate the possibility of a spectrum of phenotypes in mammary FCs, from epithelial-apocrine cells to macrophage-derived phagocytic cells.

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Introduction

Foam cells (FCs) are frequently observed both in normal and in diseased breasts, being abundant in cases with apocrine cystic changes or duct ectasia. The cells may be observed floating free within the cystic lumina, clustered in the periductal stroma or in continuity with the epithelial lining of the glandular structures. For many years there has been a continuous debate on the nature (epithelial versus histiocytic) of these cells, and many morphological and immunohistochemical studies have been proposed, supporting one or the other origin at different times [4–7, 10, 20, 21, 24, 29].

To elucidate the nature of mammary FCs in different microanatomical locations (epithelium, stroma and glandular lumina), we studied the morphological, immunohistochemical and molecular features of these cells in a series of benign breast lesions.

Materials and methods

Fifty lesions were selected for the presence of FCs from 40 breast specimens removed for benign conditions (Table 1). All material was obtained from the files of the Section of Anatomical and Cy-

Table 1 50 benign lesions selected for the presence of foam cells

Lesion	No. of cases
Epitheliosis	15
Cystic disease	12
Duct ectasia	8
Intraductal papilloma	5
Ductal adenoma	4
Sclerosing adenosis	2
Normal lobules with clear cells	2
Fibroadenoma	1
Radial scar	1

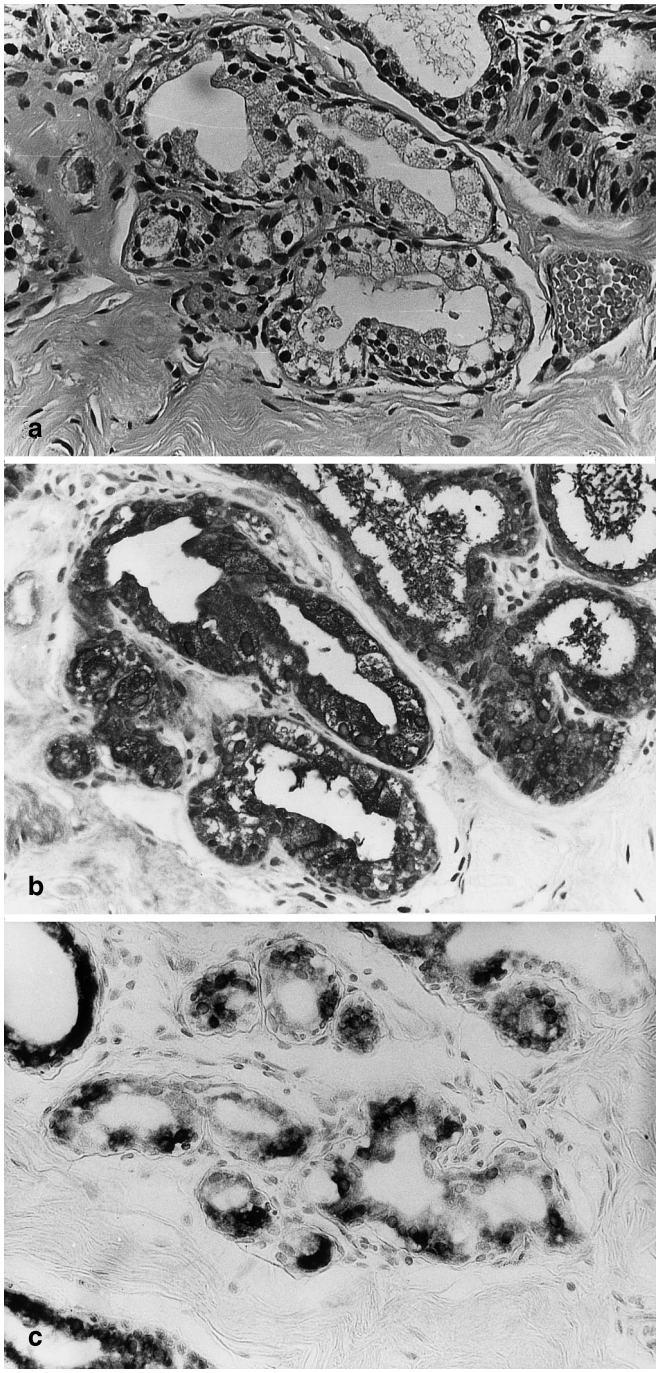

Fig. 1a–c Type A (epithelial) foam cells (FCs). **a** The ductules are lined by large, polygonal cells showing abundant, foamy cytoplasm and small, round nuclei. **b** The cells are diffusely positive with anti-gross cystic disease fluid protein 15 and **c** also express prolactin-inducible protein (PIP)/GCDFP15 mRNA on in situ hybridization, confirming the presence of apocrine differentiation. The same cells were also strongly positive with anti-cytokeratin and negative with CD68 antibody (not shown). Original magnification ×400

topathology "M. Malpighi" of the University of Bologna. Routinely stained sections from each lesion were reviewed to study the morphology of the FCs and their distribution.

The FCs were classified on the basis of their microanatomical location into four groups as follows: FCs lining the glandular lumina in an epithelial fashion (Fig. 1a), intraluminal FCs, intermingled with the luminal secretion (Figs. 2a, 3a, 4a), intraepithelial-pagetoid FCs (Figs. 3a, 4a) located within the thickness of the epithelial cells lining the glandular spaces, with a pattern simulating the pagetoid spread of lobular carcinoma, and stromal FCs, localized around ducts, ductules and/or cysts (Fig. 3a).

Consecutive sections from each lesion were stained for immunohistochemistry (IHC) using the avidin-biotin-peroxidase complex technique [18]. The antisera used are listed in Table 2 together with their respective sources and dilutions.

In some lesions, FCs had disappeared from consecutive sections. We therefore selected 20 cases in which FCs were still present for in situ hybridization (ISH) (Table 3). A 600-bp cDNA encoding human PIP (pPIP-8–3 cDNA clone supplied by Dr. R. Shiu and Y. Myal, Winnipeg, Manitoba, Canada) was used. The riboprobe was cleaved with the restriction enzyme Xbal, and antisense RNA molecules were obtained using a T7 DNA polymerase and digoxigenin-labelled nucleotide mixture (DIG RNA labelling kit, Boehringer Mannheim, Germany). The method used for ISH was that previously described by Pagani et al. [25].

Results

FCs were most prominent and numerous in lesions showing cystic dilated glands, such as apocrine cysts, duct ectasia and intraductal papilloma. In all but 7 instances, FCs were present in two or more microanatomical districts of the same lesion (for example, luminal FCs were seen associated with FCs lining the ducts in the same lesion (Fig. 2), or intraepithelial FCs were seen together with luminal FCs and stromal FCs (Figs. 3, 4); the most frequent association was luminal FCs and FCs lining the glandular spaces, which occurred in 15 instances.

The morphological features of all FCs were identical irrespective of the microanatomical district (Figs. 1a, 2a, 3a, 4a). The cells were polygonal to round in shape, had well-defined borders and abundant, clear to pale cytoplasm, filled with numerous small vacuoles. Nuclear morphology failed to yield information that would have been useful for distinguishing FCs in different locations. Nuclei, sometimes displaced peripherally by large cytoplasmic vacuoles, were small and round to oval in shape, and had inconspicuous nucleoli.

FCs were observed directly lining the glandular lumina in 22 lesions (Fig. 1a). In most cases these were seen in association and in continuity with apocrine epithelium. Some of these latter were clustered in small papillary-type projections abutting into the glandular lumen or appeared intermingled with normal epithelial cells. In most instances (15/22 cases) FCs lining the glandular spaces were found in association with FCs floating free within the lumina.

Intraluminal FCs were found in 30 lesions (Figs. 2a, 3a, 4a). FCs were present in this district almost exclusively in lesions with cystic lumina: apocrine cysts (12 cases), duct ectasia (8 cases), ductal papilloma (5 cases), ductal adenoma (4 cases) and 1 fibroadenoma. They appeared either clustered in small clumps or as single elements floating free within the lumina. Sometimes, intraluminal FCs appeared encased within the eosinophilic dense secretion, and in rare instances (3 cases) they exhibited erythrophagocytic properties.

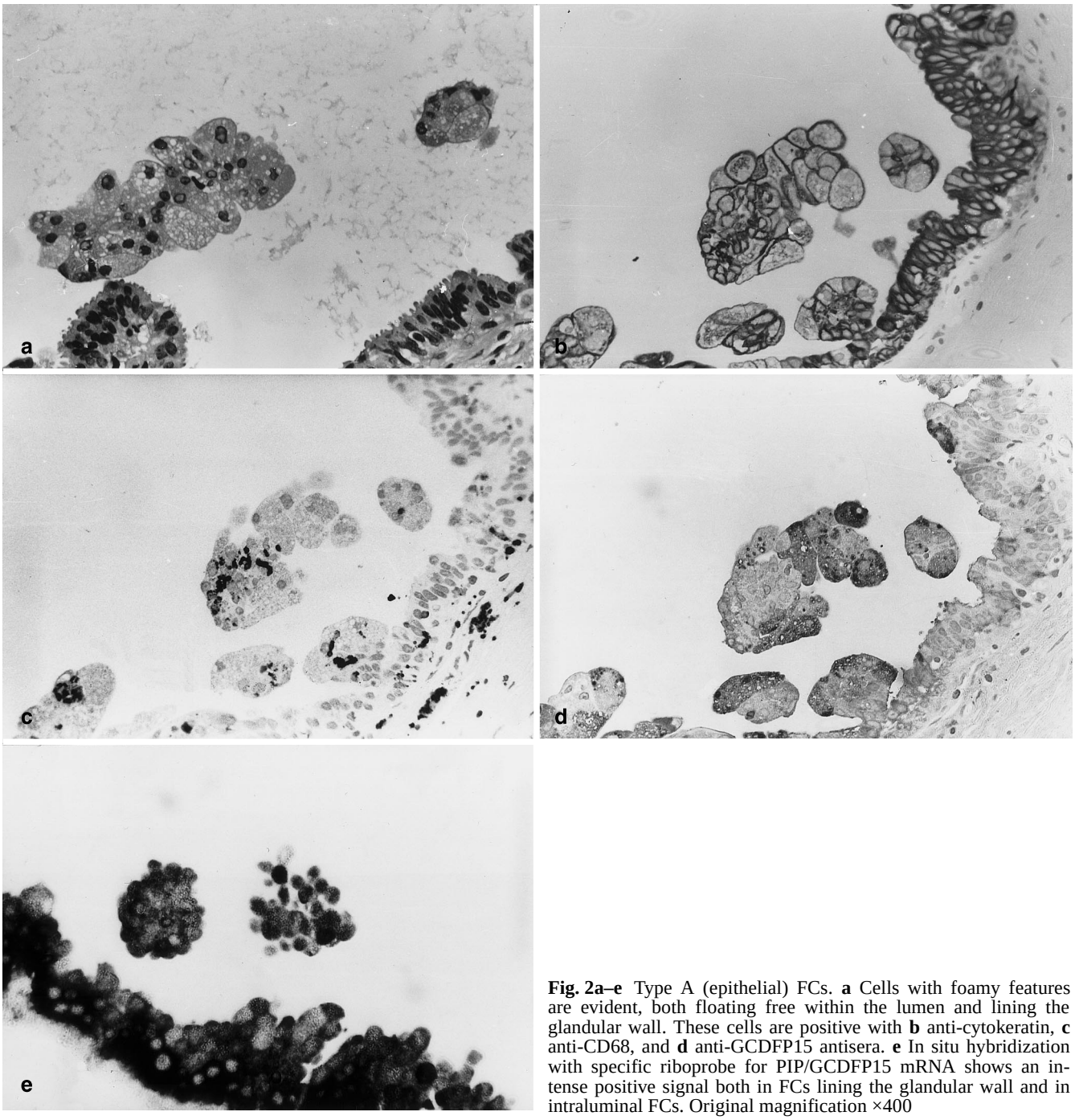

Fig. 2a–e Type A (epithelial) FCs. **a** Cells with foamy features are evident, both floating free within the lumen and lining the glandular wall. These cells are positive with **b** anti-cytokeratin, **c** anti-CD68, and **d** anti-GCDFP15 antisera. **e** In situ hybridization with specific riboprobe for PIP/GCDFP15 mRNA shows an intense positive signal both in FCs lining the glandular wall and in intraluminal FCs. Original magnification ×400

Intraepithelial-pagetoid FCs were present in 19 lesions (Figs. 3a, 4b). These were most prominent in cases of duct ectasia (8 cases), where they often appeared in association with intraluminal and/or stromal FCs. Less frequently, intraepithelial-pagetoid FCs were also observed in epitheliosis (6 cases) and in lesions showing apocrine differentiation, such as intraductal papilloma, ductal adenoma and cystic disease (5 cases).

Stromal FCs were observed surrounding the glandular (mostly duct) walls in 20 lesions (Figs. 3, 4). These were particularly prominent when the lesion was constituted by ectatic ducts, usually associated with a moderate to marked inflammatory infiltrate. In 8 cases (5 cases of duct ectasia, 2 of intraductal papilloma and 1 of epitheliosis), yellow-tan, lipofuscin-like pigment was present in the cytoplasm of FCs. These features were previously defined as ocrocytes [6]. In only 2 instances were stromal FCs seen surrounding apocrine cysts.

Immunohistochemical results are summarized in Table 4. Anti-GCDFP15 stained the FCs lining the glandular lumina in 18 out of 21 lesions stained (Figs. 1b, 2d). The positivity was diffusely cytoplasmic, and more in-

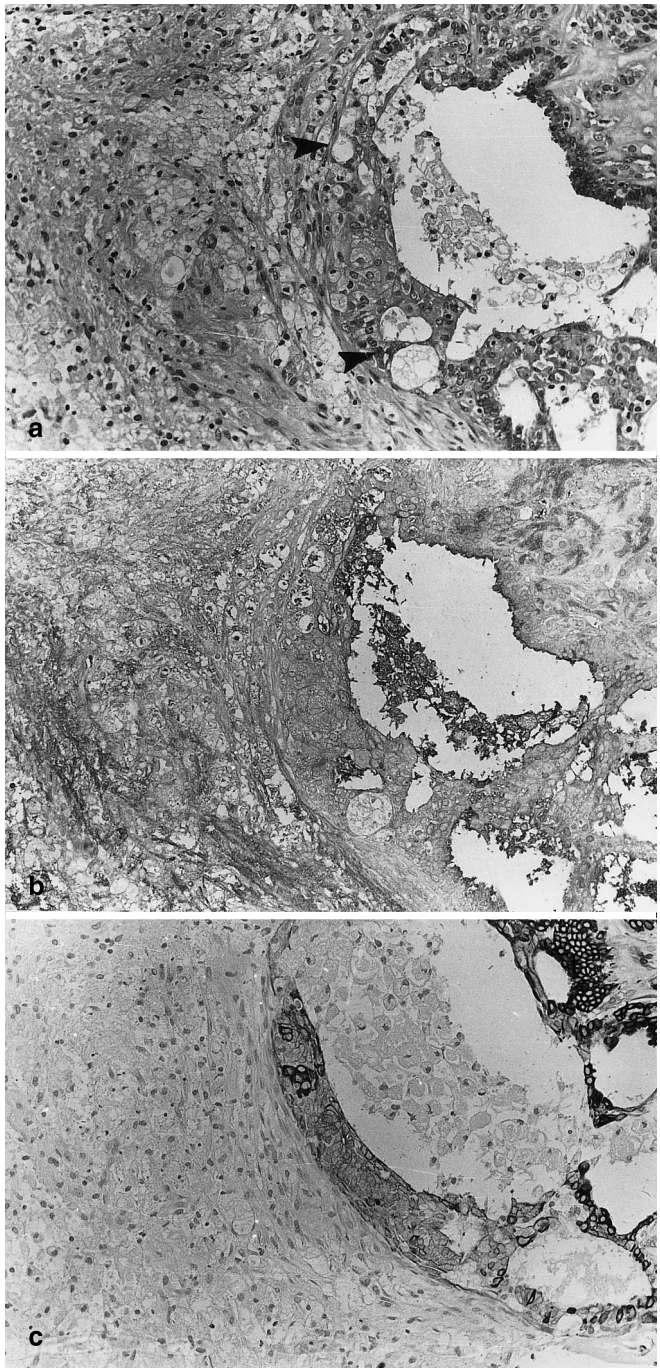

Fig. 3a–c Type B (macrophagic) FCs. **a** High power view of a ductal adenoma. In this field, numerous cells with foamy features are present both within the glandular lumen and in the stroma surrounding the ductal wall. Small nests of pagetoid-intraepithelial FCs are also evident (*arrow*). GCDFP15 antiserum diffusely stains both intraluminal and stromal foam cells (**b**), while anti-cytokeratin antiserum is positive only in the epithelial lining of the duct (**c**). The same cells were also positive with CD68 antibody and negative with in situ hybridization for PIP/GCDFP15 mRNA (not shown). Original magnification ×200

Table 2 Antisera employed for immunohistochemistry (*GCDFP15* gross cystic disease fluid protein 15, *EMA* epithelial membrane antigen, *MNF116* broad-spectrum cytokeratin, *MAC387* anti-myeloid-histiocyte antigen

Antisera	Sources	Dilutions
GCDFP 15	Dako	1:500
EMA	JP Sloane (Liverpool)	1:200
MNF116	Dako	1:100
CD68-KP1	Dako	1:100
MAC387	Dako	1:150

Table 3 Twenty lesions selected for in situ hybridization

	No. of cases
Cystic disease	5
Intraductal papilloma	5
Ductal adenoma	4
Duct ectasia	4
Normal lobules	2

tense staining was observed in the apical region of the cell. The staining also differed among the various cells of the same gland, with some more intensely stained than others; occasional cells appeared negative. The intensity of staining was inversely proportional to the degree of cytoplasmic vacuolization. The only negative cases were 3 with very large, vacuolated FCs. Anti-GCDFP15 antiserum stained intraepithelial-pagetoid FCs in 9 out of 17 cases and intraluminal FCs in 20 out of 25 cases, together with the luminal secretion (Figs. 2d, 3b, 4c). Stromal FCs were stained by anti-GCDFP15 antiserum in 8 out of 18 cases. However, in all these cases periductal staining of the stroma was also evident (Fig. 3b).

The results obtained with anti-EMA antiserum were very similar to those with anti-GCDFP15 antiserum (see Table 4). EMA positivity was seen mostly in the apical borders, and only occasional cells showed diffuse cytoplasmatic staining.

Anti-cytokeratin antiserum stained FCs lining the glandular spaces in 17 of 18 and intraluminal FCs in 6 of 26 lesions studied. The staining varied from diffuse to a peripheral cytoplasmic rim when the cells appeared extensively vacuolated (Figs. 2b, 4b). This latter staining pattern made it difficult to evaluate keratin positivity in some cases of intraepithelial-pagetoid FCs, as the cells were adjacent to normal epithelial cells and it was difficult to assess whether the thin, peripheral rim of staining belonged to the cytoplasm of the FCs. Therefore, cytokeratin expression in intraepithelial-pagetoid FCs was considered positive (5 cases) only when a distinct rim of positivity was seen along the cytoplasmic borders adjacent to other FCs (Fig. 4b). Stromal FCs were negative for keratin in all the cases tested (Fig. 3c).

Anti-MAC387 antiserum diffusely stained the cytoplasm of the stromal, intraluminal and intraepithelial-pagetoid FCs in almost all cases. FCs lining the glandular lumina were stained in 13 out of 19 cases, and in

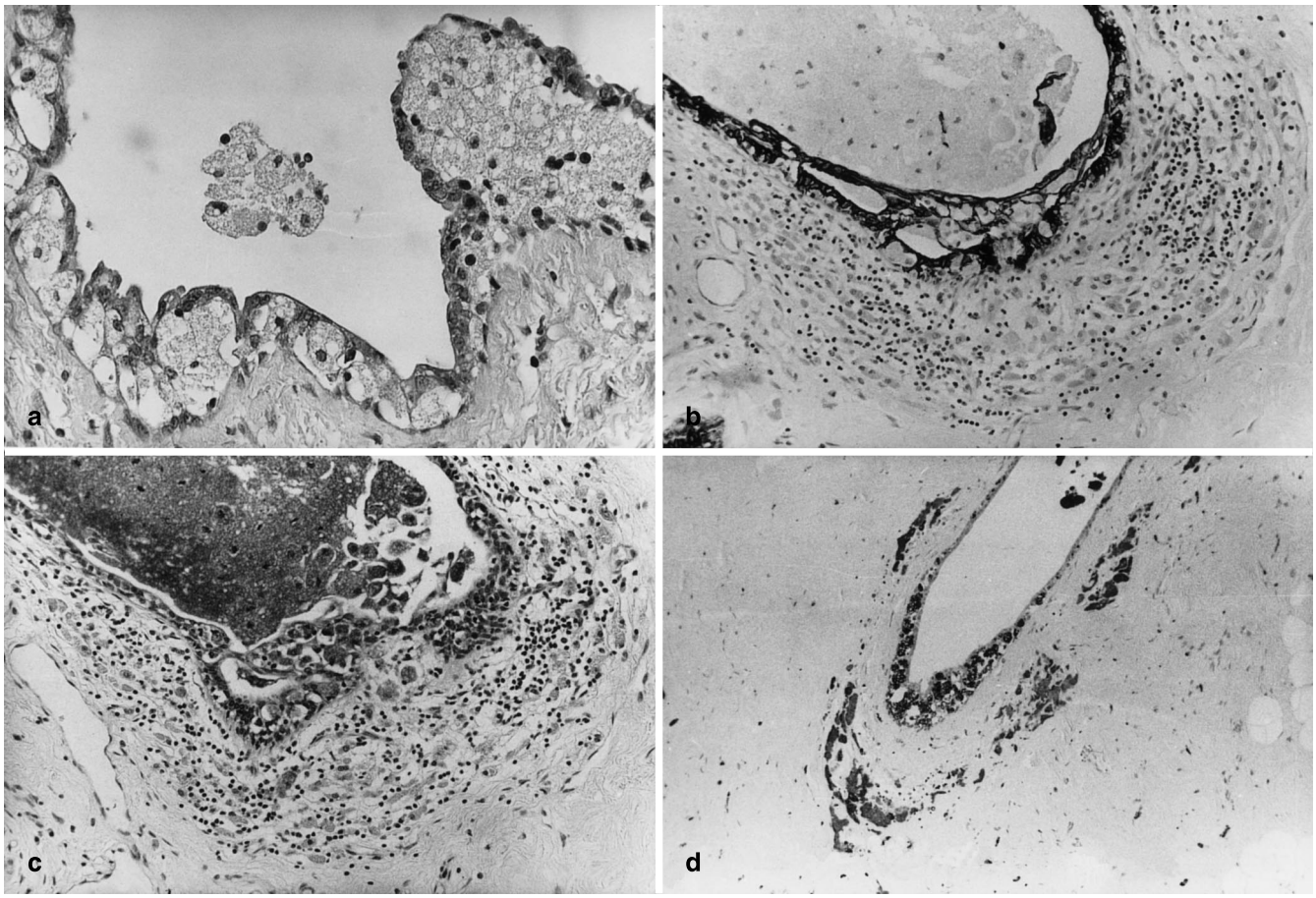

Fig. 4a–d Type B (macrophage-derived) and type C (intermediate) FCs. **a** In the haematoxylin-eosin section of this ectatic duct, FCs are evident within the thickness of the epithelial lining (pagetoid FCs) and within the lumen (intraluminal FCs). In the consecutive sections used for immunohistochemistry stromal FCs are also evident. The pagetoid FCs, in this case, show an "intermediate" profile (type C foam cells): there is **b** a peripheral cytoplasmic rim positive with anti-cytokeratin antibody and **c** strong staining with anti-GCDFP15. However, they do not express PIP/GCDFP15 mRNA (not shown) and are CD68 positive (**d**). The intraluminal and stromal FCs present in this same duct show a type B (macrophage-derived) profile: they are positive with GCDFP15 (**c**) and CD68 antisera (**d**) but completely negative with anti-cytokeratin (**b**) and with in situ hybridization for PIP/GCDFP15 mRNA (not shown). Original magnification: **a** ×400, **b**, **c** ×200, **d** ×100

Table 4 Immunoreactivity of mammary foam cells in different microanatomical districts (*FCs* foam cells, *GCDFP15* gross cystic disease fluid protein antiserum), *MNF116* wide spectrum cytokeratin, *EMA* epithelial membrane antigen, *MAC387* myeloid histiocyte antigen, *CD68* KP1 antiserum

Microanatomical locations	Antisera (No. of positive cases/no. of tested cases[b])				
	GCDFP15	MNF116	EMA	MAC387	CD68
FCs lining glandular lumina (22 cases[a])	18/21	17/18	14/16	13/19	4/19
Pagetoid FCs (19 cases[a])	9/17	5/18	8/19	15/18	11/13
Intraluminal FCs (30 cases[a])	20/25	6/26	15/22	20/24	23/25
Stromal FCs (20 cases[a])	8/18	0/19	3/20	19/19	14/14

[a] Number of cases with foci of FC seen at haematoxylin-eosin level
[b] Number of cases with FC present in consecutive sections tested with immunohistochemistry

many instances the normal breast epithelium also appeared positive.

Staining with anti-CD68 antibody appeared somewhat more selective, and only 4 out of 19 cases had positive FCs lining the glandular spaces, whereas the results in stromal, intraluminal and intraepithelial-pagetoid FCs were similar to those found with anti-MAC387 antibody. CD68 staining was diffusely granular in some cells, while in others only few cytoplasmic vacuoles appeared to be stained (Figs. 2c, 4d).

FCs bordering the glandular lumina were seen in 6 of the 20 cases selected for ISH, while intraluminal, page-

Table 5 Apocrine differentiation in mammary foam cells: comparison between immunohistochemistry (*IHC*)[a] and in situ hybridization (*ISH*)[a]. Results shown are positive cases/total cases

Foam cells	IHC	ISH
FCs lining glandular lumina	6[b]/8	6/6
Intraluminal FCs	9[c]/10	3/10
Pagetoid FCs	5[c]/8	0/8
Stromal FCs	3/7	0/7

[a] IHC performed with GCDFP15 antiserum and ISH, with PIP/GCDPF15 riboprobe
[b] Including 2 CD68-positive cases
[c] Including cases showing a thin peripheral rim of positivity for anti-cytokeratin antiserum

toid and stromal FCs were present in 10, 8, and 7, respectively (Table 5). A comparison between IHC with anti-GCDFP15 antiserum and ISH is reported in Table 5: the pattern of staining was found to be somewhat different. Staining of the intraluminal secretion was never observed with the ISH method. All 6 cases of FCs bordering the glandular lumina showed expression of GCDFP15-PIP mRNA (including 2 of the cases showing CD68 positivity), and 4 of these same cases were also positive with anti-GCDFP15 antiserum (Figs. 1, 2). The remaining 2 cases were those in which FCs were lining the ductules of normal lobules.

GCDFP15 mRNA was also found in 3 cases with intraluminal FCs (Fig. 2) that had also expressed GCDFP15 antiserum. In contrast, no staining was observed in any case of stromal and intraepithelial-pagetoid FCs. These latter cells were also negative with ISH in the 5 cases in which IHC showed the expression of both cytokeratin and GCDFP15.

Discussion

Of all the cell types present in the breast, FCs have probably been most extensively investigated. Intraluminal FCs (colostrum cells) were initially considered to be made up of aggregates of fat droplets (Donnè, 1844, cited in [33]). After Reinhardt's demonstration of the cellular nature of the foamy corpuscles in the sebaceous secretion, a similar origin was suggested for the foamy elements found within mammary ducts [33]. Since then, many controversies have been raised by the fact that the foamy cytoplasm of these cells appears to be phenotypically reminiscent of phagocytic elements derived from circulating monocytes [5–7]; hence the term ocrocyte for foamy histiocytes rich in lipofuscin granules. Subsequently, it was shown that the same cytoplasmic foaminess was a feature of both neoplastic and non-neoplastic apocrine epithelium [12–14, 26].

In the present series, luminal FCs were observed to be variably associated with epithelial or stromal FCs, as previously described by Davies [5–7]. Neither histological nor cytological findings (distribution, pattern of aggregation, nuclear and cytoplasmic features) yielded information that would have been useful for distinguishing the nature of the different types of FCs, whether epithelial or histiocytic in origin. Therefore, for an IHC investigation, we used a panel of antibodies against either epithelial or macrophage-derived antigens.

MAC387 detects a 26-kDa protein localized within the cytoplasm of the resting macrophages and myeloid cells. Immunostaining with this antiserum, however, led to many problems of interpretation, as in most instances both the intraluminal secretion and the stroma appeared stained.

The other macrophage marker used in this study was CD68. This antibody appeared to be more specifically localized within the cell cytoplasm, without staining of intraluminal fluid or periductal stroma. As expected, all cases of periductal FCs and most cases of luminal and pagetoid FCs appeared to be CD68 positive. In addition, 4 of 19 cases of FCs bordering the gland lumina were also positive, thus raising the possibility of a macrophage origin for these cells, as proposed by Dabbs [4].

CD45RB antibody, the pan-leucocyte antigen, was not used because it is frequently absent from tissue macrophages and had been found consistently negative in the series reported by Dabbs [4]. Anti-lysozyme and anti-alpha 1 chimotrypsin antisera, which are markers of tissue macrophages, were not used because these can also be present in epithelial cells [22] and are occasionally found in invasive apocrine carcinomas (V. Eusebi, personal communication).

To investigate the epithelial origin of mammary FCs, the cases were immunostained with anti-cytokeratin and anti-EMA antisera and with anti-GCDFP15 antibody. This last is a marker of apocrine differentiation [23] and was included in the epithelial panel because FCs have one of the morphological phenotypes of apocrine differentiation [12, 13, 14, 26].

The patterns of immunostaining of GCDFP15 and EMA were superimposable: both the intraluminal fluid within cysts and ducts and most FCs, wherever located, were stained by these antibodies (Table 4). In occasional instances the periductal stroma also appeared to be stained, indicating the presence of a "diffusion phenomenon" [3, 11]. This phenomenon was overcome by ISH. PIP/GCDFP15 riboprobe reveals the presence of the specific mRNA in the genetic machinery of the cell, and accordingly it shows a perinuclear localization and is obviously absent from the luminal secretion and from macrophages [25]. Therefore, it appears that the presence of EMA or GCDFP15 in a given cell per se is not indicative of apocrine differentiation, as it may be the result of protein uptake or phagocytosis [2], while the evidence of mRNA PIP/GCDFP15 appears to be a more reliable indicator of apocrine differentiation.

Cytokeratin antibody stained almost all (17/18) cases of FCs lining the glandular lumina, as well as 5 and 6 cases of pagetoid and intraluminal FCs, respectively (Table 4). The same cases were also positive with GCDFP15 antibody. In addition, these cases in which cytokeratin-positive FCs were present, when tested with the PIP-GCDFP15-specific riboprobe, showed positive

Table 6 Immunophenotypical and molecular types of mammary foam cells (*PIP* Prolactin inducible protein mRNA, *APO* gross cystic disease fluid protein 15 antiserum, *EMA* epithelial membrane antigen, *CK* broad-spectrum (MNF116) cytokeratin antiserum, *CD68* KP1 antiserum)

TYPE A Epithelial foam cells (FCs lining glandular lumina and intraluminal FCs)	TYPE B Macrophagic foam cells (Stromal, intraluminal and pagetoid FCs)	TYPE C Intermediate foam cells (Intraluminal and pagetoid FCs)
PIP+	PIP–	PIP–
APO+	APO±	APO+
EMA+	EMA±	EMA+
CK+	CK–	CK+[a]
CD68±	CD68+	CD68+

[a] thin peripheral rim of CK positivity

staining in all cases of FCs lining glandular lumina and in 3 of 6 cases of intraluminal FCs tested. In contrast, all cases of pagetoid-keratin-positive FCs were negative (Table 5). Both cytokeratin antibody and ISH with PIP/GCDFP15-specific riboprobe gave negative results in all cases of stromal FCs tested (Tables 4, 5).

By comparing the results yielded by the panel of epithelial and nonepithelial antibodies with those obtained by ISH, we were thus able to define the phenotype of most of the FCs studied, identifying three different types of mammary FCs (Table 6):

Type A FCs have an epithelial phenotype (Figs. 1, 2), being cytokeratin positive and showing apocrine differentiation confirmed by the expression of GCDFP-15 and by the presence of specific mRNA encoding the same protein. Some type A FCs were also CD68 immunoreactive (Fig. 2). This is probably a consequence of the lysosome richness of a cell rather than its histiocytic origin. In fact, the detection of CD68 within lysosome-rich cells of various types (granular cells, neural and myoid tumours, normal hepatocytes, renal glomerules and tubules) [8, 27, 28, 31] is indicative of a lysosome-directed specificity rather than of a true macrophage-derived antigen.

Type B FCs are consistent with a macrophage lineage (Figs. 3, 4). They are lysosome-rich cells that lack cytokeratin, and they do not show apocrine differentiation, being negative by the ISH method.

Finally, type C FCs are those with an “intermediate” IHC and molecular profile, between epithelial and macrophage elements. Type C FCs are lysosome-rich cells (CD68 positive), have a peripheral cytoplasmic rim that is positive with anti-cytokeratin antiserum, and show EMA and GCDFP15 staining (Fig. 4). Nevertheless, they lack PIP/GCDFP15 mRNA expression.

Our results indicate that, at least in some cases, mammary FCs are not of monocytic origin, but are epithelial cells showing apocrine differentiation. Prominent cytoplasmic foaminess is a well-known feature of apocrine cells and is related to the ultrastructural finding of numerous empty vesicles, which entirely fill the cytoplasm [14]. The empty vesicles can occasionally coalesce and produce diffuse clearing of the cytoplasm, which is indistinguishable at the H-E level from other types of clear cell change that are encountered in the breast [9].

The finding of intermediate-type FCs (type C) is intriguing. The presence of numerous lysosomes in nonmonocytic cells usually reflects increased cell turnover. It is well known that degenerating cells are characterized by a considerable increase in the content of lysosomes (autophagic vacuoles), and thus, for example, in ancient schwannomas the number of lysosome-rich (CD68+) Schwann cells is much higher than in ordinary schwannomas [8]. Alternatively, the high content of lysosomes suggests the possibility of phagocytic properties.

In vertebrates, two types of phagocytes can take part in the elimination of necrotic debris: those derived from circulating monocytes and those that are resident tissue cells [1]. Phagocytosis of necrotic cells by neighbouring resident cells is an essential aspect of the process of apoptosis [32]. Constitutively, cells of nonphagocytic lineage that can become phagocytes have been described in cardiac myocytes and in various ectodermal cells in animals [16, 19]. Recently, this possibility has also been reported in adult human muscle cells [15]. Finally, fragments of red blood cells have been observed within the cytoplasm of adrenal cortical cells, both in normal and in pathologic conditions [17, 30]. Therefore, the identification of a subset of FCs (type C) showing numerous lysosomes and faint epithelial markers might indicate a degenerative phenomenon or, alternatively, the possibility that under specific stimuli such as the accumulation of intraluminal secretion, epithelial mammary cells can take part in the elimination of the debris, acquiring phagocytic properties. This action occurs in other specialized, constitutively nonphagocytic cells. Although further ultrastructural and in vitro studies appear to be necessary to confirm our results, these findings may help to enhance our understanding of physiological and pathological mechanisms in the breast.

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References

1. Beaulaton J, Lockshin RA (1982) The relation of programmed cell death to development and reproduction: comparative studies and an attempt at classification. Int Rev Cytol 79:215
2. Bussolati G, Gugliotta P, Morra I, Pietribiasi F, Berardengo E (1986) The immunohistochemical detection of lymph node metastases from infiltrating lobular carcinoma of the breast. Br J Cancer 54:631–636

3. Carcangiu ML, Sibley RK, Rosai J (1985) Clear cell changes in primary thyroid tumors. A study of 38 cases. Am J Surg Pathol 9:705–722
4. Dabbs JD (1993) Mammary ductal foam cells: macrophage immunophenotype. Hum Pathol 24:977–981
5. Davies JD (1973) Hyperelastosis, obliteration and fibrous plaques in major ducts of the human breast. J Pathol (Lond) 110:13
6. Davies JD (1974) Human colostrum cells: their relation to periductal mononuclear inflammation. J Pathol (Lond) 112: 153–160
7. Davies JD (1975) Periductal foam cells in mammary dysplasia. J Pathol (Lond) 117:39–45
8. Dei Tos AP, Doglioni C, Laurino L, Fletcher CDM (1993) KP1 (CD68) expression in benign neural tumours. Further evidence of its low specificity as a histiocytic/myeloid marker. Histopathology 23:185–187
9. Dina R, Eusebi V (1997) Clear cell tumors of the breast. Semin Diagn Pathol 14:175–182
10. Engel S (1953) An investigation of the origin of the colostrum cells. J Anat 87:362
11. Eusebi V, Bondi A and Rosai J (1984) Immunohistochemical localization of myoglobin in nonmuscular cells. Am J Surg Pathol 8:51–55
12. Eusebi V, Millis RR, Cattani MG, et al (1986) Apocrine carcinoma of breast. Am J Pathol 123:532–541
13. Eusebi V, Foschini MP, Bussolati G, Rosen PP (1995) Myoblastomatoid (histiocytoid) carcinoma of the breast. A type of apocrine carcinoma. Am J Surg Pathol 19:553–562
14. Eusebi V, Damiani S, Losi L, Millis RR (1997) Apocrine differentiation in breast epithelium. Adv Anat Pathol 4:139–155
15. Fidzianska A and Goebel HH (1992) Phagocytic capability of human muscle fibers. Hum Pathol 23:1044–1047
16. Garcia-Porrero JA, Collado JA, Ojeda JL (1979) Cell death during detachment of the lens rudiment from ectoderm in the chick embryo. Anat Rec 193:791
17. Glasgow BJ, Steinsapir KD, Anders K, Layfield LJ (1985) Adrenal pathology in the acquired immune deficiency syndrome. Am J Clin Pathol 84:594–597
18. Hsu SM, Raine L, Fanger H (1981) Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques. A comparison between ABC and unlabeled antibody (PAP) procedures. J Histochem Cytochem 29:557–580
19. Hurle JM, Lafarga M, Ojeda JL (1977) Cytological and cytochemical studies of the necrotic area of the bulbus of of the chick embryo heart: phagocytosis by developing myocardial cells. J Embryol Exp Morphol 41:161
20. King EB, Duarte LA, Thor A, Petrakis NL (1991) Characterization of foam cells in breast tissue using monoclonal antibodies for epithelial and non-epithelial antigens. Acta Cytol 35:583–656
21. Lee CS, McDowell GH, Lascelles AK (1969) The importance of macrophages in the removal of fat from the involuting mammary gland. Res Vet Sci 10:34
22. Mason DY, Taylor CR (1975) The distribution of muramidase (lysozyme) in human tissues. J Clin Pathol 28:124–132
23. Mazoujian G, Pinkus GS, Davis S, Haagensen DE Jr (1983) Immunohistochemistry of a gross cystic disease fluid protein (GCDFP15) of the breast. A marker of apocrine epithelium and breast carcinomas. Am J Pathol 110:105–112
24. McGillivray JB (1969) The problem of “chronic mastitis” with epitheliosis. J Clin Pathol 22:340
25. Pagani A, Eusebi V, Bussolati G (1993) Detection of PIP-GCDFP15 gene expression in apocrine epithelium of the breast and salivary glands. Appl Immunohistochem 2:29–35
26. Pagani A, Sapino A, Eusebi V, Bergnolo P, Bussolati G (1994) PIP/GCDFP15 gene expression and apocrine differentiation in carcinomas of the breast. Virchows Arch [A] 425:459–465
27. Pulford KA, Sipos A, Cordell JL, Stross WP, Mason DY (1990) Distribution of the CD68 macrophage/myeloid associated antigen. Int Immunol 2:973–980
28. Roncaroli F, Rossi R, Severi B, Martinelli GN, Eusebi V (1993) Epithelioid leiomyoma of the breast with granular cell changes: a case report. Hum Pathol 24:1260–1263
29. Sandison AT, Walker JC (1962) Inflammatory mastitis, mammary duct ectasia and mammillary fistula. Br J Surg 50:57
30. Symmington T (1969) Functional pathology of the human adrenal gland. Williams & Wilkins, Baltimore, pp 68–86
31. Tsang WYW, Chan JKC (1992) KP1 (CD68) staining of granular cell neoplasms: is KP1 a marker for lysosomes rather than the histiocytic lineage? Histopathology 21:84–86
32. Wyllie AE, Kerr JFR, Currie AR (1980) Cell death: the significance of apoptosis. Int Rev Cytol 68:251
33. Virchow R (1858) Fünfzehnte Vorlesung, 10 April 1858. Passive Vorgänge. Fettige Degeneration. In: Die Cellularpathologie. August Hirschwald, Berlin, pp 285–307