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Phenotypic expression of immune secretory function in Focal Pregnancy-like Change of the human breast

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Summary. Fibrocystic disease of the breast in middle-aged women characteristically shows focal epithelial lesions of a very varied nature. The functional immunohistochemical changes in such lesions have been little studied. Focal Pregnancy-like Change in the breast has a striking morphological similarity to the secretory breast lobules in pregnancy and in lactation. We show that the epithelial cells in all the lesions of Focal Pregnancy-like Change studied simultaneously express secretory component, Ig A and J chain in their cytoplasm. Additionally these epithelial cells, unlike those in resting breast lobules, contain lysozyme and lactoferrin. All these phenotypic immunohistochemical changes in Focal Pregnancy-like Change resemble the breast lobules of late pregnancy and lactation. Possibly, the very focality of Focal Pregnancy-like Change reflects a peculiar and local sensitivity of 'resting' breast lobules to unidentified hormonal stimulation.

Key words: Breast – Focal Pregnancy-like Change – Secretory component – Ig A – J chain – Lysozyme – Lactation – Lactoferrin

Introduction

Focal Pregnancy-like Change in the breast is an unusually localised epithelial lesion, the morphology of which on light microscopy resembles the more diffuse epithelial changes seen in the breast during pregnancy and lactation. (Kiaer and Anderson 1977). The lesions were first recognised and fully described by McFarland (1922). They are composed of foci of oval and columnar, vacuolated epithelium arranged in acini which resemble the lobules in pregnancy. McFarland himself thought that they were caused by delayed involution of lactating acini, and accordingly called them Residual Lactational Acini. However their occurrence in nulliparous women and in men on oestrogen therapy render this hypothesis invalid (Sandison 1962, Schwartz and Wilens 1963, Kiaer and Anderson 1977). For these reasons it has been suggested that the lesions represent a local susceptibility of the glandular tissue of the breast to oestrogens (Kiaer and Anderson 1977). A later ultrastructural study indicated that Focal Pregnancy-like Change may result from both a lactational stimulus and an involutional defect (Mills and Fraite 1981). Between three and five percent of normal breasts examined *post mortem* contain these focal lesions (Frantz et al. 1951, Sandison 1962).

The aim of the present study was to investigate the functional capacity of Focal Pregnancy-like Change by means of immunohistochemistry. We describe the expression of the three principal components (Tourville et al. 1969, Isaacson 1982, Brandtzaeg 1983) of the immune secretory transport system (i.e. secretory component, Ig A and J chain), together with other unrelated secretory proteins in the form of lysozyme and lactoferrin. Our findings suggest that Focal Pregnancy-like Change in the human breast closely resembles the phenotypic epithelial alterations of pregnancy and lactation in both the functional and morphological aspects.

Materials and methods

Patients. A total of 531 cases of fibrocystic disease of breast, as defined by the World Health Organization (1981), diagnosed in the University Department of Pathology at the Bristol Royal Infirmary in a four-year period (Jan 1973–Dec1976) was screened retrospectively for the presence of Focal Pregnancy-

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like Change by examination of stored haematoxylin and eosin-stained sections. The modal age of these patients was 41 to 50 years, range 19 to 72 years. From the relevant paraffin blocks 10 serial sections, 5 μm thick, were cut for immunohistochemistry. Both the lesions of Focal Pregnancy-like Change and 'normal' resting lobules unaffected by fibrocystic disease were studied immunohistochemically. Additionally, paraffin blocks from normal breast tissue of three lactating women (aged 29 to 35 years) and one woman (aged 26 years) who was 21 weeks pregnant were used. The breast biopsies in these women were undertaken for fibroadenoma (two cases), and galactocoele (two cases), one of which was clinically thought to be malignant.

All the tissues had been fixed in freshly prepared unbuffered formal saline and processed as described before (Davies et al. 1983).

Immunoperoxidase method. Immunolocalisation of secretory component, Ig A, J chain, lysozyme, lactoferrin, Ig G and Ig M was performed by means of an indirect peroxidase-antiperoxidase technique. The paraffin sections were dewaxed and hydrated. Endogenous peroxidase was inactivated by treatment with 0.5% H_2O_2 in methanol for 30 min. The sections were then submitted to preliminary trypsinisation (Mera et al. 1985), using 0.1% general-purpose grade trypsin (Flow Laboratories) solution for 30 min at 37° C. The treated sections were incubated for 45 min, having been flooded with 100 μl per section of the primary antisera (all from Messrs Dako), diluted 1 in 200 with 0.05 M tris-HCl buffer pH 7.6. After washing twice in this buffer for a total of 10 min, the sections were then immersed in swine anti-rabbit Ig (Dako) diluted 1 in 60 with tris-HCl for 40 min. After rinsing in the same buffer they were incubated for 30 min with 100 ml of rabbit horseradish peroxidase-antiperoxidase (Dako) diluted 1 in 40 with tris-HCl.

After two rinses in tris-HCl, a 0.05% solution of 3,3 diaminobenzidine HCl (Sigma St. Louis, MO, USA) in tris-HCl buffer pH 7.6 containing 0.01% hydrogen peroxide as substrate, added immediately before use, was then applied to the sections for five minutes. The sections were counterstained with Meyer's haematoxylin, dehydrated, and mounted in DPX. Controls for immunospecificity included omission of the primary antiserum and absorption of the primary antisera with their appropriate immunogens (obtained from Messrs Behring, FRG, or in the case of lysozyme from Dr. E.F. Osserman, New York) in a series of progressive dolutions (Mera et al. 1985). These absorbtions were performed at room temperature for 16 h as described earlier (Davies et al. 1982).

Results

Examination of the 531 cases of benign fibrocystic disease of breast revealed 10 (1.9%) in which Focal Pregnancy-like Change was found. All 10 lesions were extremely focal, being restricted to a single paraffin block. The cases of fibrocystic disease showed an expectedly wide age range, and those in which Focal Pregnancy-like Change was found did not depart from this general age distribution. Similarly, there were Focal Pregnancy-like Change lesions in all classes of parity in the series (3: para 1; 3: para 2; 3: para 3+). The single nulliparous woman with Focal Pregnancy-like Change had received no oral contraceptives, oestrogen or progesterone therapy.

For a very focal lesion it might be expected that the thoroughness of examination would bear some relationship to the frequency of detection, and this proved the case for Focal Pregnancy-like Change in our material. The number of paraffin blocks examined in the cases ranged from 1 to 35. Unlike the distribution of Focal Pregnancy-like Change when compared with age and parity, there was a positive skew when the cases and the number of paraffin blocks were considered. With increasing numbers of blocks the frequency of detection increased. No instance of Focal Pregnancy-like Change was found in any case from which only one or two blocks were examined; conversely seven of the ten cases of Focal Pregnancy-like Change were discovered in fibrocystic mastopathies with five or more blocks examined, which constituted only 39.5% of the mastopathic lesions ($0.1 > P > 0.05$).

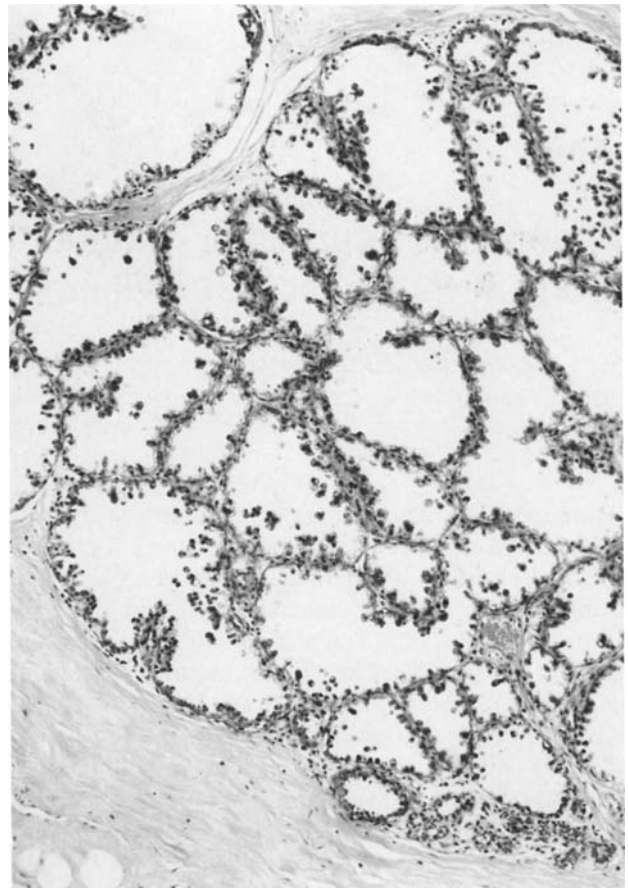

Fig. 1. Part of a lobule showing Focal Pregnancy-like Change. The acinar dilatation is a prominent feature. Haematoxylin and eosin. ×71

Morphological findings

Although the light-microscopic appearances of the epithelium in Focal Pregnancy-like Change bore

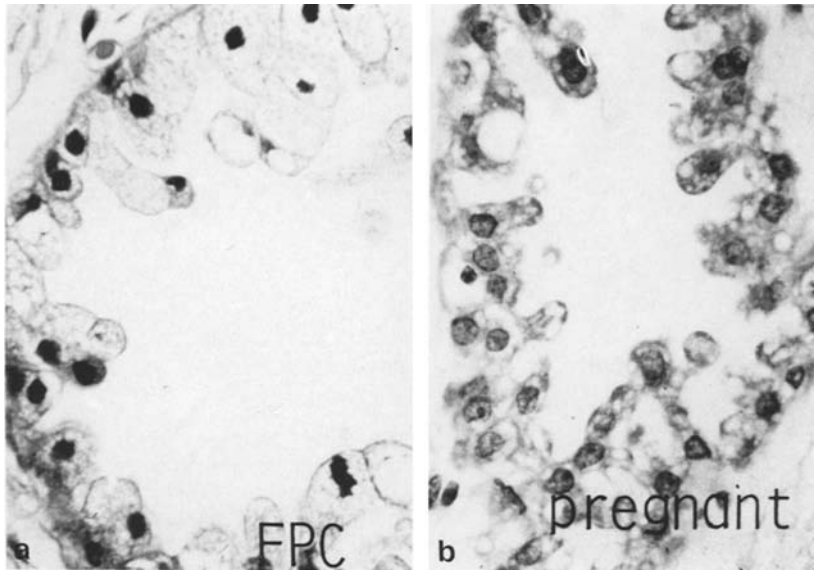

Fig. 2. The epithelium in Focal Pregnancy-like Change (FPC, *left* **a**) resembles pregnant breast (*right* **b**) in the cytoplasmic vacuolation. The nuclear condensation of chromatin in Focal Pregnancy-like Change contrasts with the open network in pregnancy (*right* **b**). Haematoxylin and eosin. ×355

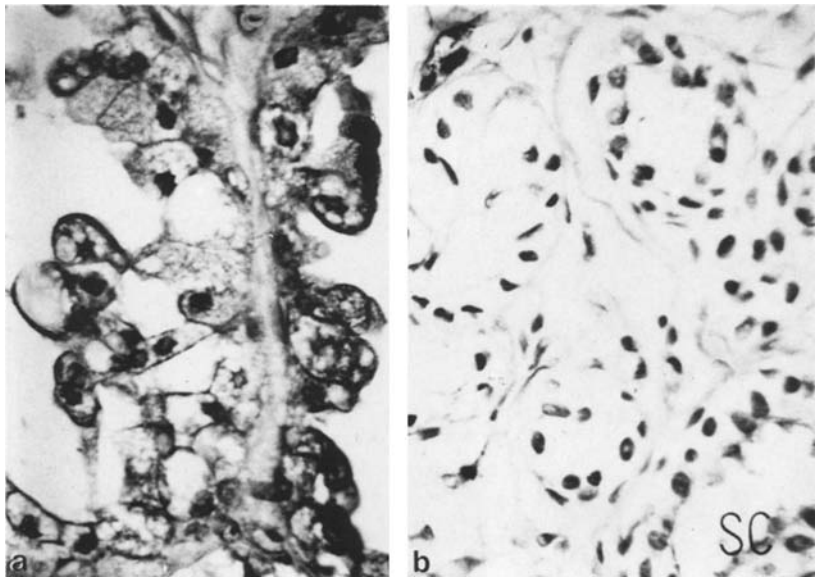

Fig. 3. Immunostaining for secretory component (SC) in, *left* **a**, Focal Pregnancy-like Change, and (*right* **b**) a resting breast lobule. The general cytoplasmic staining of the Focal Pregnancy-like Change (*left*) contrasts with the lack of staining in the normal lobule (*right*). PAP and nuclear counterstain. ×355

a distinct similarity to the more widespread alterations in late pregnancy and lactation, the change in our cases was confined to one single lobule. The affected lobules were distended (Fig. 1), with granular material in the dilated acini. The epithelium of the lobules was cuboidal and its cytoplasm displayed conspicuous vacuolation (Fig. 2a), which more pronounced than in pregnancy (Fig. 2b). The nuclei of the cells in Focal Pregnancy-like Change also exhibited a rather more condensed chromatin than in pregnant or lactating lobules (Fig. 2). The microvacuolar appearance of the epithelium of the Focal Pregnancy-like Change differed sharply from the 'resting' lobules in the other parts of the breast biopsies.

Immunohistochemistry

Seven immunoreactive proteins, namely, secretory component, Ig A, J chain, lysozyme, lactoferrin, Ig G and IgM were sought in the lesions of Focal Pregnancy-like Change, the lobules and ducts of the resting breasts, and those of pregnancy and lactation. The differences in their distribution in these classes of breast are detailed below.

Focal Pregnancy-like Change

All 10 cases displayed similar histochemical reactions. The epithelial cytoplasm in these lesions uniformly showed the presence of secretory component (Fig. 3), IgA (Fig. 4) and J chain. In most

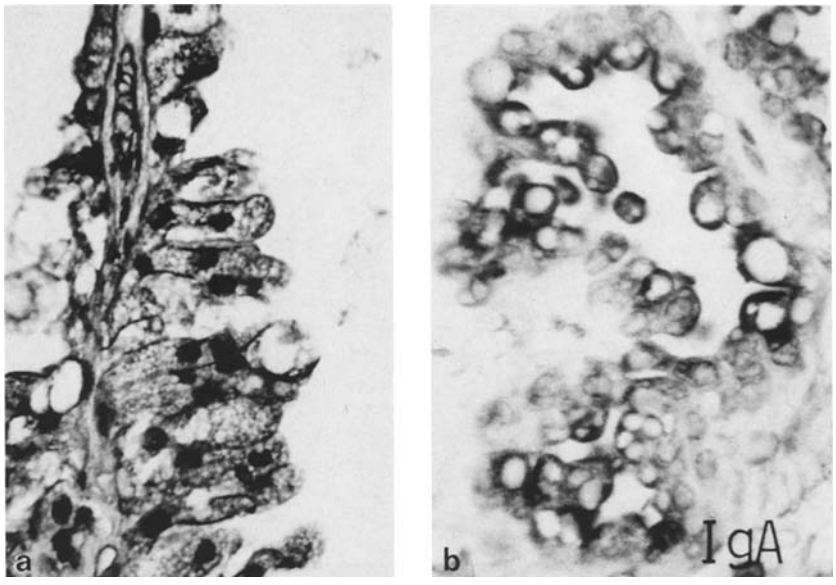

Fig. 4. Similar distribution of cytoplasmic immunostaining for Ig A in the epithelium of Focal Pregnancy-like Change (*left* **a**) and a pregnant breast lobule (*right* **b**). PAP and nuclear counterstain. ×355

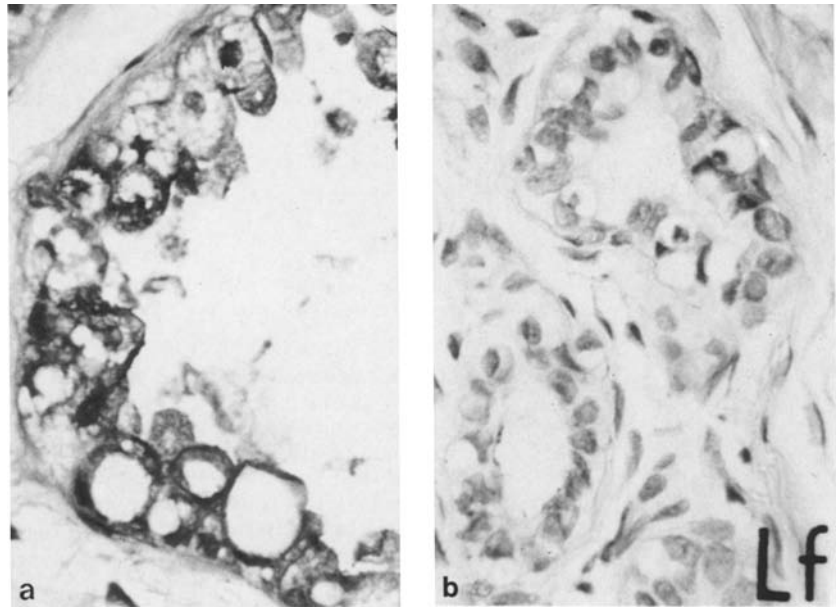

Fig. 5. Marked immunostaining in the cytoplasm of all the epithelial cells in Focal Pregnancy-like Change, *left* **a**, for lactoferrin (Lf) contrasts with complete absence of immunoreactivity in a resting lobule, **b**, on the *right*. PAP and nuclear counterstain. ×355

cases, although these proteins were widely distributed throughout the cytoplasm, the immunoreactivity was especially marked towards the luminal margins of the cells (Figs. 3a, 4a). In addition all the lesions of Focal Pregnancy-like Change showed epithelial immunostaining for both lysozyme and lactoferrin (Fig. 5). This pattern of immunohistochemical staining was universal within the lesions: all epithelial cells displayed these changes.

Resting breast ducts and lobules

Resting breast lobules from the 10 cases of Focal Pregnancy-like Change, and 27 others chosen at random from the series of 531 fibrocystic mastopathies, were also uniform in their immunohistochemical reactions. None exhibited any immunoreactivity in their central cytoplasm for secretory component, Ig A, J chain, lysozyme, lactoferrin, Ig G or Ig M. A few lobules contained Ig A within their lumina, and the inner margin of a few epithelial cells was stained for secretory component. In general, however, the immunostaining for secretory component of resting lobules differed markedly from Focal Pregnancy-like Change (Fig. 3). Scattered epithelial cells in the resting ducts showed weak immunoreactivity for secretory component, Ig A, J chain, lysozyme and lactoferrin. In addi-

tion, the coagulated secretion within the lumina of the lobules in the resting breasts did occasionally show immunoreactivity for all the proteins studied, with the notable exceptions of the immunoglobulins Ig G and Ig M.

Breasts in pregnancy and lactation

Breast tissue from all four pregnant or lactating women showed uniform immunohistochemical reactions in the lobular epithelium. The cytoplasm of these cells was diffusely immunoreactive for secretory component, Ig A (Fig. 4b), J chain, lysozyme, and lactoferrin (Fig. 5). None of the epithelial cells contained Ig G or Ig M. Although the secretion of lactating or pregnant breasts was uniformly unstained by the antiserum for Ig G, there was focal immunostaining for Ig M in the secretion of some lobules.

Interstitial connective tissue

The luminal coagula in blood vessels were usually immunostained for Ig G in all types of breast tissue studied. Scattered plasma cells containing Ig G or Ig A were also seen in all types of breast. The Focal Pregnancy-like Change lesions differed from pregnancy and lactation in showing only occasional Ig A-containing plasma cells, whereas Ig A plasma cells predominated in pregnancy and lactation.

Discussion

Many changes take place in the morphogenesis of the human breast (Raynaud 1961). Their hormonal control is only gradually being understood (Kratochwil 1986). Pregnancy is a further major morphological and functional change, and many of its modifications are reflected in the immunohistochemical changes in phenotype described in this paper. In non-pregnant states, or early in pregnancy, focal secretory changes may occur (Fechner 1970; Taylor 1971; Craigmyle 1984). Later in life Focal Pregnancy-like Change is seen occasionally, even long after the menopause (McFarland 1922; Sandison 1962). The biological significance of Focal Pregnancy-like Change is uncertain. It may occur in hyperprolactinaemic states (Brown et al. 1982), but does not show any association with breast carcinoma. Furthermore it differs from the vast majority of breast carcinomas in expressing the three proteins of the immune secretory transport system, which are not seen in most breast carcinomas (Rossiello et al. 1984; Al Sam and Davies 1985), with the exception of medullary carcinomas (Hsu et al. 1981).

Functionally the lactating breast appears to be a member of the immune secretory transport system (Hurlimann et al. 1976; Brandtzaeg 1983). As with the mammae of other species the breast confers protection on the neonate, via its secretions, and has local tissue defences (Watson 1980). The protective mechanisms are effected by a combination of leucocytes, various classes of immunoglobulin, lysozyme and lactoferrin (Watson 1980; McCarty et al. 1982; Brandtzaeg 1983). Our immunohistochemical findings in Focal Pregnancy-like Change closely resemble earlier studies of the lactating mamma in man and other animals (Brandtzaeg 1983).

Secretory component is a glycoprotein synthesised by epithelial cells lining the gastroinestinal tract, the respiratory mucosa, the urinary bladder and various exocrine glands (Tourville et al. 1969; Brandtzaeg 1974, 1977; Weisz-Carrington et al. 1976, 1978; Kirkham et al. 1983). The expression of secretory component in Focal Pregnancy-like Change, as demonstrated in our study, resembles late pregnancy and lactation, but clearly differs from resting lobules in the non-pregnant state.

Ig A and J chain, which are also part of the immune secretory system (Tourville et al. 1969; Brandtzaeg 1974, 1977), are codistributed with secretory component in the lesions of Focal Pregnancy-like Change. Both Ig A and J chain are synthesised by plasma cells (Isaacson 1979), and are covalently bonded before uptake by the epithelial cells of the breast. Their immunohistochemical presence in the cytoplasm of epithelium reflects active transport across the cells towards the lumen and ultimate excretion into the luminal contents (Sletten et al. 1975; Isaacson 1982).

Lysozyme, otherwise known as muramidase, and lactoferrin (Mason and Taylor 1975, 1978) are well recognised components of a variety of human mucosal secretions with local and neonatal protective functions (Bullen et al. 1972; Watson 1980; Brock 1980). Although we found both these proteins in the secretions of some resting breasts, neither we nor others (Mason and Taylor 1975, 1978; Brock 1980) have detected them in the epithelium of resting breast lobules. Possibly their presence, revealed in the present study in occasional cells of medium-sized ducts accounts for the immunohistochemical detection of lysozyme and lactoferrin in the luminal secretion of resting breasts. Such observations would accord with the observed accumulation of fluid secretion in non-pregnant breasts with duct ectasia (Dossett 1958; Thomas et al.

1982), a condition especially prevalent in women after the reproductive era (Davies 1971) when lobular atrophy is usually well advanced (Hutson et al. 1985). In addition, Weisz-Carrington et al. (1978) have shown that the immune secretory system displays most activity in the lobules of pregnant and lactating mice, with little activity in virgin animals.

The age distribution of our cases of Focal Pregnancy-like Change did not differ from the parent population of fibrocystic disease of the breast, nor was there any significant departure from the parity of such cases. There is no reason to believe that taking contraceptive hormones leads to Focal Pregnancy-like Change (Kiaer and Anderson 1977; Mills and Fechner 1980; Mills and Fraite 1981). A possible explanation of the change is that there is a very localised responsiveness of the breast parenchyma to subtle differences in the hormonal milieu. Perhaps prolactin (Brown et al. 1982) is responsible.

The essentially localised nature of Focal Pregnancy-like Change is confirmed in our study. The slightly lower incidence of the lesions in our series is probably explained by the fewer blocks examined in otherwise routine surgical biopsies. Other series (Sandison 1962; Kiaer and Anderson 1977) with more blocks per case found a higher incidence. Our immunohistochemical findings resemble those of Bailey et al. (1982) studying α-lactalbumin in pregnancy, lactation and in Focal Pregnancy-like Change. It is evident that functional change in epithelial phenotype with the biosynthesis of a wide variety of secretory proteins, including activation of the immune secretory transport system, may occur in the human breast in circumstances beyond pregnancy and lactation.

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