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Intraepithelial lymphocytes and macrophages in the normal breast

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Summary. In this study the presence of intraepithelial cells within the normal breast parenchyma was investigated by electron microscopy and immunocytochemistry. Cells were observed which could be differentiated from the epithelial and myoepithelial cells by their cytoplasmic and nuclear morphology and the absence of cell junctions. Two cell types (lymphocytes and macrophages) were identified ultrastructurally and the bone marrow origin of the cells was confirmed by immunocytochemistry. The intraepithelial lymphocytes and macrophages were present in all samples irrespective of the physiological state. In the "resting", pregnant, and lactating breast the majority of cells were lymphocytes while in the involuting breast there was a marked increase in the proportion of macrophages. The rarity of lymphoma of the breast may be related to the relatively small amount of lymphoid tissue present and the passive nature of the environment.

Key words: Breast – Lymphocytes – Macrophages – Ultrastructure – Immunocytochemistry

Introduction

There have been a number of ultrastructural studies on the normal breast (Ahmed 1978; Fanger and Ree 1974; Ozzello 1971; Salazar and Tobon 1974; Smith et al. 1984; Stirling and Chandler 1976 and 1977; Strum et al. 1983; Tannenbaum et al. 1969; Toker 1967; Waugh and van Der Hoeven 1962). Although these reports have universally described the parenchyma as containing epithelial and myoepithelial cells there have been numerous inconsistencies in the identification of subpopulations of these cells and the existence of additional cell types. The main confusion concerns the identity of electron-lucent cells situated between the epithelial and myoepithelial cells. These basal clear cells have been thought to represent either epithelial cells (Toker 1967), possibly stem cells (Salazar and Tobon 1974)

or lymphocytes and macrophages (Stirling and Chandler 1976). In a recent paper, Smith et al. (1984) identified the cells as epithelial cells leaving the existence of intraepithelial lymphocytes and macrophages unresolved.

In this paper the basal clear cells will be characterized using electron microscopy and immunocytochemistry. In addition the distribution of these cells within the "resting", pregnant, lactating, and involuting breast will be described.

Materials and methods

The normal tissue examined in this study consisted of samples from biopsies of "resting" (30 patients), pregnant (9), lactating (6), and involuting (2) breast. The samples were taken from normal areas of biopsies in which the final diagnosis was normal breast or fibroadenoma except for the two samples of involuting breast which contained carcinoma. The preparation of the tissue for electron microscopy was as described previously (Ferguson and Anderson 1981a and b) but can be summarised as follows: the tissue was fixed in glutaraldehyde, post fixed in osmium tetroxide, and embedded in Araldite or Emix. The thin sections were examined with either a Philips 301 or Jeol lOOCX electron microscope after staining with uranyl acetate and lead citrate.

Immunocytochemistry. The cell lineage of the intraepithelial cells was examined using the monoclonal antibodies PD7/26 which react only with cells of bone marrow origin (Warke et al. 1983) and E29 which reacts with cells of epithelial origin (Cordell et al., submitted). Sections of formalin fixed/paraffin embedded samples were reacted with the antibodies by the PAP method using suitable controls which included the omission of the primary antibody.

Quantitation. The approximate number of intraepithelial cells to total number of parenchymal cells was quantified using the immunocytochemical stained sections. The results were based on the average obtained for five randomly selected lobules of each case. The ratio of lymphocyte to macrophages was obtained from the ultrastructural examination based on thin sections from three different blocks.

Results

The parenchyma of the breast was formed by a single layer of epithelial cells with underlying myoepithelial cells (Fig. 1). The ultrastructural appearances of the epithelial and myoepithelial cells were as described previously for the "resting" (Stirling and Chandler 1976), pregnant (Ferguson and Anderson 1983a) and lactating (Ferguson and Anderson 1983b) breast.

In addition to the epithelial and myoepithelial cells a number of basally positioned clear cells with dense nuclei were observed by light microscopy in all physiological states (Fig. 2). Ultrastructurally the electron-lucent cells were observed situated between the epithelial and myoepithelial cells (Figs. 3 and 6). They were differentiated from the epithelial and myoepithelial cells by their light staining cytoplasm and the absence of cell junctions (Fig. 4). Two types of intraepithelial cells were identified.

(i) *Lymphocytes.* These cells had a large nuclear to cytoplasmic ratio. They had a characteristic nuclear morphology with large amounts of peripherally located dense heterochromatin (Figs. 1, 3 and 4). The light staining cytoplasm contained few organelles. A small Golgi apparatus, centrioles, a few

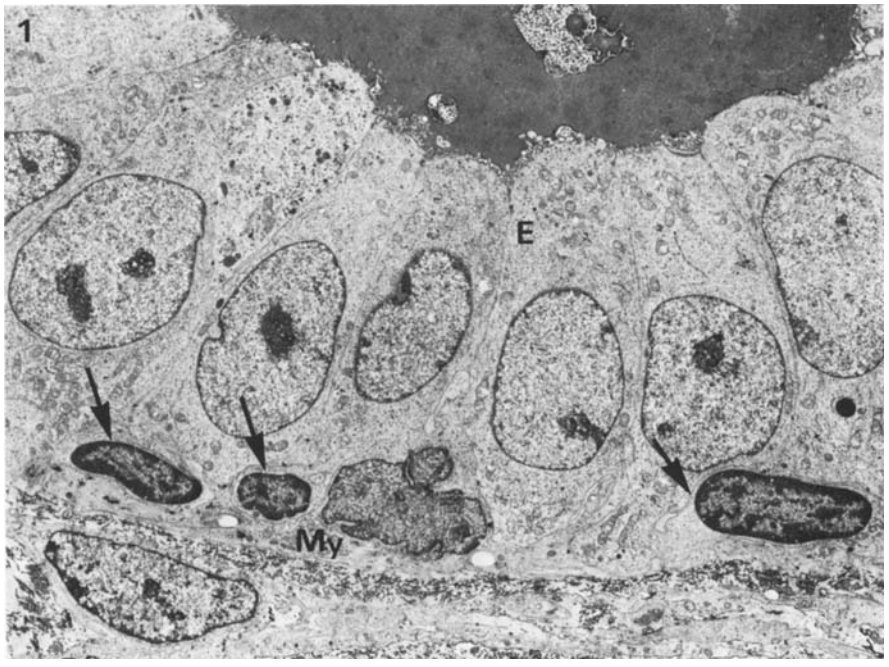

Fig. 1. Electron micrograph of part of a ductule from a "resting" breast. Note the three intraepithelial lymphocytes (*arrows*) between the epithelial (*E*) and myoepithelial cells (*My*). ×3,600

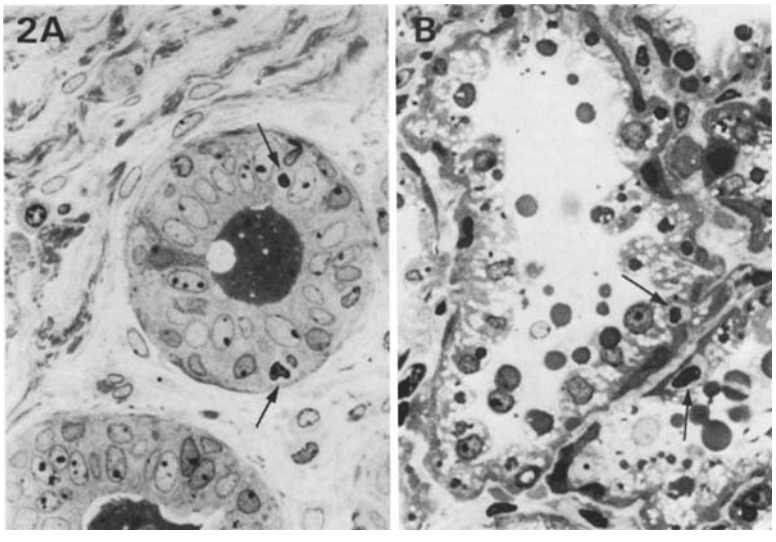

Fig. 2 A, B. Light micrographs of ductules or acini from "resting" (**A**) and lactating (**B**) breasts illustrating the presence of basal clear cells with dense staining nuclei (*arrows*) (Araldite embedded/toludine blue stained). ×400

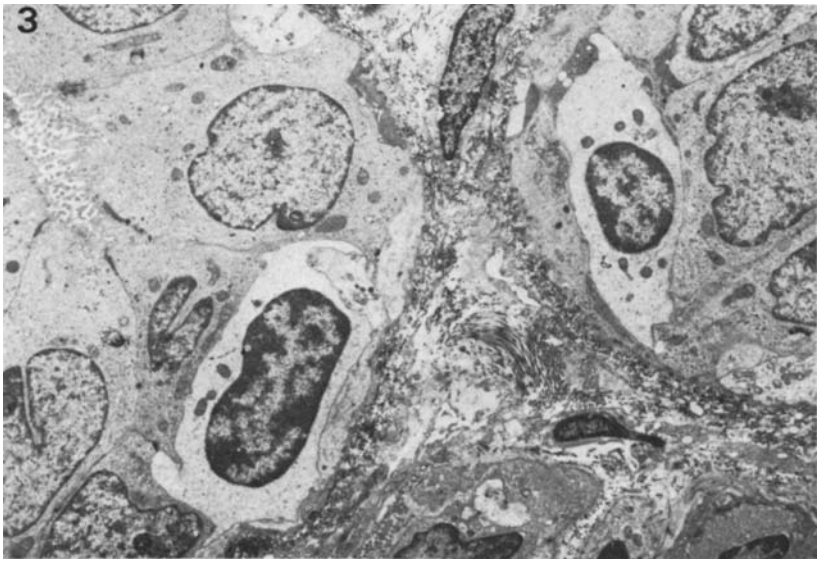

Fig. 3. Electron micrograph of parts of two ductules with electron-lucent IEL. ×3,300

mitochondria and strands of rough endoplasmic reticulum (Fig. 4) and 2–3 lysosomes were observed. There was some variation in the size and shape of the lymphocytes (Fig. 1).

(ii) *Macrophages*. These cells were invariably larger than the lymphocytes and the nucleus contained electron-lucent amorphous chromatin. The cytoplasm contained a large Golgi apparatus, a number of mitochondria and strands of rER and a few lysosomes (Fig. 5). Many of the cells contained single or multiple heterophagosomes. The contents of the phagosomes showed varying degrees of degradation (Fig. 6).

Light microscopic examination of the immunocytochemical stained sections showed a proportion of basally positioned cells which were positive with the leukocyte specific antibody (Fig. 7) and negative with the epithelial specific antibody. Controls in which the primary antibody have been omitted were also negative. The leukocyte positive cells were located in the same position as the intraepithelial lymphocytes and macrophages identified ultrastructurally. In addition, the few lymphocytes and plasma cells present in the intra-lobular connective tissue were positively stained by the anti-leukocyte antibody.

In the "resting" breast the number of intraepithelial cells varied from 5% to 15% of the total cell population of the parenchyma (Fig. 7a). The majority of these cells were identified as lymphocytes with lower numbers of macrophages.

During pregnancy and lactation the proportion of intraepithelial cells was similar to the "resting" breast constituting approximately 10% of the total cell population (Fig. 7b). However, this represents a marked increase

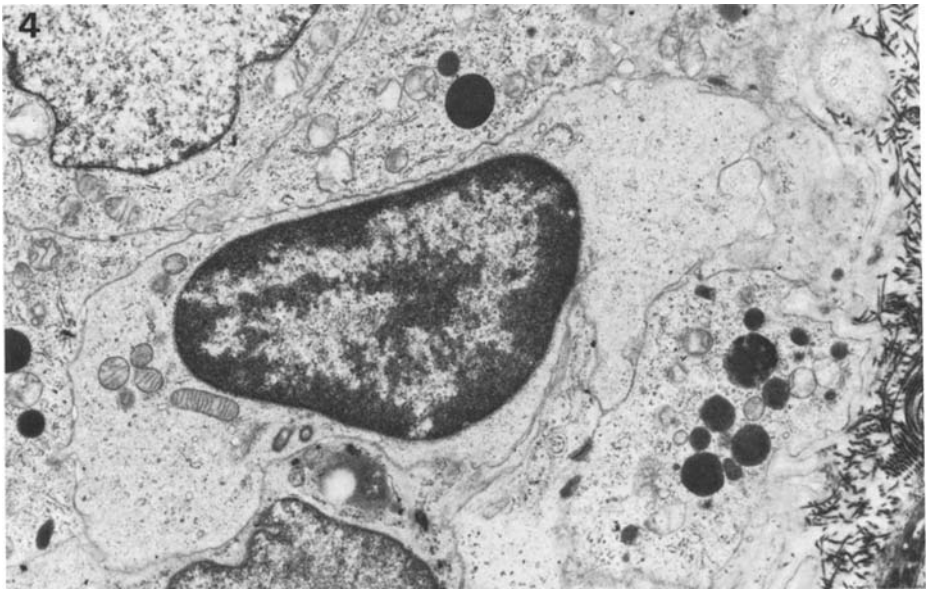

Fig. 4. IEL illustrating the nucleus with peripherally located heterochromatin. Note the paucity of cytoplasmic organelles. ×9,000

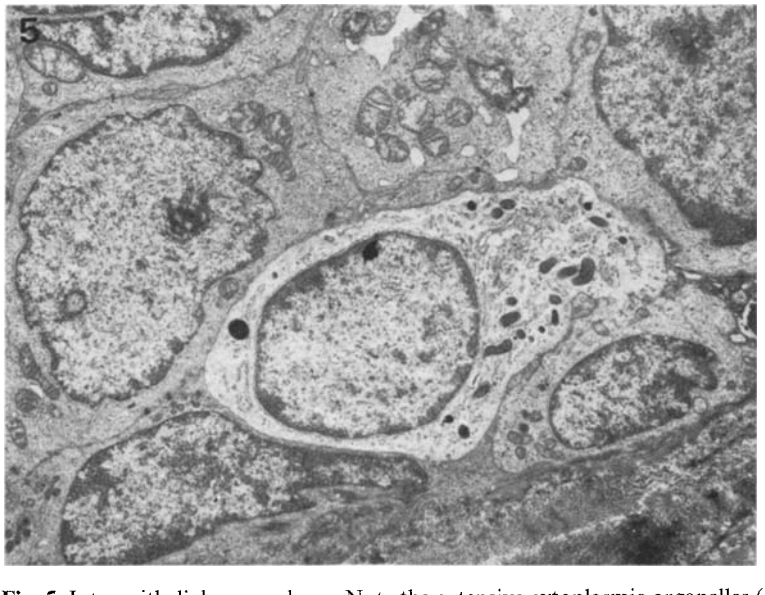

Fig. 5. Intraepithelial macrophage. Note the extensive cytoplasmic organelles (cf Figs. 3 and 4). ×4,800

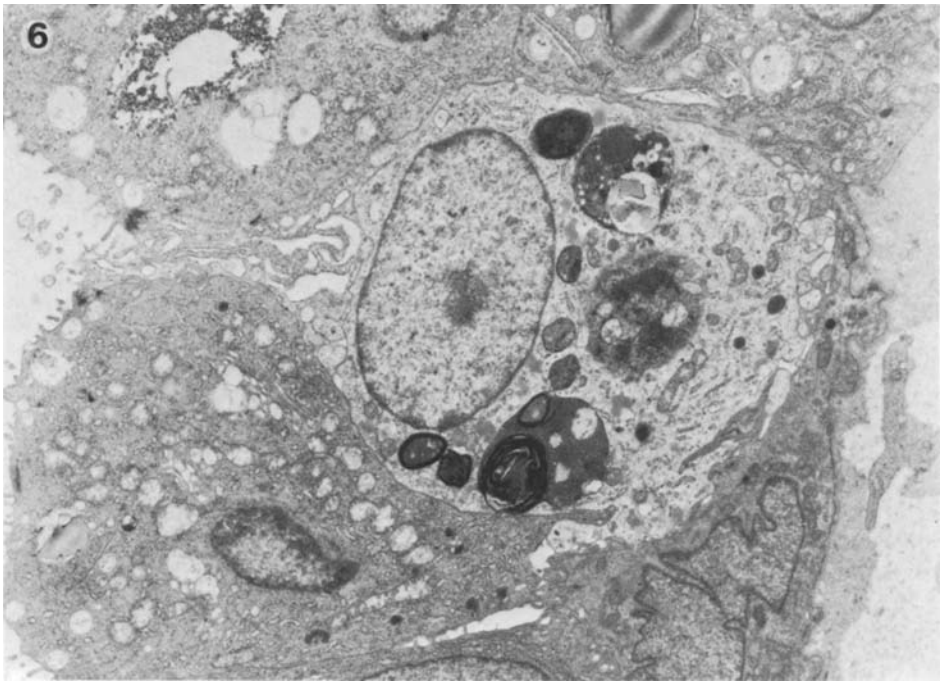

Fig. 6. Intraepithelial macrophage possessing a number of heterophagosomes from an involuting breast. × 6,000

in the total number of intraepithelial cells since the total amount of breast parenchyma increases markedly during pregnancy and lactation. The ratio of lymphocytes to macrophages was similar to that in the "resting" breast.

In the involuting breast (3 and 5 days after stopping breast feeding) there was a marked increase in the number of intraepithelial cells reaching approximately 20% of the total cell population (Fig. 7c). The ultrastructural examination of the lobules showed that this was the result of an increase in the number of macrophages. These macrophages were phagocytosing dead and dying epithelial cells.

Discussion

In this study by combining electron microscopy and immunocytochemistry the basal clear cells have been identified as intraepithelial lymphocytes (IEL) and macrophages. This is at variance with a recent study in which it was reported that the basal clear cells were electron-lucent epithelial or myoepithelial cells (Smith et al. 1984). In the present study variations in the electron density of the epithelial and myoepithelial cells were observed in certain biopsies. However, it has been noted in a number of tissues that variation in the cytoplasmic density is a result of changes associated with fixation (Ghadially 1982). In the present study a few electron-lucent epithelial and

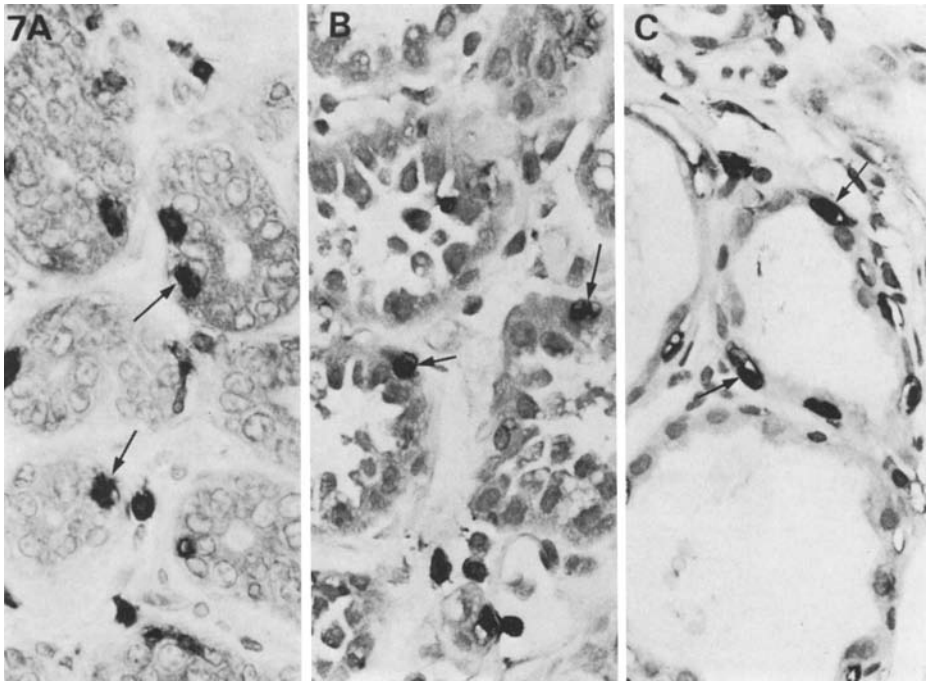

Fig. 7. Ductules or acini from "resting" (**A**), lactating (**B**) and involuting (**C**) breasts immunostained with the anti-leukocyte antibody. Note the positive cells within the epithelium (*arrows*). × 500

myoepithelial cells similar to those described by Smith et al. (1984), were observed particularly in biopsies which by other criteria (organelle preservation) were poorly fixed. However, these cells could be easily differentiated from the intraepithelial cells.

In the present study the intraepithelial lymphocytes and macrophages formed a significant component of the parenchyma in all the biopsies examined irrespective of the physiological state of the breast. There has been only one previous ultrastructural description of these cells in the normal human "resting" breast (Stirling and Chandler 1976) and one report of IEL in the pregnant breast (Seelig and Beer 1981), although macrophages form an integral component in animals (Wooding 1977; Helminen and Ericsson 1968a and b). The finding of intraepithelial cells within the breast is similar to that described for a number of other epithelial lined tissues such as the intestine (Toner and Ferguson 1971), prostate (Helminen and Ericsson 1972) and endometrium (Hopwood and Levison 1976). It is surprising that these cells have not been reported more extensively in the breast although in the intestine the IEL "are often overlooked or referred to as undifferentiated stem cells" (Toner and Ferguson 1971). A similar situation exists in the breast where IEL have been identified as stem cells (Salazar and Tobon 1974) or have been apparently overlooked as in the recent study by Smith et al. (1984), where they can be seen in Fig. 3 of their illustrations.

The intraepithelial macrophages are probably acting as scavengers phagocytosing dead or dying cells (Ferguson and Anderson 1981 a). The increase in the number of macrophages during involution is similar to that described for the mouse (Helminen and Ericsson 1968 a and b) and is probably related to the increase in cell debris associated with epithelial cell degeneration during involution.

The IEL represent one component of what has been termed the mucosa-associated lymphoid tissue (MALT). MALT has been extensively studied in the gut and bronchus (reviewed Bienenstock and Befus 1980) and it has been proposed that there is a "common mucosal immune system" which includes the breast (Bienenstock et al. 1979). However, the role of MALT in the breast differs from that of the gut and bronchus in that the mucosa of the breast is sterile and not repeatedly exposed to foreign antigens. MALT is probably involved in the transfer of immunological agents from mother to offspring via the milk. The means by which the lymphocytes become sensitised to produce specific IgA is not completely resolved although there is evidence for the transfer of lymphocytes from the gut to the breast resulting in local synthesis of IgA (Goldblum et al. 1975) and subcutaneous vaccination with cholera resulted in the appearance of specific IgA antibodies in milk (Svennerholm et al. 1977).

Recently a distinct histological type of lymphoma has been described in MALT associated organs (Herbert et al. 1984; Isaccson and Wright 1983 and 1984; Moore and Wright 1984) and included amongst these have been thyroid lymphomas (Anscombe and Wright 1985). The apparent discrepancy that the thyroid which does not normally possess MALT should develop a similar lymphoma may be because thyroid lymphoma is associated with inflammation or autoimmune disease and so arises in antigenically stimulated tissue (Anscombe and Wright 1985). Despite the presence of MALT in the breast, primary lymphoma of the breast is extremely rare in comparison to the gut, lung or thyroid. Lymphomas only account for between 0.05–0.5% of the total breast neoplasms (DeCosse et al. 1962; Wiseman and Lias 1972; Mambo et al. 1977). From this examination of the normal breast it could be proposed that the rarity of breast lymphoma is related to the relatively small amount of lymphoid tissue present in comparison to the gut or lung and also, in the "resting" breast, MALT is in a quiescent state and not undergoing repeated antigen stimulation. The absence of autoimmune disease within the breast may be a further factor.

In conclusion, this study shows conclusively that the parenchyma of the normal breast consists of epithelial and myoepithelial cells plus a number of intraepithelial lymphocytes and macrophages.

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