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Intracellular Storage of IgA and Secretory Component in Carcinomas of the Female Breast

Gerhard Syré and Maria Sehn
Institut für Pathologische Anatomie der Universität Wien
(Vorstand: Prof. Dr. Johann-Heinrich Holzner), Wien, Austria

Summary. This study reports ten cases of mammary carcinoma with intracytoplasmic lumina and inclusion bodies visible by light microscopy; five tumors were classified as lobular in type and four as different forms of infiltrating ductular carcinomas. One tumor showed a lobular growth in combination with ductular structures. For the identification of the inclusion bodies, indirect immunofluorescence on paraffin embedded material was performed, which revealed IgA as well as secretory component within the intracytoplasmic lumina. It was concluded that the production of secretory component and the uptake of IgA is possible even in carcinomas without glandular structures, and that immunomorphology should supplement histological and histochemical evaluation in order to define the contents of intracytoplasmic lumina.

Key words: Intracytoplasmic lumina in carcinoma of the breast – Secretory IgA and breast carcinoma.

Introduction

The existence of intracytoplasmic lumina (IL) and inclusion bodies (IB) in mammary cancer cells has been revealed by light (Delbet and Mendaro 1925, Battifora 1975, Steinbrecher and Silverberg 1976) as well as electron microscopy (Wellings and Roberts 1963; Fisher 1976). These structures have been used as cytologic markers of mammary carcinoma cells, obtained either directly from neoplastic tissue (Tschubel and Helpap 1976) or from pleural effusions in metastatic breast cancer (Spriggs and Jerrome 1975). Some of the histochemical features of IL and IB, e.g. PAS positivity retained after diastase digestion, alcianophilia and loss of alcianophilia after sialidase digestion, led to the opinion that IL and IB consisted of mucinous material (Spicer et al. 1962; Cooper 1974), especially sialomucin (Gad and Azzopardi 1975). The purpose of this

Offprint requests to: G. Syré, Institut für Pathologische Anatomie der Universität Wien, Spitalgasse 4, A-1090 Wien, Austria

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study was to assess the immunoglobulin nature of IB by immunofluorescence microscopy on paraffin embedded breast cancer specimens.

Material and Methods

Material was obtained from patients who had undergone radical mastectomy because of breast cancer. Ten cases were selected in which IL and IB were visible by light microscopy. All the specimens were fixed in 8% neutral formalin (pH 7.4) embedded in Paraplast plus (Sherwood Medical Industries Inc., St. Louis, MO, USA). Sections (4 μm thickness) were stained with hematoxylin-eosin, periodic acid-Schiff reagent (PAS) with and without diastase pretreatment, and acid fuchsin orange G (AFOG, Zollinger and Mihatsch 1978). For immunofluorescence studies, parallel sections from the same block were used. Unstained dewaxed (xylene) sections were pretreated with 0.1% pronase (Pronase type VII, Sigma Chem. Comp., St. Louis, MO, USA) according to the method described elsewhere (Denk et al. 1976) and washed with phosphate-buffered saline (pH 7.4). For indirect immunofluorescence, slides were then incubated in a moist chamber at room temperature for 45 min first with unlabeled, then with FITC-labeled antisera. In each case the following unlabeled antibodies were used as the first layer: antihuman IgG, IgM, IgA and secretory component antibodies, all raised in rabbits (Behring Werke AG, Marburg, West Germany). Fluorescein-conjugated antisera against rabbit IgG, raised in goats (Behring Werke AG) were used as second layer lafter a buffer wash. Thereafter, the slides were washed in buffered saline and mounted in buffered glycerol. For examination a Leitz Ortholux microscope equipped with Ploem Opak epiillumination with a filter combination 3/3 was used.

In addition, electron microscopic studies were performed in one case. A small portion of fresh material was immediately fixed in 2.5% cacodylate buffered glutaraldehyde (pH 7.4), postfixed in osmium ferrocyanide solution and embedded in Epon 812. Ultrathin sections were cut on a LKB ultramicrotome, double stained with methanolic uranyl acetate and basic lead citrate, and examined in a Zeiss EM 9S electron microscope.

Results

Light Microscopy. According to Azzopardi's (1978) classification of mammary carcinomas, four cases were diagnosed as different forms of infiltrating ductular carcinomas, whereas five cases were of the lobular type. One tumor showed a lobular growth in combination with ductular structures.

In all cases a varying number of tumor cells contained IL ranging in size from tiny vacuoles to large cavities occupying half the cell and displacing and indenting the nucleus. Usually a cell contained only one IL, but occasionally two or more were seen. One or more globules were enclosed in each IL, which, in hematoxylin-eosin stained sections, may be confused with erythrocytes. PAS-staining gave positive results even after diastase pretreatment, and the IB appeared as vivid red bodies after AFOG staining (Fig. 1b). In rapidly fixed specimens, the IL were completely filled with PAS-positive material, while AFOG staining revealed a vivid red core surrounded by a thin, pale blue rim. The distribution of IL and IB within different parts of the tumor was inconstant. Most IL and IB were found in peripheral segments of the tumor, especially in slender tumor trabeculae, whereas central solid areas were devoid of IL and IB; the distribution of these structures is similar to that of plasma-cells which surround the tumor and are seldom found in central tumor areas. In non-neoplastic mammary glandular tissue away from the tumor, the glandular lumina were filled with homogeneous eosinophilic and PAS-positive material which also stained a vivid red with AFOG.

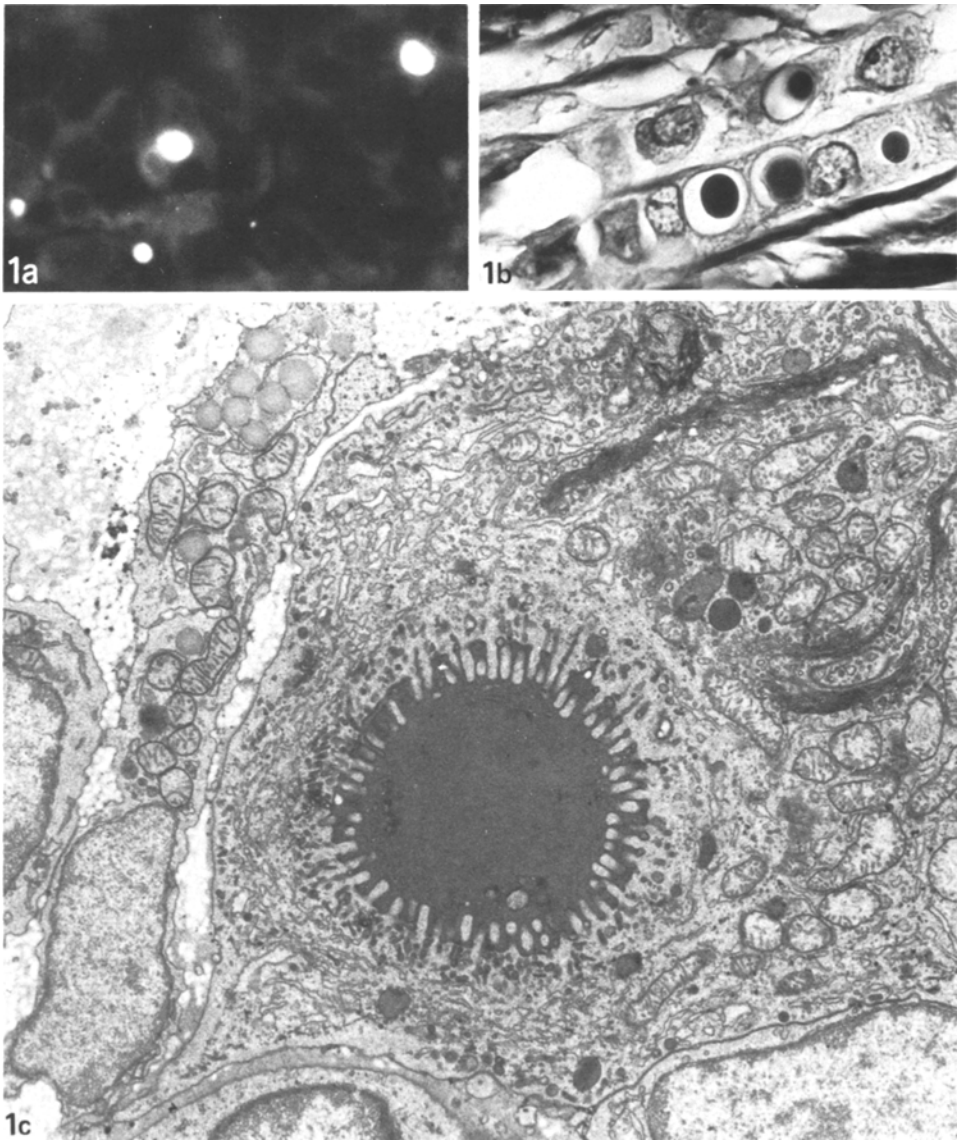

Fig. 1 a–c. Breast carcinoma of lobular type. All figures are from the same case. **a** Intracytoplasmic inclusion bodies containing IgA (indirect immunofluorescence, anti-human IgA, 63×). **b** Intracytoplasmic lumina indenting the nucleus. Note inclusion bodies which do not completely fill the vacuoles. (AFOG-staining, 63×). **c** The electron micrograph shows part of a tumor cell with a Golgi apparatus and a closely related intracytoplasmic vacuole with electron dense content. Microvilli are protruding into the lumen. (5,000×)

Immunofluorescence Microscopy. Indirect immunofluorescence microscopy on paraffin embedded pronase-pretreated sections revealed IgA as well as secretory component within IL (Fig. 1 a). Sometimes only an empty vacuole was seen surrounded by a rim of fluorescence. No specific reaction was obtained with antibodies to human IgG and IgM. Extracellular deposits containing immuno-

globulins or secretory component (SC) could not be demonstrated in connective tissue or blood vessel walls, whereas IgA-containing plasma-cells surrounded the tumor. The lumina of nonneoplastic mammary glands were sometimes filled with homogeneous IgA- and SC-positive material, and tiny IgA-positive granules were dispersed in the cytoplasm of epithelial cells, located mainly at the apical cell membrane. Sometimes epithelial cells were negative, although IgA and SC were found in the lumina of lobules and ducts.

Electron Microscopy. The case studied by electron microscopy showed intracytoplasmic vacuoles filled with homogeneous electron dense material and located close to the Golgi apparatus (Fig. 1c). They varied considerably in size, and their inner surface was lined by microvilli projecting into the lumen of the vacuoles. Tiny electron dense granules surrounded the vacuoles in a rim, and some of them seemed to fuse with the vacuole membranes. Similar electron dense granules appeared along the neighboring cell membrane. In less well fixed portions of the tumor, only the central part of the luminal content showed a homogenous appearance, whilst the microvilli had apparantly retracted; the remaining space was filled with numerous electron dense granules.

Discussion

Quantitatively, IgA is the major immunoglobulin in the external body fluids of mammals, and secretory IgA is the major immunoglobulin secreted by the human mammary gland during late pregnancy and lactation, as well as in the resting state. IgA is secreted as a dimer linked by a polypeptide (J) chain complexed with a glycoprotein, termed the secretory component (Tomasi and Grey 1972). IgA and J-chain are derived from plasma-cells, whereas SC is a product of epithelial cells (Kraehenbuhl and Kühn 1978). Transport of IgA through epithelial cells depends on the intracellular production of SC (Tomasi and Grey 1972) first secreted into the glandular lumen. SC adheres to the apical cell membrane, is then taken up by endocytosis, and transported through the cell in vesicles, which finally fuse with the basolateral cell membrane (Kraehenbuhl and Kühn 1978). In this location SC becomes exposed to the interstitial fluid and is now able to bind dimeric IgA produced by local plasma-cells. After seizing the immunoglobulin, SC mediates the transport through the epithelial cell into the gland lumen. There is some evidence that this mechanism is maintained even in neoplastic cells (Nagura et al. 1979).

An extensive description of IL and IB has been given by Battifora (1975) who reported aggregation of microvilli and periluminal condensation of the cytoplasm as distinct features. He restricted his morphologic description to IL not larger than the nucleus, since in larger vacuoles the morphology was altered due to the close proximity to the plasma membrane. However, delayed fixation, sometimes inevitable in routine material, may also significantly influence the morphologic pattern in that the IL are distended with loss of microvilli, and IB are made up of a central core and many surrounding small globules.

In the past, many attempts have been made to elucidate the histochemical nature of IB. These investigations led to the opinion that IB consisted of mucin-

ous material, especially sialomucin (Spicer et al. 1962; Cooper 1974; Gad and Azzopardi 1975). The present study, however, suggests that AFOG-staining may be helpful to distinguish between mucinous substances and globulins, but since AFOG-staining is not specific for IgA and also gives positive results with fibrin and some other proteins (Zollinger and Mihatsch 1978), immunomorphological methods should be performed to confirm the presence of IgA.

The ultrastructural features of IL are similar to those of the apical cell membrane of normal mammary epithelial cells, as described by Fisher (1976). These similarities support the notion that IL arise by infolding of the cell membrane (Fisher 1976). However, if IL actually communicated with the interstitial space one would expect discharge of their contents; however, neither IgA or SC could be detected extracellularly by immunofluorescence microscopy. Considering the similarities of IL and the apical cell membrane, as well as the fact that most of the carcinomas investigated were composed of solid tumor trabeculae, with only scanty glandlike structures, it may be that IL result from the lack of polarization frequently observed in tumors. This is supported further when the secretion mechanism of IgA outlined above, is considered, since it seems to depend on the presence of mature and polarized cells. However, the uptake of dimeric IgA still takes place even in a non-polarized tumor cells and this suggests that apparent loss of polarity does not necessarily result in the failure of SC production and that IL membranes may act like apical cell membranes. On the other hand, the storage of IgA and SC within IL could also be the result of cell polarization towards the IL lumen.

Despite the small number of cases investigated, IgA-containing IL are present in ductal as well as lobular carcinomas, but seem to be more abundant the latter, as described in earlier reports (Gad and Azzopardi 1975; Steinbrecher and Silverberg 1976; van Bogaert and Maldague 1980). Moreover, they have also been reported in various other carcinomas such undifferentiated adenocarcinoma of the bronchus (Ghadially 1978) and prostatic adenocarcinomas (Kastendieck 1977). In addition, IgA-containing tumor cells were found in gastric adenocarcinomas (Ohta et al. 1979).

Thus, it may be concluded that carcinomas arising from epithelia able to transport IgA under normal conditions, may contain IL and IB. Since at least some of them produce mucus, immunomorphology should supplement histochemical procedures in order to define the contents of IL.

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