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# Ultrastructural identification of Ia positive dendritic cells in the lactating rat mammary gland

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**Summary.** Dendritic cells which express Ia antigen have been demonstrated for the first time in the lactating rat mammary gland. Ultrastructurally, the dendritic cells appear as electron-lucent pale cells interspersed among the epithelial cells of the alveoli, forming a cell population distinct from classical macrophages. They show morphological resemblance to the dendritic cells of lymphoid organs as well as the Langerhans cells of skin. The Ia antigen has been localised by electron microscopic immunocytochemistry on the cell membrane and endocytotic vesicles and tubules. Ia positive cells are also seen in the stroma of the mammary gland. It is proposed that the dendritic cells of the mammary gland belong to the lineage of epidermal Langerhans cells and lymphoid dendritic cells, subserving an immunological role in the lactating breast.

**Key words:** Ia antigen – Dendritic cell – Rat mammary gland – Lactation

The "dendritic cell" was first described morphologically and characterised by Steinman and Cohn (1973) in the lymphoid organs of the mouse. These cells are potent stimulators of T lymphocyte division in both allogeneic and syngeneic cultures (Steinman and Witmer 1978; Nussenzweig and Steinman 1980) and play an important role in antigen presentation previously ascribed to macrophages (Nussenzweig et al. 1980).

Immune response associated (Ia) antigens, are polymorphic membrane glycoproteins, coded for by genes linked to the Ir genes within the major histocompatibility complex. The presence of surface Ia antigen was found to be an important marker for dendritic cells (Steinman et al. 1979).

Dendritic cells which express Ia antigens are now known to have a widespread distribution in the body and occur in the interstitial connective tissue of almost all organs except the brain (Hart and Fabres 1981). Of special interest, however, is the Langerhans cell of skin which has morphology, Ia positivity and immunological function similar to those of dendritic cells, but in addition, some distinctive ultrastructural features.

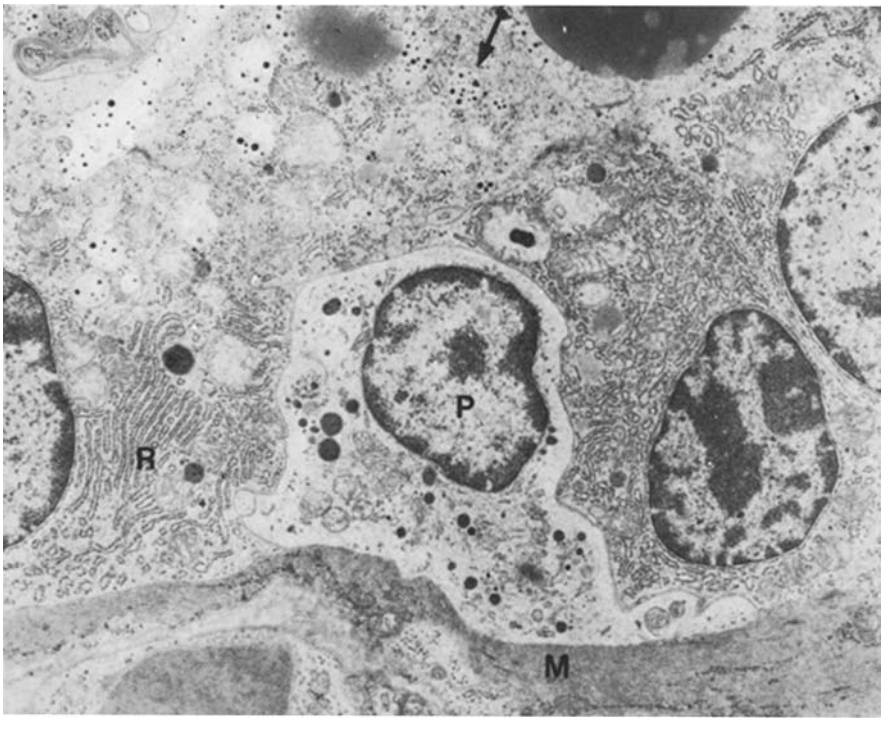


**Fig. 1.** Electron micrograph of an alveolus of a lactating rat mammary gland. A "pale" electronlucent cell (*P*) is seen between a myoepithelial cell process (*M*) and secretory epithelial cells containing parallel arrays of rough endoplasmic reticulum (*R*) and secretory protein granules (*arrow*). Mag. × 7,500

This communication describes the presence of intraepithelial, dendritic Ia positive cells, similar to the Langerhans cell of skin, in the lactating rat mammary gland. The presence of such cells in the mammary gland has not been previously reported.

## Materials and methods

Female Ludwig Wistar rats obtained from Olac Ltd., (Bicester, Oxfordshire, U.K.) and sacrificed on the third day of lactation were used in this study. Freshly dissected mammary glands were viewed under a dissecting microscope, when the lobuloalveolar units were carefully stripped of all stroma and cut into tiny pieces of less than 1 mm cube. Routine electron microscopy was conducted on some of the samples. For electron immunocytochemistry, tissue pieces were incubated with monoclonal anti-rat Ia antibodies (MAS 028 and MAS 043, Sera Lab, U.K., diluted 1:10 with 0.5% bovine serum albumin in phosphate buffered saline (BSA-PBS)) at room temperature for 2 h, washed for 15 min with several changes of PBS and then incubated with peroxidase conjugated $F(ab)_2$ fragments of anti-mouse IgG (Sigma, 1:25 BSA-PBS) for 1 h. The samples were again rinsed with PBS and incubated with freshly prepared 0.05% diaminobenzidine and 0.03% hydrogen peroxide in PBS for 5 min. The tissues were then fixed in 2% glutaraldehyde for 12 h and post fixed in osmium tetroxide for 4 h at 4° C. Both fixatives were prepared with 0.05M phosphate buffer (pH 7.2–7.4) and the osmotic pressure was adjusted to 330 mOsM by addition of sucrose. For controls, the first antibody was

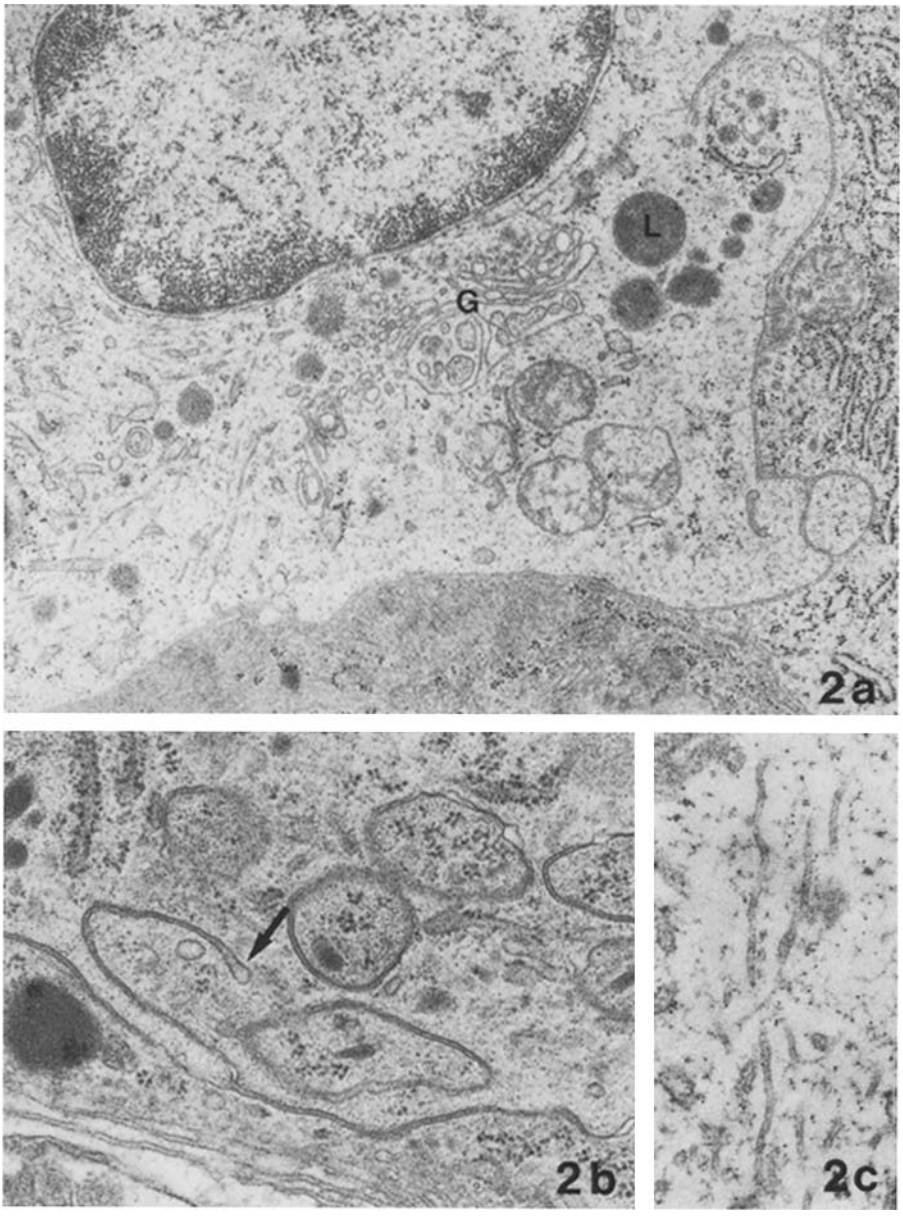


**Fig. 2.** **a** Electron micrograph of a dendritic cell showing Golgi body (*G*) and few lysosomes (*L*) in the perinuclear region. Mag. × 12,000. **b** The plasma membrane of a dendritic cell shows numerous invaginations terminating in bulbous ends (*arrow*). Mag. × 37,000. **c** Microtubules in the cytoplasm of a dendritic cell. Mag. × 50,000

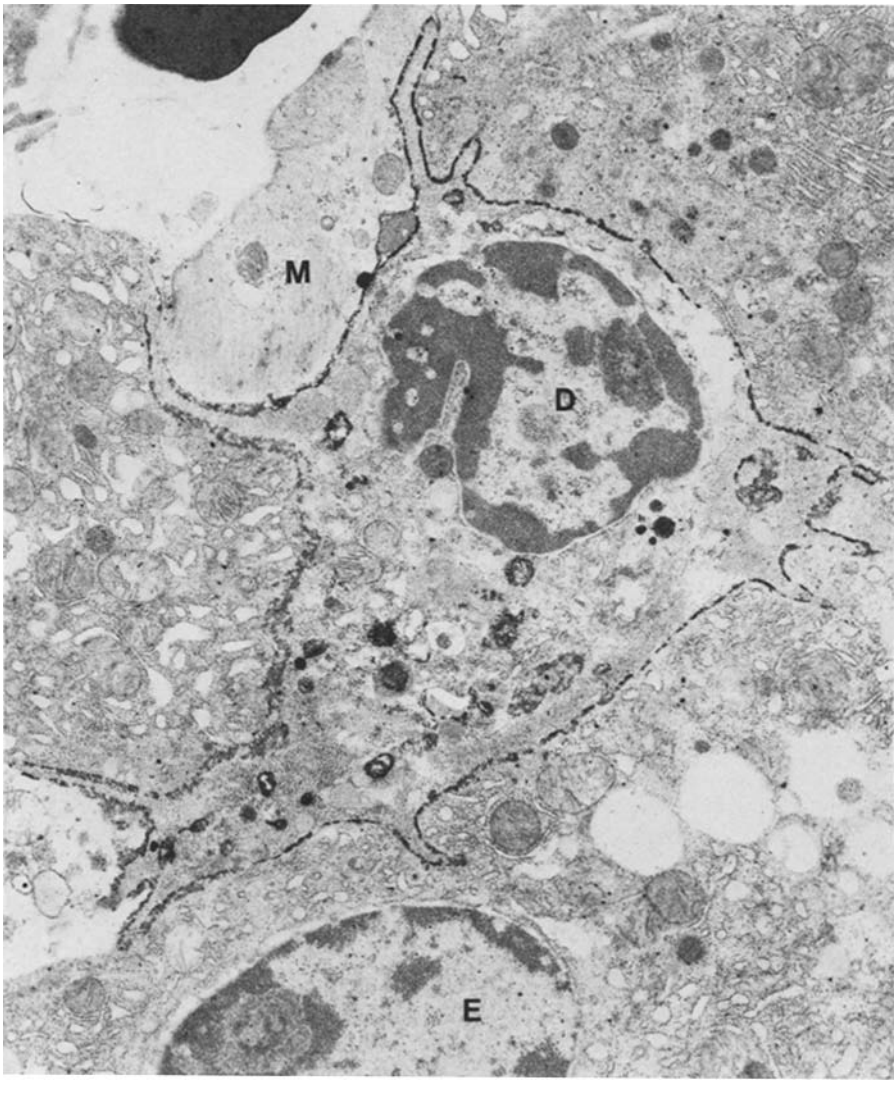


**Fig. 3.** Immunoelectron micrograph of a dendritic cell (*D*), stained with monoclonal anti-rat Ia antibodies, lying between the myoepithelial cell process (*M*) and epithelial cells (*E*). The electron dense reaction product is localised chiefly on the cell membrane, thus clearly defining the "dendritic" morphology of the cell. Mag. ×12,000

replaced by normal mouse immunoglobulins. Tissue pieces were processed for electron microscopy. Thin sections were lightly stained with uranyl acetate and lead citrate prior to viewing in a Philips EM 400 electron microscope.

## Results

The lobuloalveoli of the lactating rat mammary gland consist of a single layer of epithelial cells surrounded by a discontinuous layer of myoepithelial cell processes. The epithelial cells are characterised by abundant rough endo-

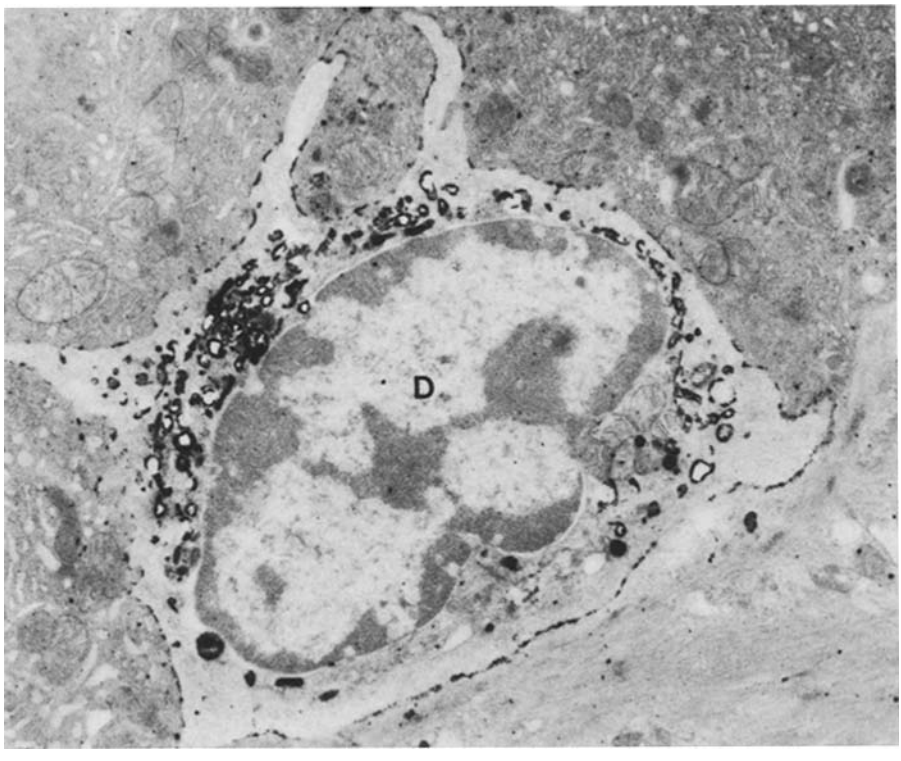


**Fig. 4.** Immunoelectron micrograph of a dendritic cell (*D*), stained with anti-rat Ia antibodies. The reaction product outlines the cell membrane as well as intracytoplasmic vesicles. Mag. ×11,000

plasmic reticulum (RER) in parallel arrays or vesicular forms, intracytoplasmic lipid and membrane bound protein granules. The myoepithelial cell cytoplasm is packed with myofilaments showing focal densities. Interspersed between the epithelial cells are 'pale' electron-lucent cells (Fig. 1). Basal clear cells (Radnor 1972) are readily distinguished from them by their location and the presence of cytoplasmic myofilaments.

These "pale" cells can be further subdivided into classical macrophages and dendritic cells. The dendritic cell has an irregular shape and abundant pale or clear cytoplasm. It has large mitochondria with well developed cristae, short stretches of RER, and few membrane bound lipid droplets. The perinuclear region contains Golgi apparatus, centrioles, rough and smooth surfaced vesicles, multivesicular bodies and few lysosomes. It also contains long microtubules and few microfilaments. At the periphery of the cell, plasmalemmal projections of variable lengths extend into the cytoplasm and have bulbous ends (Fig. 2). The classical macrophage, on the other hand, differs from a dendritic cell by having a large number of lysosomes in its perinuclear region.

Immunocytochemical staining with Ia antibodies shows localisation of the stain on the cell membrane and outlines the endocytotic vesicles and tubules (Figs. 3 and 4). The dendritic nature and irregular cell processes

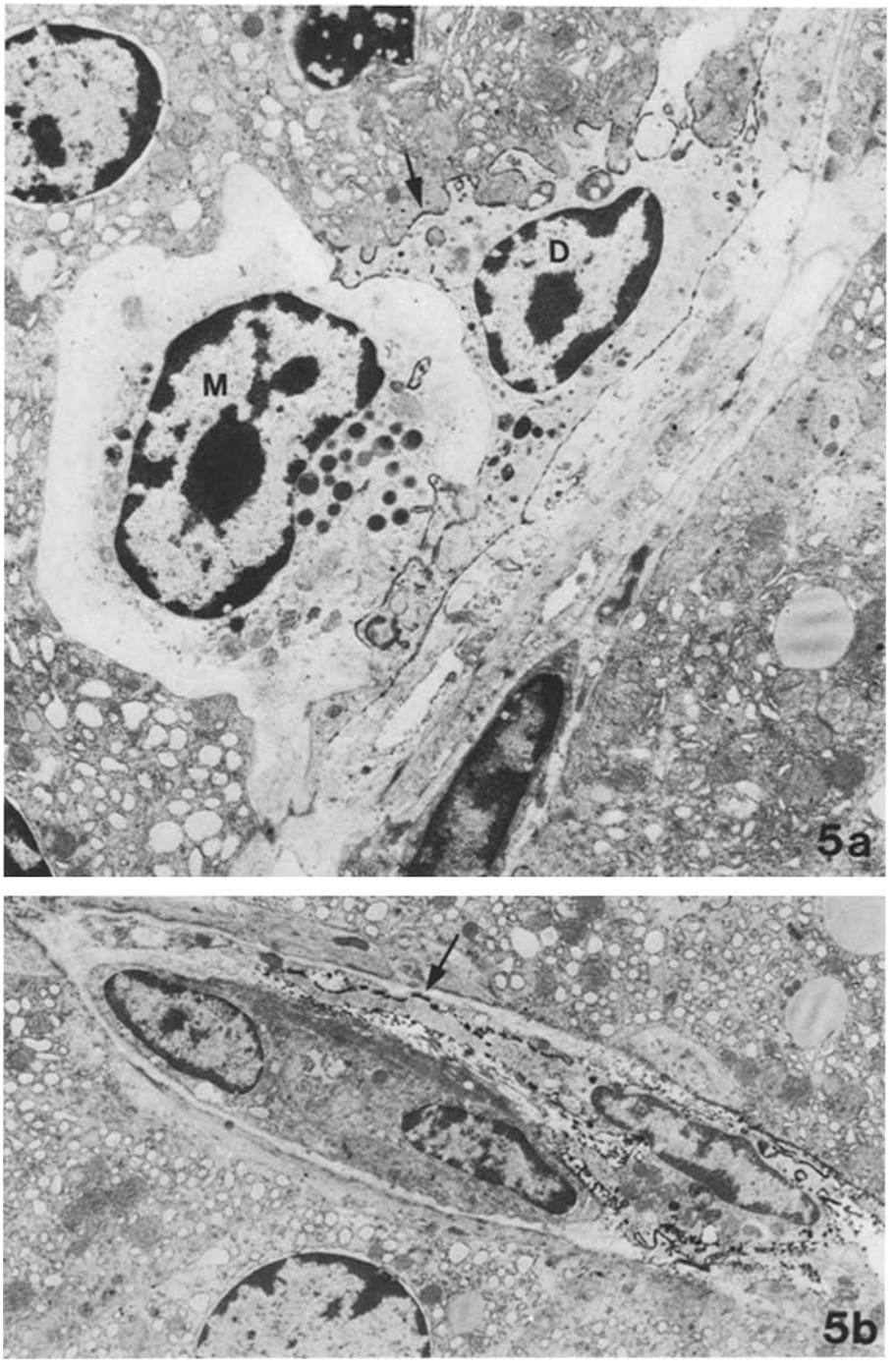


**Fig. 5. a** Immunoelectron micrograph of a dendritic cell (*D*), lying close to a macrophage (*M*). Surface Ia antigen is expressed by the dendritic cell along its cell membrane (*arrow*), but not by the macrophage. Note a large number of lysosomes in the perinuclear region of the macrophage. Mag. ×10,000. **b** An Ia positive stromal cell. The antigen is localised on the cell surface (*arrow*) and a few vesicles. Mag. ×6,200

are better visualised with electron microscopic immunocytochemistry than with routine electron microscopy. The dendritic cells often show close proximity to classical macrophages (Fig. 5a). The macrophages show variable expression of Ia antigen, some being negative and others weakly positive. Elongated Ia positive cells are also identified in the stroma (Fig. 5b).

## Discussion

The mammary gland assumes an immunological role during pregnancy and lactation. Lymphoblasts committed to IgA synthesis migrate from mesenteric lymph nodes to the mammary gland (Roux et al. 1977; McDermott and Bienenstock 1979) and there is transfer of immunoreactive lymphoid cells from mother's milk to neonate during suckling (Beer et al. 1975). In an ultrastructural study of lactating rat mammary gland, Helminen and Ericsson (1968) described intraepithelial pale cells, and postulated them to be large lymphocytes. Seelig (1980) showed that the number of leukocytes within the ductal epithelium increased from 3.5% at the beginning of pregnancy to 8.7% at the onset of lactation.

The dendritic cells form an important component of the immune system (Steinman and Cohn 1973). They are known to occur not only in the lymphoid organs, but have a generalised distribution in the connective tissue of almost all organs with the exception of the brain (Hart and Fabres 1981). Expression of surface Ia antigens appears to be a consistent observation in antigen presenting cells. It was reported that almost all rat dendritic cells bind mouse monoclonal anti-rat Ia antibodies strongly whereas most macrophages do so only weakly (Klinkert et al. 1982).

Epidermal Langerhans cells are known to have a dendritic morphology and distinctive ultrastructural features in the form of Birbeck granules (Birbeck et al. 1961). Langerhans cells from mouse, guinea pig and man also carry Ia antigens and function as stimulator cells for allogeneic lymphocytes (Rowden 1980). Dendritic cells in lymphoid tissues and Langerhans cells have similar morphology, and perhaps belong to the same class of cells. This suggestion is strengthened by the fact that an occasional Birbeck granule is also present in dendritic cells in the lymph nodes (Klinkert et al. 1982).

Langerhans cells are also found in the dermis and form a cell population which migrates between skin and lymph nodes. Some of the Langerhans cells have a prominent network of microfilaments, occasionally of the dimensions of actin filaments, as well as a prominent system of microtubules, both features signifying active cell movement (Silberberg-Sinakin et al. 1978).

Langerhans cells are also known to occur in other epithelia – the oral mucosa, and cervix (Hutchens et al. 1971 and Younes et al. 1968). In the intestine, Ia positive cells are known to occur, not only in the Peyer's patches, but also among the villus epithelial cells of the mucosa (Mayrhofer et al. 1983). However, the morphology of the intraepithelial Ia positive cells of the intestine is not clear. Using a polyclonal antibody, Klareskog et al.

(1980) have shown an increase in the expression of Ia antigen in rat breast epithelium during pregnancy and lactation. The antibody appears to stain all epithelial cells of the lobuloalveolus uniformly and it is possible that the antibody recognises an Ia-like molecule on the milk fat globule membrane (Wiman et al. 1979), not recognised by the more specific monoclonal antibodies. The monoclonal antibodies used in this study do not stain the epithelial or myoepithelial cells of the lactating rat breast. The stain is specifically localised on the plasma membrane of the dendritic cells and a few macrophages. It also outlines the endocytotic vesicles and tubules of the dendritic cells. A few Ia positive cells are, however, present in the stroma.

The Langerhans cell granules have at times been seen in continuity with the cell membrane and are thought to be a unique form of endocytosis (Hashimoto 1970). Although classical Langerhans cell granules have not been found in the dendritic cells of rat breast, plasmalemmal projections ending in bulbous ends have often been seen. In view of the ultrastructural similarities and Ia positivity – it is proposed that the dendritic cells of the lactating rat breast described here, are akin to the dendritic cells of the lymphoid tissue and the Langerhans cell of the skin.

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