Short Report

Demonstration of Birbeck (Langerhans cells) granules in the normal chicken epidermis

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ABSTRACT

Mammalian Langerhans cells (LC) are epidermal dendritic cells which originate in bone marrow and migrate toward the T cell area of lymph nodes, where they act as professional antigen-presenting cells. A variety of cell surface markers, such as the ectoenzyme adenosine triphosphatase (ATPase), Ia and CD1a antigens, have been used extensively to identify LC. Ultrastructural identification of this cell type in the mammalian epidermis is made by the demonstration of a typical and unique cytoplasmic organelle, the Birbeck granule (BG). Although we had earlier demonstrated the coexpression of ATPase and Ia antigens on epidermal dendritic cells of the chicken epidermis, the presence of the BG has not previously been documented. The aim of the present study was to investigate whether chicken epidermal LC-like cells possess an organelle similar to the BG, and thus to complete their identification. Our findings are the first demonstration of characteristic rod-shaped, racket-shaped and disc-shaped intracytoplasmic organelles, morphologically similar to the mammalian BG, in avian LC.

Key words: Dendritic cells; birds; skin.

INTRODUCTION

Dendritic cells (DC) of the immune system are bone marrow-derived leukocytes that comprise a family of professional antigen-presenting cells involved in the effective generation of antigen-specific T-cell responses (Steinman, 1991). In mammalian skin, epidermal Langerhans cells (LC) are considered to be immature DC that pick up antigens by pinocytosis and phagocytosis (Reis e Sousa et al. 1993; Sallusto et al. 1995); subsequently they migrate via afferent lymphatic vessels in the papillary layer of the dermis to regional lymph nodes, where they arrive as mature cells homing to the T-cell-dependent areas where they activate resting T cells (Macatonia et al. 1987; Kripke et al. 1990).

At the light microscopic level, the classic markers to identify LC in normal mammalian skin are the cell surface expression of the enzyme adenosine triphosphatase (ATPase) (Wolff & Winkelmann, 1967), class II (Ia) molecules encoded by major histocompatibility complex (MHC) (Klareskog et al. 1977; Rowden et al. 1977) and CD1a antigen (Fithian et al. 1981). At the ultrastructural level, the only reliable criterion to designate a cell as an LC is the presence of the typical and unique intracellular organelle termed the Birbeck (Langerhans cell) granule (BG) (Birbeck et al. 1961). Three-dimensional reconstructions showed that the BG may assume a variety of shapes, from a disc shape to a cup shape (Sagebiel & Reed, 1968). The most common sectional profile of the BG is a rod-shaped structure of variable length with a central linear density between 2 limiting membranes; occasionally an electron-lucent vesicular portion is found at one end which gives the BG a tennis racket-like appearance (Wolff, 1967). If the granule is cut tangentially it appears as a rounded or oval body containing a square lattice of particles, with a vesicle located in the periphery (Wolff, 1967; Sagebiel & Reed, 1968).

Although we had demonstrated the presence of epidermal Langerhans-like cells in the chicken epi-

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dermis using ultrastructural histochemistry for ATPase (Carrillo-Farga et al. 1991) that also expresses MHC class II (Ia) antigens (Pérez-Torres & Millán, 1994), the presence of organelles similar to the BG was not investigated. The purpose of this study is to make an extensive ultrastructural search for the BG in epidermal LC-like cells of the normal chicken skin.

MATERIALS AND METHODS

Animals

Three 8-wk-old male chickens were used. The apparently normal skin of pectoral apterias were cleaned with 70% ethanol and anaesthetised with 1 ml of 1% subcutaneous lidocaine (Xylocaine); 4 mm punch biopsies were obtained and immediately rinsed with sterile distilled water, cut manually into 1–2 mm pieces and processed for transmission electron microscopy.

Transmission electron microscopy

Biopsy samples were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) for 2.5 h at 4 °C. After 3 washes in 0.15 M cacodylate buffer (pH 7.4) at 4 °C, skin fragments were postfixed with 1% osmium tetroxide in 0.2 M cacodylate buffer (pH 7.4) during 45 minutes at 4 °C, dehydrated in gradually increasing concentrations of ethanol, transferred to propylene oxide and embedded in Araldite 6005. Ultrathin sections, obtained with a diamond knife and contrasted with uranyl acetate and lead citrate, were examined with a Zeiss EM-109 electron microscope.

At least 50 images of the cell body and dendritic processes of epidermal clear dendritic cells per animal were examined.

RESULTS

Clear dendritic cells identical to those described before (Carrillo-Farga et al. 1991) were observed among basal keratinocytes of epidermal stratified squamous epithelium and consequently identified as LC-like cells.

BG-like organelles were recognised inside of many cytoplasmic processes and occasionally in the cell body. They have a characteristic rod-shaped profile made of parallel membranes enclosing a central electron-dense line (Fig. 1, insert *a–e*), of variable length and about 13–15 nm in thickness. The longer BG-like organelles resembled more a tubular structure but were quite similar to the cross-section of a disc-shaped BG (Fig. 1, insert *c–e*). No continuity or attachment between plasma membrane and BG-like organelles was observed.

The cell bodies of LC-like cells were practically devoid of BG-like structures. Sometimes, a few contained racket-shaped organelles located in the vicinity of the nuclei (Fig. 2). The rod portion of these organelles presented two differences with respect to rod-shaped BG-like organelles (Fig. 1, insert *a–e*): the absence of the electron-dense central line and a slightly increased thickness (20–22 nm). This racket-like appearance was the most rare image of all BG-like structures observed, but also uncommon were images of tangential sections of these organelles. If such sections occurred (as they did just 3 times), they appeared as rounded or oval bodies with a maximum diameter of about 200 nm, formed by an eccentric vesicle attached to a discoid or curved portion (Fig. 3, insert), which never had a clearly evident square-lattice arrangement of small particles.

Finally, no BG-like structures were observed in the keratinocytes or in other cell type different from clear dendritic cells.

DISCUSSION

Previous studies in several avian species (Carrillo-Farga et al. 1991; Akhter et al. 1993) and in the frog (Carrillo-Farga et al. 1990) have demonstrated the presence of ATPase-positive epidermal DC, ultrastructurally similar to mammalian LC except for the lack of BG. When sequential immunofluorescence to MHC class II (Ia) antigens and ATPase enzyme histochemistry is employed, a complete overlap of these reliable LC markers is observed on the epidermal DC in the chicken (Pérez-Torres & Millán, 1994) and in the frog (Castell et al. 1999). However, until now, ATPase-positive LC with BG only has been identified in the epidermis of the turtle (Pérez-Torres et al. 1995).

The present results reveal that chicken epidermal LC-like cells also possess BG-like organelles which appear as rod-shaped, racket-shaped and flat, disc-shaped structures, depending on the plane of section. These profiles correspond to the most frequently described BG in mammalian LC. However, some differences were noted: 1) the rod-shaped BG-like organelles described in the present study had a thickness of 13–15 nm, whereas the BG are about 43 nm in mice (Kobayashi & Hoshino, 1987) and 30–35 nm in human (Elofsson et al. 1981); the significance of the thickness of BG in regard to immunological function of LC has not been analysed; 2) the BG-like organelles (the rod-shaped and the

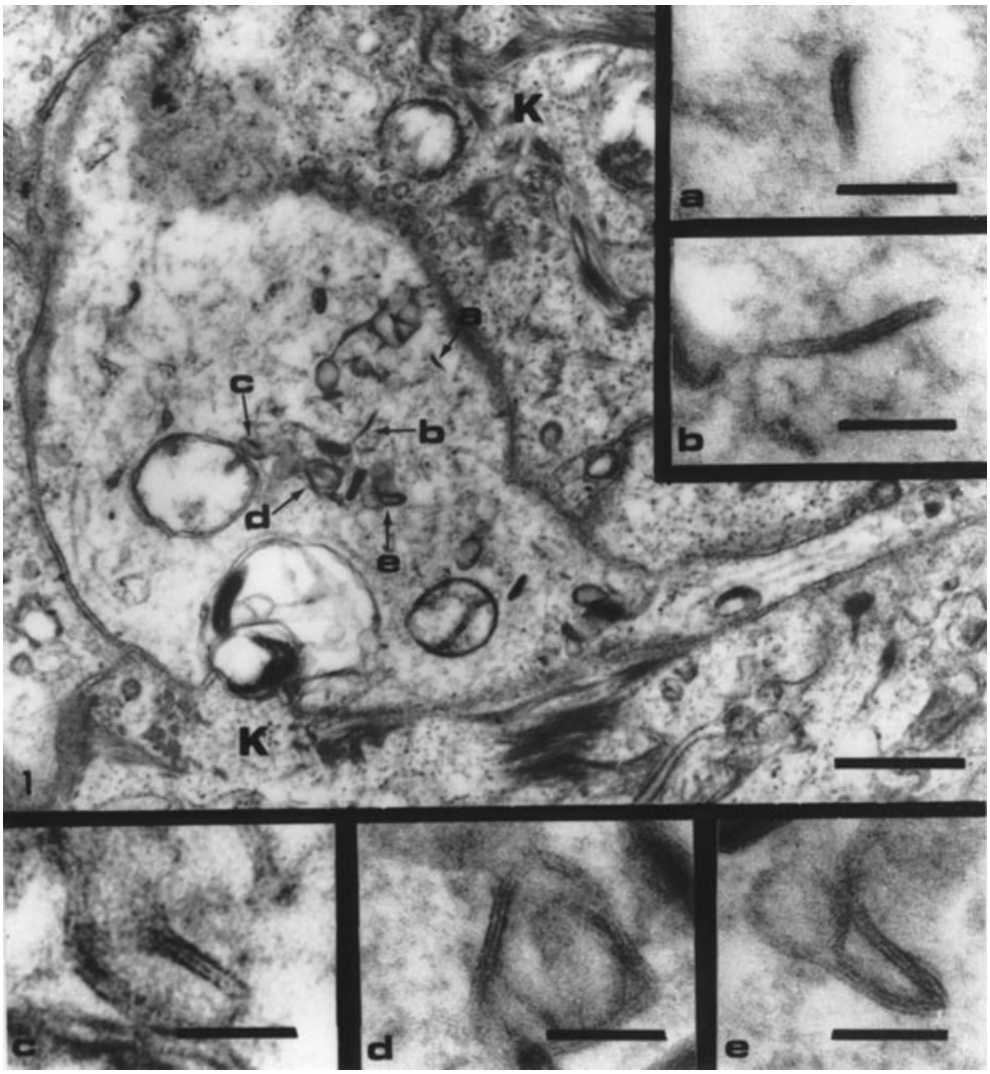

Fig. 1. Transmission electron micrograph of chicken normal skin showing a Langerhans cell dendritic process. Several rod-shaped Birbeck granule-like organelles with typical electron-dense central lamella are denoted by letters (*a–e*) and magnified in the corresponding gallery of inserts. The longer organelles (inserts *c–e*) display a cored tubule appearance. K, keratinocytes with tonofilaments. Bar, 5 μm (Fig. 1); bars, 100 nm (inserts *a–e*).

disc-shaped) were found more frequently in dendritic processes than in the cell body of chicken LC-like cells, which is opposite to the localisation of these structures in mammalian LC (Wolff, 1972), and 3) the 'atypical' aspect of the rod portion of the racket-shaped and disc-shaped organelles (increase of thickness, absence of electron-dense central line and square-lattice arrangement, respectively) probably is due to the unzipping of limiting membranes causing a disintegration of the central electron-dense lamella, a phenomenon that reflects the fusion between a BG disc and an acidic compartment like an endosomal vesicle (Bartosik, 1992). According to this proposal, several features suggest that BG derive from cell membrane invaginations (Takahashi & Hashimoto, 1985) and are involved in a specialised form of adsorptive endocytosis, transporting ligand-receptor complexes from the cell surface to primary lysosomes (Takigawa et al. 1985; Hanau et al. 1987; Bartosik, 1992). However, for the present, the nature of the signals that trigger BG formation is still unknown, and the real significance of BG in the antigen-trapping and antigen-presenting function of LC is a controversial theme (Mommaas et al. 1994).

Recently it has been reported that only CD34+ progenitor cells from human peripheral blood that express the Lag antigen (Caux et al. 1996), a specific glycoprotein on BG (Kashihara et al. 1986), and the skin-homing receptor CLA (cutaneous lymphocyte-associated antigen) differentiate into LC with BG (Strunk et al. 1997). These new aspects concerning the BG suggest that their presence may be related with the differentiation and maturation pathways of the LC (Mackensen et al. 1995; Strunk et al. 1996).

The results obtained in the present study are the first evidence of the presence of BG-like organelles in

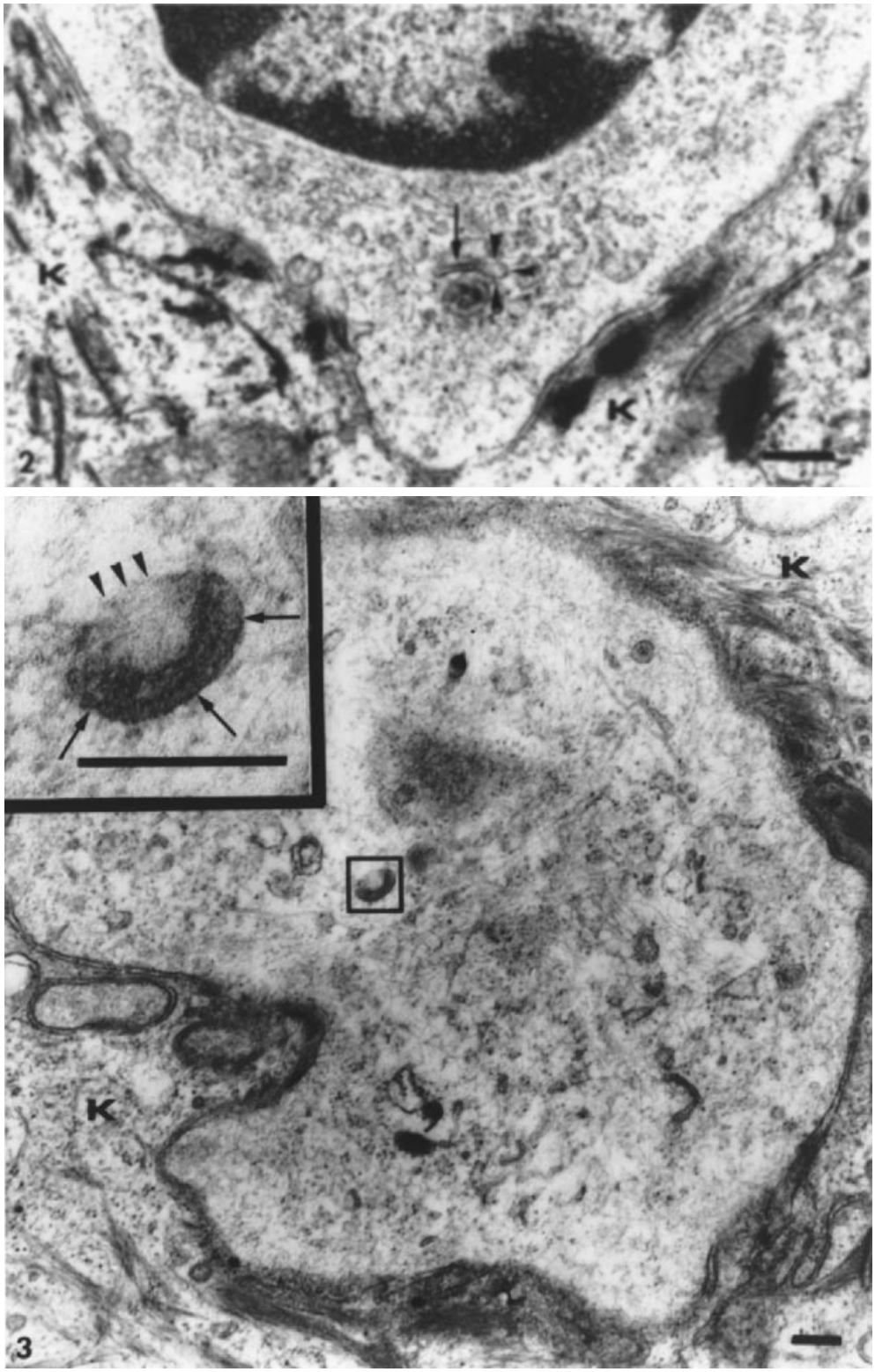

Fig. 2. Transmission electron micrograph of chicken normal skin showing an epidermal Langerhans cell in the basal layer. Note the rod (arrow) and the vesicular expansion (arrowheads) of a racket-shaped Birbeck granules-like organelle. The rod portion lacks of the electron-dense central lamella. K, basal keratinocytes. Bar, 250 nm.

Fig. 3. Transmission electron micrograph of chicken normal skin showing a large cytoplasmic process with ultrastructural features of Langerhans cells including a disc-shaped Birbeck granule-like organelle, which has been cut tangentially (frame). The insert represents a higher magnification of this structure formed by a flat disc (arrows) with an electron-lucent vesicle attached in the periphery (arrowheads). Correlate this aspect of Birbeck granule-like organelle with its cross-section that gives rise to the racket-shaped profile (Fig. 2). K, keratinocytes. Bar, 200 nm.

normal chicken skin and thus complete the identification of the LC in this animals.

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