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Original Articles

Vitamin A Deficiency in Rats Induces Langerhans Cells in the Iliac Lymph Nodes Draining to the Squamous Metaplastic Urinary Bladder

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We previously observed Langerhans cells (LC) in the squamous metaplastic urinary bladder in rats fed a vitamin A-deficient diet. Here we report the finding of LC in the iliac lymph nodes, one of the abdominal lymph nodes draining to the urinary bladder. LC had previously only been observed in the superficial and hilar lymph nodes, but never in the abdominal lymph nodes. This is also the first observation of Birbeck granule (BG)-positive LC in the iliac lymph nodes. LC were not found in the transitional mucosa of the urinary bladder. In the present experiments, the LC were observed in both the squamous metaplastic mucosa and lamina propria of the urinary bladder, and also in the iliac lymph nodes, which drain into the urinary bladder. BG-positive LC may mature not only in the squamous epithelia, but also in squamous metaplastic mucosa. LC in the lamina propria of the urinary bladder might migrate to the iliac lymph nodes, which are regional lymph nodes, where they could function in antigen presentation. LC in the skin play a role in antigen-antibody interactions, and the squamous metaplastic mucosa of the urinary bladder may be a similar environment, in which LC in the mucosa can migrate to the regional lymph nodes.
Key words vitamin A deficiency, Langerhans cell, iliac lymph node, squamous metaplasia

INTRODUCTION

In a previous report, we observed Langerhans cells (LC) in the superficial and hilar lymph nodes, but not in the mesenteric lymph nodes and spleen[1]. We speculated that LC needed to contact the squamous epithelia in order to form Birbeck granules (BG). LC can be observed not only in normal squamous epithelia, but also in metaplastic squamous epithelia. Hosokawa *et al*. investigated the migration and maturation of LC in rat tracheal squamous epithelia induced by vitamin A deficiency[2]. Furthermore, LC were also observed in urinary bladder squamous epithelia of vitamin A-deficient rats[3].

In the present study, we examined whether BG-positive LC exist in the internal iliac lymph nodes, which are regional lymph nodes draining from the squamous metaplastic urinary bladder, in vitamin A-deficient rats, as opposed to normal rats in which LC do not exist in that location.

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MATERIALS AND METHODS

3-week-old male Sprague-Dawley rats (Japan SLC, Shizuoka, Japan) were used for this study. Animals were maintained in plastic cages with a 12/12-hour light/dark cycle in an air-conditioned room at 21-25°C. Twenty rats were fed a vitamin A-deficient diet (Oriental Yeast Co., Tokyo, Japan) with water *ad libitum*. Ten control rats were kept under the same conditions and fed a commercial basal diet (Oriental Yeast Co., Tokyo, Japan). Animals were weighed weekly. The experimental rats were sacrificed under pentobarbital anesthesia at the stage of weight plateau and/or the during the weight loss stage from a peak weight of 10 g over normal body weight. The duration of the study was 16 to 20 weeks.

The urinary bladder was removed after ligating the urethra and injecting PBS into its cavity. The iliac lymph nodes were also removed. After being cut into several pieces, some of the specimens were processed by the AMeX (acetone, methyl benzoate, and xylene) method[4], and paraffin sections were prepared for routine staining with hematoxylin and eosin (H-E) and for immunohistochemical demonstration of mouse anti-protein kinase C type Ⅱ β (PKC Ⅱ) antigen (Seikagaku Co., Tokyo, Japan).

AMeX-paraffin sections were treated with normal goat serum and anti-PKC Ⅱ (1 : 200). They were then treated with alkaline phosphate-conjugated goat anti-mouse IgG (1 : 100 ; Cappel Co., Durham, NC, USA), according to an indirect method. The sections were incubated with levamisole containing fast blue substrate solution (Vector Laboratories Inc., Burlingame, CA, USA).

For electron microscopic study, other portions of the urinary bladder and iliac lymph nodes were fixed initially in a mixture of 2.5% glutaraldehyde and 2% paraformaldehyde in phosphate buffer. They were then postfixed in 2% osmium tetroxide buffered with phosphate. Ultrathin sections were stained with uranyl acetate and lead compounds and examined with a Jeolco 100 CX-electron microscope.

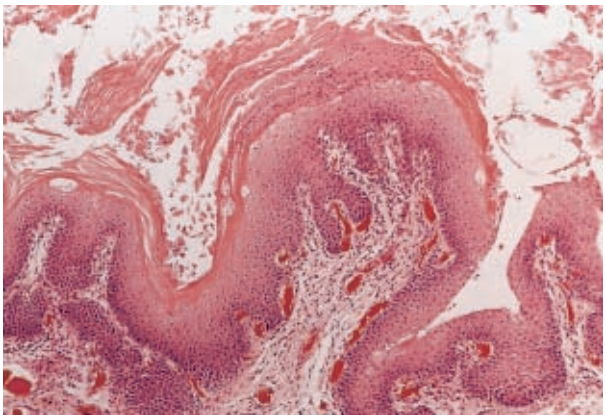

Fig. 1. Urinary bladder mucosa with transformation into keratinizing squamous metaplasia can be observed. (Hematoxylin-eosin staining) ×80

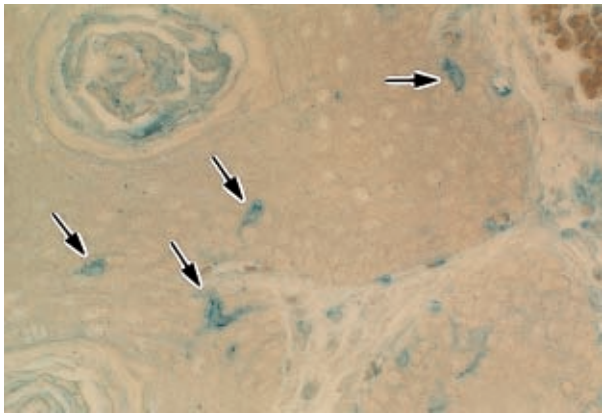

Fig. 2. Several PKC Ⅱ-positive cells can be seen in the mucosa. Arrows show the LC. ×160

RESULTS

About half of the experimental animals showed early stage squamous metaplasia in the urinary bladder. In the early stage, the covering cells were still found on the surface of the basal cells. A few PKC Ⅱ-positive cells were observed among these basal cells. However, BG-positive LC could not be ultrastructurally observed in the mucosa. The urinary bladder in half of another group of rats progressed to the stage of cornification, and covering cells could not be found in the urinary mucosa, indicating that keratinization of the mucosa had occurred (Fig. 1). PKC Ⅱ-positive cells, which were large and had dendritic processes, were scattered in the mucosa (Fig. 2). Under electron microscopy, these dendritic cells contained BG in the cytoplasm. A few BG-positive LC were also observed in the lamina propria (Fig. 3). The majority of BG were rod-shaped, but some were tennis racket-shaped. Furthermore, previously reported atypical granules[2] were also found in the cytoplasm. Some LC possessed multilamellar major histocompatibility complex (MHC) class α compartment (MαC)-like structures, which were found relatively often in human Langerhans cell histiocytosis and previously called fingerprint-like structures (Fig. 4).

All internal iliac lymph nodes, in which squamous metaplasia of the urinary bladder was completed, were 2 to 3 times greater in diameter than those of control rats. Normal structure was preserved in these large lymph nodes, but the paracortex was rather hyperplastic and sinus histiocytosis was also observed. Under light microscopy, we could not detect LC in the lymph node parenchyma, although large cells containing dendritic processes and coffee bean-like nuclei were scattered in the paracortical areas and in the marginal sinuses (Fig. 5).PKC Ⅱ-positive cells were immunohistochemically scattered, and

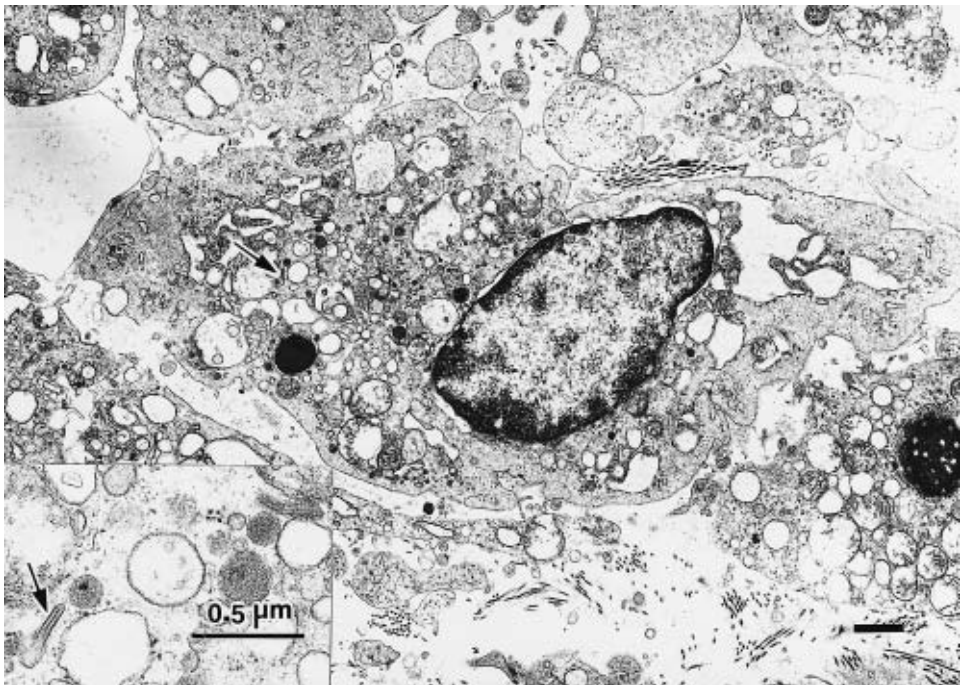

Fig. 3. A Langerhans cell is found in the lamina propria. An arrow points to the BG. ×6,700, bar=1 μm, Inset: High magnification of the BG. ×29,200, bar=0.5μm

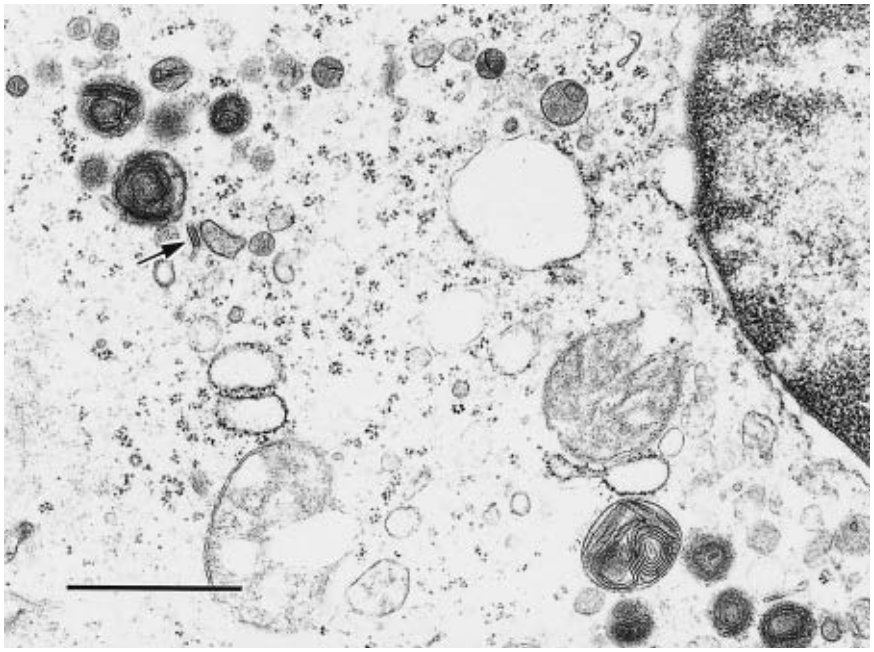

Fig. 4. Fingerprint-like structures are found in a Langerhans cell. An arrow shows a BG. ×23,400, bar=1 μm

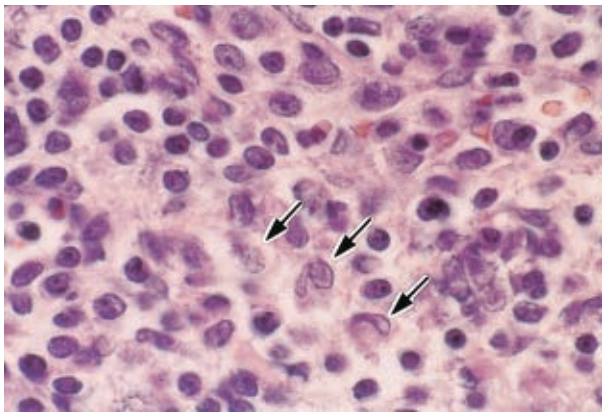

Fig. 5. Dendritic cells are observed in the iliac node. Arrows point to the LC. (Hematoxylin-eosin staining) ×770

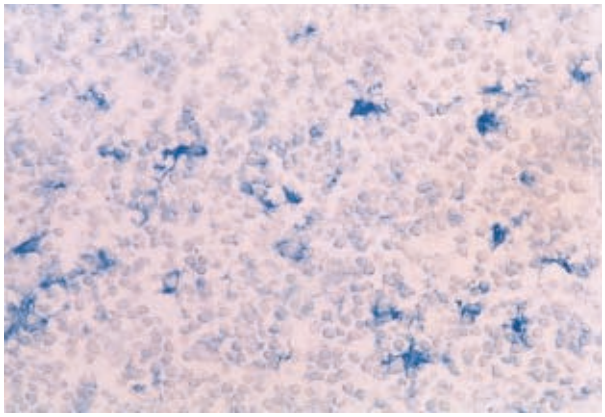

Fig. 6. A few PKC Ⅱ-positive cells are observed in the iliac node. ×320

sometimes, several assembled in these areas (Fig. 6). Only a few PKC Ⅱ-positive cells were observed in the lymph nodes in which squamous metaplasia was incomplete.

We selected the iliac nodes from five cases, in which complete squamous metaplasia of the urinary bladder was noted, for electron microscopic observation. Ultrastructurally, LC were found in the iliac node parenchyma in all five cases. The LC were large and contacted neighboring lymphocytes by dendritic processes. Their cytoplasm was clear and their nuclei were irregular in outline. Cytoplasmic organelles such as the mitochondria, rough endoplasmic reticulum, and Golgi apparatus were well developed. BG which were rod-shaped and sometimes tennis racket-shaped were observed in the cytoplasm, especially near the Golgi apparatus (Fig. 7). However, no BG attached to the plasma membrane could be found. Atypical granules were also observed.

In control rats, the urinary bladder was lined by transitional epithelium, but never possessed squamous metaplastic epithelium. No PKC Ⅱ-positive cells were observed in the urinary bladder epithelium or in the iliac nodes, which were normal in size and had normal architecture.

DISCUSSION

We have reported that LC are found in human superficial and hilar lymph nodes, which drain from squamous epithelia, but are not found in the mesenteric lymph nodes and the spleen. On the other hand, interdigitating cells (IDC), exist in all human organs[1]. Hosokawa *et al*. found that vitamin A deficiency induces BG-positive LC in the metaplastic squamous epithelia of rat trachea[2]. They suggested that LC may need to contact the squamous epithelia in order to form BG.

Caux *et al*. reported that CD34+ cells in the human umbilical cord acquired BG when cultured in the presence of GM-CSF and TNF-α[5]. The human epidermis has been shown to produce several cytokines, such as TNF-α[6]. We surmise, therefore, that cytokines such as TNF-α in the squamous epithelia may play a role in the production of BG. On the other hand, Groves *et al*. reported that TNF-α causes decreased CD1a+ LC in the epidermis and increased CD1a+ cells in the dermis[7]. IL-1β and/or IL-18 may be necessary for migration of epidermal LC to draining lymph nodes[8,9].

In the studies here, we observed BG-positive LC in the metaplastic squamous epithelia of the urinary bladder as well as in the lamina propria of the urinary bladder of vitamin A-deficient rats. The most conspicuous finding was the observation of BG-positive LC in the internal iliac lymph nodes, which drain from the metaplastic squamous epithelia of the urinary bladder in these rats. These LC showed the same profile as those in normal skin, both morphologically and immunohistochemically. To our knowledge, this is the first observation of BG-positive LC in the iliac lymph nodes, which are one of the abdominal lymph nodes. We speculate that IDC, which may also be referred to as immature LC without BG, may migrate into the metaplastic squamous epithelium of the urinary bladder and

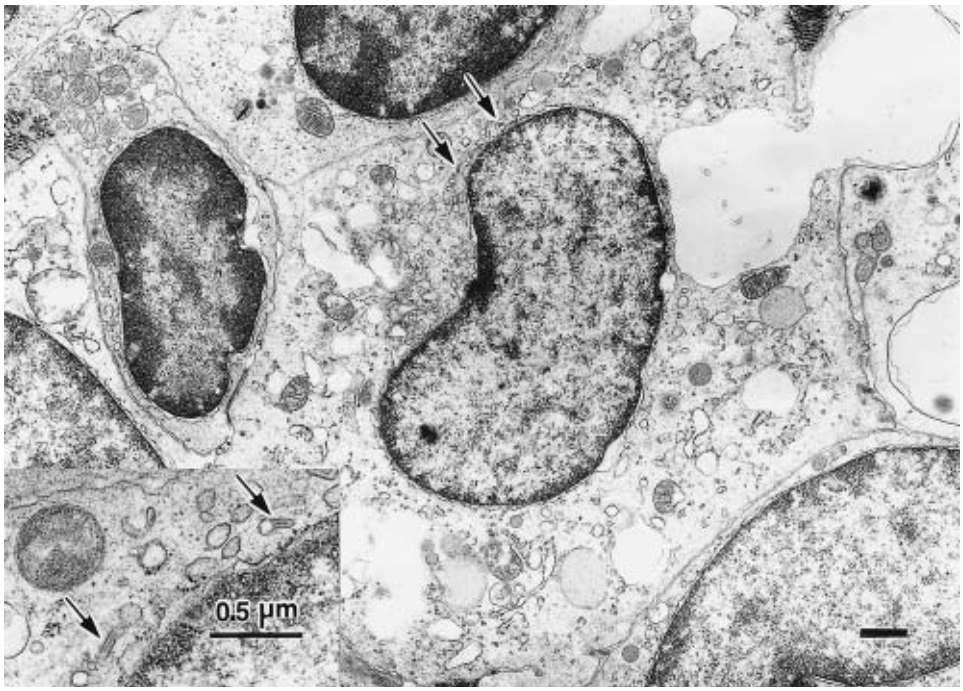

Fig. 7. A Langerhans cell contains some Birbeck granules in the iliac node. Arrows point to the BG. X 6,700, bar=1μm, Inset: High magnification of the BG. ×23,400, bar=0.5μm

become mature BG-positive LC. Namely, exposure to the squamous epithelium microenvironment, which is similar to the skin, may be needed for BG formation. Then, LC should migrate to the draining iliac lymph nodes for antigen presentation. BG-positive LC in the lamina propria may also be migrating to the iliac lymph nodes. Sugiura *et al*. have recently observed both CD1a and S-100 protein-positive cells with dendritic processes, which should be LC, in the lymph vessels of the dermis in patients with seborrheic keratosis[10]. The squamous metaplastic epithelium of the urinary bladder may produce cytokines such as TNF-α, as does the skin, and they may function in BG production and LC migration. Herbst *et al*. reported that MαC-like structures were observed in BG-positive LC[11]. The existence of MαC-like structures in BG-positive LC suggests that these LC express MHC classα antigen and present antigens to T cells.

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