Ultrastructural Study of Langerhans Cells in Equine Insect Hypersensitivity "Kasen"

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ABSTRACT. Ultrastructural features of Langerhans cells (LCs) of equine "Kasen" were studied. Electron microscopic observation revealed that LCs were dendritic and had irregular nuclear membranes. A number of Birbeck granules (Bgs) of various types were observed in the cytoplasm of LCs. In LCs in the upper stratum spinosum, many Bgs were observed (Type 2 LC). LCs in the epidermo-dermal junction (EDJ) had a few Bgs, vesicles (multivesicular bodies) and highly electron-dense granular endosomes in the cytoplasm (Type 3 LC). Inactive LCs were also observed between the keratinocytes (Type 1 LC). Various types of LCs observed in the skin lesions of equine "Kasen" were interpreted as representing those that recognize, intake and process antigens.

KEY WORDS: equine, insect hypersensitivity, Langerhans cell.

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We have shown that equine "Kasen" had the characteristics of Type I and Type IV allergic dermatitis [3] and that the Langerhans cells (LCs), which are skin accessory cells, had an important role in the pathogenesis of equine "Kasen" [4]. In the present study, we investigated the ultrastructural features of the LCs in equine "Kasen" lesions.

The investigation was conducted on the same specimens as used in a previous study [4].

The specimens, measuring approximately 1 cm², were biopsied from skin lesions of the tail base, neck and mane using a dermatome. Thin-sliced sections of the skin lesions were fixed in 1.5% paraformaldehyde-0.5% glutaraldehyde solution in phosphate buffer (pH 7.4) and post-fixed with 1% osmium tetroxide, then dehydrated according to the routine method, and embedded in epoxy resin (Epok 812). After 1- μ m-thick sections were stained with 0.5% toluidine blue and observed by light microscopy, ultra-thin sections were double-stained with uranyl acetate and lead citrate, then examined by a transmission electron microscopy (Hitachi H-7000).

The LCs widely expanded their dendritic processes among the keratinocytes, the nuclear membrane was irregular, and the broad cytoplasm had the characteristics of Birbeck granules (Bgs) and a rich content of organelles including mitochondria, central bodies, Golgi complex, rough endoplasmic reticula, ribosomes or tonofilament-like structures (Fig. 1). Bgs had a variety of shapes depending on the combination of vesicular and rod-like structures, such as a representative rod-like shape having a central linear structure measuring 94–470 nm in length and 42 nm in width (Fig. 1) and a tennis racket-like shape with a dilated vesicular tip (Figs. 2, 3). LCs were classified into 3 types according to the morphology of

these components. LCs in the upper stratum spinosum were found to have a number of Bgs associated with endocytosis caused by a cell membrane depression (Fig. 4). In other words, LCs with a number of Bgs of various shapes and that were associated with endocytosis were classified as Type 2. Vesicles (multivesicular bodies) of various sizes having a marginal membrane and highly electron-dense granular endosomes of various sizes were markedly increased in the cytoplasm of LCs located in the epidermo-dermal junction (EDJ), in addition to a few Bgs (Fig. 5). In other words, these cells were classified as Type 3. On the other hand, we observed inactive LCs with a narrow and lucid cytoplasm containing a small number of mitochondria and ribosomes, a poorly depressed nuclear membrane, and a scarce number of Bgs at sites in the epidermal layer; we classified these cells as Type 1.

A notable morphological change occurring in the LCs of equine "Kasen" was the presence of distinct Bgs observed by electron microscopy. Bgs varied in shape, from rod-like to tennis racket-like or presenting with a depression continuous with the cell membrane. The various morphological forms of Bgs in LCs are closely similar to those of human skin [7].

Hosokawa *et al.* [2] investigated the migration and maturation of LCs in rat tracheal squamous metaplasia due to vitamin A deficiency. Although LCs with Bgs were not found in the early stage of metaplasia, small numbers of LCs were recognized in the stage of stratification. Ultrastructurally, they have only a few Bgs with ovoid nuclei and few dendrites. Many LCs with Bgs were found in the early stage of cornification. Most of LCs matured typically with lobulated nuclei and prominent dendritic processes. However, in epithelium showing mature squamous metaplasia, the numbers of Bgs had decreased compared with the LCs recognized in the early stage of cornification. These electron microscopic findings of LCs are closely similar to those of the skin lesions of

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Fig. 1. Typical Langerhans cell (LC) in epidermo-dermal junction (EDJ). The broad cytoplasm has Bgs with representative rod-like shapes (arrowheads, inset), mitochondria (arrows), central body (open arrow) and tonofilament-like structures (t). Transmission electron microscopy (TEM). Uranyl acetate and lead citrate. $\times 13,500$, inset: $\times 40,500$.

Figs. 2, 3. Tennis racket-like Bg observed in the cytoplasm of LC. TEM. Uranyl acetate and lead citrate. Fig. 2: $\times 54,000$. Fig. 3: $\times 45,000$.

Fig. 4. Type 2 LC in upper stratum spinosum. Bg associates with endocytosis caused by a cell membrane depression (arrowheads), and widely expanded dendritic processes (arrows). TEM. Uranyl acetate and lead citrate. $\times 42,500$.

Fig. 5. Type 3 LC in EDJ. LCs in the EDJ has a few Bgs (arrowheads), vesicles (inset A) and highly electron-dense granular endosomes (inset B) in the cytoplasm. TEM. Uranyl acetate and lead citrate. $\times 13,600$; inset A: $\times 40,000$; inset B: $\times 40,000$.

"Kasen". In addition, it has been suggested that Bgs function in the intracellular trafficking of surface antigens [1, 6]. Bgs and highly electron-dense granular endosomes were acidic organs exhibiting endocytosis and were critical for antigen

processing [8]. In conclusion, we classified LCs in lesions affected by "Kasen" into 3 types on the site of manifestation, ultrastructural findings of Bgs and findings in the literature [1, 2, 6, 8]. Specifically, LCs of Type 1 to Type 3 represent

the morphology of functional activities. Type 1 LC, which was confirmed by the presence of a small number of Bgs in narrow cytoplasm, represent the morphology before the intake of the antigen. Type 2 LC, which involves endocytosis and a number of Bgs of various shapes, represents the morphology at the time of antigen intake. Type 3 LC, whose cytoplasm had a remarkable number of vesicles (multivesicular bodies) and highly electron-dense granular endosomes of various sizes, represents the morphology after the intake of the antigen. LCs is trigger of the allergic contact dermatitis (delayed type hypersensitivity) in human [5]. These findings indicate that LCs may trigger the onset of type IV allergy in equine "Kasen".

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