Localization of herpes simplex virus type 1 in sebaceous glands of mice

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Summary. The distribution of HSV-1 during the development of zosteriform skin lesions in SCID mice was analyzed by immunofluorescence and electron microscopy. The virus initially appeared within certain keratinocytes, sometimes surrounded by keratinocytes whose surfaces were also positive for the antigens, in the lower epidermal layers including the hair follicles, and then extended upward to the entire epidermis and downward to the sebaceous glands 1–2 days later, when no macroscopic skin lesion was seen. The affected epidermal cells subsequently degenerated and lost their viral antigens within a day, when the zosteriform lesion then became evident. This was followed by a degeneration of the dermis. The sebaceous glands eventually degenerated in 10 days, but some glands in the necrotic skin areas preferentially retained HSV-1. The horizontal spread of the virus in the epidermis beyond the first invaded dermatome occurred much later. In mice passively immunized with specific immune serum, viral antigens were observed even 20 days after the infection in sebaceous glands in necrotized areas. Therefore, HSV-1 appears to spread first via the extracellular fluid among the keratinocytes after being shed from nerve endings, and then produces a successive degeneration of the affected keratinocytes which may prevent any further extension of horizontal viral spread. The pilosebaceous apparatus is possibly acting as a site not only for the replication of HSV-1 with a delayed cytopathic effect, but also as an area that is temporarily sheltered from host defense mechanisms.

Introduction

Herpes simplex viruses (HSV), family Herpesviridae, genus Simplexvirus, enter the peripheral terminals of the neurons at an early stage of primary lytic infection with and/or without proliferation at the primary site, and then travel with the axonal flow to the ganglia where they proliferate. Thereafter, they again travel...
down the axon to produce zosteriform lesions of the dermatome. This process has been well demonstrated in mouse models by both macroscopic observation and virus titration [15, 18, 19]. However, few studies on acute cutaneous infection have identified the precise sites of viral proliferation; HSV appeared in the epidermis, which suggests a shedding of the virus from the peripheral nerve endings with a successive viral spread into the dermis [19].

Along with the latent ganglionic infection [20], the persistence of HSV in the skin has been well demonstrated in animals [16]. Moreover, some investigators have reported details of cutaneous latency in mice and guinea pigs [5, 9]. Therefore, a more detailed histological analysis of these cutaneous lesions would also be valuable for a better understanding of the pathogenesis as well as the clinical manifestations of HSV-1.

Murine skin consists of the epidermis, pilosebaceous appendages, and the dermal connective tissue containing vascular and neural networks. Epidermal basal cells proliferate and differentiate to produce either a keratinized layer of the skin surface or pilosebaceous structures. Recent reports describe the localization of stem cells in the bulge area of hair follicles; derivatives of these dormant cells are presumed to regulate the hair cycle [7]. Fibroblasts are present in the connective tissue which forms a physical barrier against extracorporeal organisms [10, 11]. Nevertheless, it is not completely understood how these structures and cell functions are related to the pathogenesis of HSV infection.

Mice with severe combined immunodeficiency (BALB/c C57BL/Ka-Igh-1b/Icr, SCID mice) carry no detectable immunoglobulin [2]. Therefore, clearer results can be obtained by the immunohistological examinations of tissues from this strain. Syngeneic murine immunoglobulins (Ig) can also be traced histologically when administered to such mice. In addition, we have preliminarily reported that the scid mutation did not influence the proliferation of HSV-1 [14]. Thus, because of defects in the development of T- and B-cells (for reviews, see [4]), one would expect to observe the in vivo behaviour of viruses under conditions in which no specific immune response takes place.

In the present study, using SCID mice, we demonstrated that the histological localization of HSV-1 in skin during the development of the zosteriform skin lesion was unexpectedly different from the corresponding morphological changes, and that HSV-1 was predominantly localized in the sebaceous glands, sometimes until after a total cutaneous degeneration had occurred.

**Materials and methods**

**Virus**

HSV-1, strain 7401H, isolated from the vesicular skin lesions of an adult patient with herpes labialis and formerly designated as Hayashida strain [15], was passaged 7 times in Vero cells. The virus was propagated by infecting the Vero cells at 0.1 PFU per cell. The infected cultures were incubated at 37 °C for 3 days until a complete cytopathic effect (CPE) was observed. The cells and medium were then harvested, sonicated at 150 W for 5 min (Insonator MR 590, Kubota, Tokyo, Japan) to release the cell-associated virus and centrifuged at