Herpes simplex virus type-1 and -2 pathogenesis is restricted by the epidermal basement membrane

Brief Report

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Summary. Murine flank scarification with HSV-1 and -2 results in primary lesions at the site of inoculation within three days and lesions at secondary sites within four days. The severity of the infection can be given a numerical value or “score” which is derived from the number and size of these lesions. Using this model, we investigated the role of the epidermal basement membrane in HSV pathogenesis. We exposed murine epidermis to $5 \times 10^4$ plaque forming units of HSV-1 and -2, which by day 8 produced inoculation site (primary site) disease scores of 27 and 12.4 respectively, and secondary site disease scores of 29 and 30 respectively. In contrast, intradermal injection of HSV below the epidermal basement membrane did not cause disease. To determine if the basement membrane restricts HSV spread in vitro, Vero cells were cultured in the lower well of a dual well system. The upper well was separated from the lower well by a filter coated with the artificial basement membrane, matrigel. Addition of virus to the upper well failed to result in either viral accumulation in the lower well or infection of the cells in the lower well. These data suggest that the basement membrane is a barrier to the passage and spread of HSV.

Herpes simplex viruses -1 and -2 (HSV) are two of eight herpes viruses which infect humans [11, 30]. Both of these viruses infect epithelial surfaces and then move into nervous tissue [7, 30]. Having entered the epithelium, they undergo replication and infect and destroy neighboring cells. This produces a primary lesion at the initial site of infection [28]. During the initial infection, viruses infect sensory nerve endings in the skin or mucosa and travel to the associated nerve dorsal root ganglion via retrograde axonal transport [2]. It is in the ganglia
where the herpes virus become latent [30]. Viral reactivation can occur due to various stimuli such as stress, fever, and local tissue injury [5, 17, 18, 23]. During reactivation the virus undergoes anterograde transport from the ganglion [3, 16] and reappears in the epithelial tissues at, or near, the initial site of infection where a recurrent lesion forms [5, 22].

HSV pathogenesis in vivo has been investigated by several routes of infection, including ocular, vaginal, footpad, intranasal, peritoneal and flank epidermal inoculation of mice [3, 5, 22, 24, 26, 27]. In the model of epidermal inoculation, the murine flank is scarified in the presence of virus by scratching the skin at the site of exposure [3, 22, 24]. By day three post-scarification a lesion forms on the murine flank at the site of inoculation and is referred to as primary site disease. Within four to seven days, secondary lesions form at sites distant from the inoculation site. This secondary site disease does not result from transdermal spread of the virus, but rather from retrograde transport of virus from the inoculation site to the associated ganglion and subsequent antero-grade transport to new sites in the epidermis [22, 24]. With this murine flank scarification model, the three day period for the formation of primary site disease and the subsequent formation of secondary lesions is similar in time-course and histopathology to that observed in human infection with HSV-1 and -2 [22, 24]. Here, we use the flank scarification murine model to study the pathogenesis associated with HSV-1 and -2 epidermal and intradermal inoculation.

The mechanisms of HSV pathogenesis in the skin is in part related to the structure and biology of the epidermis and dermis [13, 24, 25]. For example, an acellular matrix of proteins known as the basement membrane which is composed of laminin, type IV collagen, heparan sulfate proteoglycan and entactin separates the epidermal and dermal layers of the skin [8, 9]. Basement membranes also surround all peripheral nerves [8, 9] from the ganglion to the dermal basement membrane where the free nerve ends erupts above the dermal basement membrane and are susceptible to viral entry [12]. The basement membrane protein laminin has been shown to limit HSV-1 cell to cell spread in human skin keratinocytes by stimulating the formation of tight junctions in these cells [29]. Further, basement membrane heparan sulfate binds to HSV and may act as a receptor for the virus or perhaps through this binding the basement membrane acts as a trap or barrier to free HSV [31]. Therefore, the skin basement membrane provides several potential mechanisms which can alter and restrict the spread of HSV in the body [29, 31].

The route of HSV infection is an important determinant of pathogenesis and disease severity [1, 7, 19]. Here we expose mice to HSV-1 and -2 through two different routes and compare the resulting pathogenesis, disease severity and development of protective immunity. The first route involves epidermal inoculation by scarification of the skin on the murine flank. The second route involves intradermal inoculation of mice through intradermal injection of HSV beneath the basement membrane. We also examine the ability of cell free virus to cross an artificial basement membrane barrier, matrigel. Our data indicate that the basement membrane is a barrier to HSV passage and demonstrate a role for the basement