Langerhans cells in vaccinia virus infection in mouse skin

Brief Report

Y. Becker and E. Sprecher

Department of Molecular Virology, Faculty of Medicine, Hebrew University, Jerusalem, Israel

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Summary. Langerhans cells function as potent antigen-presenting cells in the epidermis. They were shown to play an essential role in the mechanisms of defense of the skin against viral infections. In the present study, the response of Langerhans cells to infection of the skin with vaccinia virus was investigated. Decrease in Langerhans cell density in the skin was accompanied by an increase in the pathogenicity of the WR and Noguchi but not of the Lister strain of vaccinia virus. Langerhans cell density was shown to increase rapidly at the site of inoculation with the two pathogenic strains of vaccinia virus.

Langerhans cells (LC) function as potent antigen-presenting cells in the epidermis [2, 16]. They express high levels of Ia antigens and display strong membranal ATPase activity [1, 23]. Evidence suggesting that skin LC might play a role in immunization against smallpox came from studies by Nagao and Inaba [18]. These authors demonstrated the presence of vaccinia virus (VV) particles in LC following intradermal inoculation of smallpox vaccine. During recent years much information has accumulated which indicates that LC play a cardinal role in the mechanisms of defense against viral infections in the skin [26]. Particularly, they were shown to play a critical role in the mechanisms of defense of the skin against herpes simplex virus type 1 (HSV-1) infection. A correlation was found between decrease in LC number and increase in HSV-1 pathogenicity [24, 25]. In analogy with what was shown for VV [18], LC were shown to take up HSV-1 antigens in the skin during virus infection [24] and to present these antigens to HSV-1 sensitized T cells [7, 14, 19, 25, 29, 30]. It was also found that infection with virulent, but not avirulent, HSV-1 strains in the footpad skin induced a significant increase in epidermal LC density [24].

VV which is used as a smallpox vaccine has become a center of interest regarding its potential as a vector for genetically engineered vaccines [20]. For
this reason, attention is being directed to the possibility that residual pathogenicity still exists in this virus [3, 9]. Since LC density and function were shown to control HSV-1 pathogenicity [19, 24, 25, 29], it was of interest to examine the severity of VV infection in normal skin and in skin which was depleted of LC by local steroid treatment.

Three strains of VV were used: the Lister strain (obtained from Prof. E. Katz, Department of Virology, our medical school), WR strain [5], and Noguchi strain (obtained from Prof. F. Fenner, The Australian National University, Canberra) were grown and titrated on BSC-1 cells maintained in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% calf serum. Titer of the three viruses was $3 \times 10^7$ pfu/ml. Male, 4-8-week-old mice of the C57BL/6 and A strains were obtained from the Hebrew University animal facilities. Mice were injected in the footpad or through the peritoneum (i.p.) with 0.05 ml of virus using a 25 gauge syringe.

The relative pathogenicity to mice of the three virus strains used was tested in a murine model of VV infection in the footpad skin and by the intraperitoneal (i.p.) route [9]. Six week-old male A and C57BL/6 mice were injected with the Lister strain of VV at $1.5 \times 10^6$ pfu/mouse in the right footpad or i.p. and mortality was recorded. The Noguchi and WR strains were injected either i.p. or in the footpad skin of A strain mice with the same amount of infectious virus. The Lister and WR strain were found to be totally avirulent for normal mice (Table 1). The Noguchi strain killed about 12% of the A mice when injected i.p. or through the footpad (Table 1).

Infection with HSV-1 virulent strains induced an increase in LC density in the footpad skin while avirulent virus strains did not [24]. Thus, the effect of VV infection on epidermal density of LC at the site of virus inoculation was examined. A strain mice were inoculated with $1.5 \times 10^6$ pfu/mouse of the three vaccinia strains in the right footpad and the skin was removed at different times post-infection (p.i.) and stained for ATPase activity [6]. Mice were killed by CO$_2$ asphyxiation immediately prior to excision of skin from the rear footpads. After separation from the epidermis in buffered EDTA solution, epidermal sheets were washed three times in Tris maleate buffer (containing 6.85% sucrose) at 4°C for 20 min and then fixed for 20 min at 4°C in 4% cacodylate-buffered formaldehyde. The sheets were then washed three times in Tris maleate buffer (6.85% sucrose) for 30 min at 4°C and stained with a solution containing 10 mg of ATP, 3 ml of 2% PbNO$_4$, 5 ml of 5% MgSO$_4$, and 92 ml of Tris maleate buffer (8.53% sucrose) at 37°C for 20 min. The preparations were then washed again three times in Tris maleate buffer at 23°C and put into a 1% solution of ammonium sulfide for 5 min. They were finally washed twice with distilled water, mounted in a 9:1 solution of glycerol-PBS and counted with the aid of an eyepiece grid (0.046 mm$^2$ = one field; 20 fields chosen at random counted for each specimen).

Two strains of VV (Noguchi and WR) were found to induce a significant increase in LC density (Fig. 1). Infection with the Lister strain caused a smaller