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Herpes Simplex Virus Type 1 Pathogenicity in Footpad and Ear Skin of Mice Depends on Langerhans Cell Density, Mouse Genetics, and Virus Strain

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Skin Langerhans cells have been shown to be very efficient in presenting antigens to T-helper cells and stimulating the immune response. The present study demonstrates their essential role in the control of primary herpetic infections in the skin. Two unrelated stimuli (abrasion and steroids) were shown to cause depletion of the Langerhans cells in the murine epidermis, and both caused enhancement of the virulence of herpes simplex type 1 (HSV-1) in the skin. The Langerhans cell density was found to be lower in the skin of the ear than in the footpad. HSV-1 was consistently more virulent when injected into the ear epidermis than in the footpad. Thus, HSV-1 pathogenicity in mouse skin depends on the mouse age and strain, the virus strain, and the state of the epidermal Langerhans cells. These findings are discussed in relation to the antigen-presenting cell function of the Langerhans cells.

Two models of herpes simplex virus type 1 (HSV-1) murine skin infection have been developed. Hill et al. (19) injected the virus into the pinna of the mouse ear and showed that the virus spreads to the cervical ganglia and central nervous system, causing encephalitis and death. They also demonstrated that the virulence, latency, and propensity of HSV-1 to cause recurrent infections were dependent on both the mouse and the virus strains (16, 19). Tape-stripping (18) and UV light (8) caused recurrence of the virus in the primarily infected ear. Latent virus could be recovered by a cocultivation technique from the ganglia innervating the ear (19). Cook and Stevens (11) inoculated the virus into the footpad of mice and studied the route of virus entry from the periphery along the sciatic nerve to the spinal ganglia and from there to the brain. Only by using very severe immunosuppression were they able to induce recurrence of the virus (34). On the other hand, by using a cocultivation technique, latent virus was recovered from the peripheral ganglia (33). Subak-Sharpe et al. (37), using the footpad route of HSV-1 infection, demonstrated the presence of latent virus in the skin of the infected footpad.

Langerhans cells have already been known for more than a century (21), but only in the past few years have their functional and phenotypic properties been elucidated (reviewed in reference 36). The murine Langerhans cells are derived from the bone marrow (20) and bear Ia (38), Ly.5 (23), Mac.2 and Mac.3 (15), and S-100 (30) antigens as well as C3 and Fc receptors (35). Langerhans cells display membranal ATPase activity (27) and contain cytoplasmic Birbeck granules, detected by electron microscopy (7). They have been shown to function as highly efficient antigen-presenting cells (10), able to interact with T lymphocytes in local lymph nodes (31). They belong to a family of bone marrow-derived, dendritic, and Ia-positive cells distributed in lymphoid and epithelial tissues all over the body (1).

We previously reported (32) that Langerhans cell density significantly increased after infection of the footpad skin with a pathogenic strain of HSV-1. Infection with a nonpathogenic strain of HSV-1 did not lead to such an increase in Langerhans cell number. We also demonstrated that intradermal inoculation of the footpad with a hypertonic saline solution followed by abrasion led to depletion of Langerhans cells in the footpad skin. In the absence of the cells, the virulence of both pathogenic and nonpathogenic strains of HSV-1 was enhanced. Progressive increase in Langerhans cell density in the skin during recovery from abrasion was correlated with a gradual increase in resistance of the mouse to infection with HSV-1. This study on the role of Langerhans cells in the skin defense has now been extended to four strains of mice and 11 strains of HSV-1. The effect of prednisolone on the Langerhans cells and the local immune response of the mouse skin to HSV-1 infection in the footpad were examined. The present study demonstrates that HSV-1 virulence upon murine skin infection is dependent on four different factors: pathogenicity of virus strain, genetic strain of mice, age of mice, and density of the epidermal Langerhans cells in the skin.

MATERIALS AND METHODS

Mice. Inbred strains C3H, A, and C57BL/6 and the outbred Sabra mouse strain (The Hebrew University) were used. The mice were all female and 4 weeks of age when used, except where indicated otherwise.

Virus strains and infection of mice. Eleven strains of HSV-1 were used, as follows. The LP, SP11, and SP18 strains were plaque purified and selected on the basis of plaque size from NIH strain 11124 of HSV-1 (13). The HSV-1 Justin strain was obtained from Hila Locker, Department of Molecular Genetics, The Hebrew University; HSV-1 strains R19, F, and HFEM were obtained from G. Darai, University of Heidelberg, Heidelberg, Federal Republic of Germany; and strain KOS was obtained from F. Rapp, Pennsylvania State University, Hershey. The three TK^- mutant strains were obtained from the corresponding strains grown in the presence of bromodeoxyuridine. All virus strains were propagated in BSC-1 cell cultures, and the titers were adjusted to $10^{7.0}$ PFU/ml by adequate dilution by Dulbecco modified Eagle medium before infection of mice. Mice were anesthesized with ether and then injected in the right hind footpad or right ear pinna with 0.05 ml of virus,

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using a 25-gauge syringe needle. Mice were observed daily for clinical signs and survival for at least 6 weeks. To recover virus, tissues were taken from moribund mice and ground in phosphate-buffered saline (PBS) containing 5% fetal calf serum, and 100 μl of the fluid was inoculated into BSC-1 cell cultures.

Depletion of Langerhans cells in the skin epidermis. Two methods were used. (i) The right footpad of each mouse was inoculated with 0.1 ml of a 10% aqueous NaCl solution as described by Cook and Stevens (11). After 6 h, the skin of the footpad was rubbed with sandpaper to remove the stratum corneum without causing any severe lesion. One drop of virus was placed on the abraded area with a Pasteur pipette, followed by four or five light strokes with the point of a 19-gauge syringe needle. When footpad bleeding occurred, mice were discarded. (ii) Mice were injected with 0.05 ml of different dilutions (1:2, 1:4, 1:8) of a 50-mg/ml sterile prednisolone acetate suspension (Dell Laboratories, Inc., Teaneck, N.J.).

Separation of the epidermis and histochemical staining. The number of Langerhans cells in the footpad was determined by the ATPase staining method (27). The epidermis was separated from the dermis after 2.5 h of incubation in a buffered solution of EDTA at 37°C. Preparations 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 a 4% cacodylate-buffered solution of 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 (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 eyegrid piece ($0.015\ mm^2$ = one field).

Cell suspensions. To obtain epidermal cells, animals were sacrificed by cervical dislocation, and their ears were carefully shaved, removed, and washed in PBS. They were then transferred to a solution of 95% ethanol for 2 min and washed again. The ears were split, and the cartilage was removed. The ears were then put in a sterile solution of 0.3% trypsin (Sigma) in GNK buffer (0.8% NaCl, 0.04% KCl, and 0.1% glucose, adjusted to pH 7.6 with 0.5% $NaHCO_3$) for 30 min in 5% CO_2 at 37°C. RPMI 1640, containing antibiotics, 2 mM glutamine, 10% fetal calf serum, and 0.1% DNase I (Sigma), was then added to the ears, and the dermis was separated mechanically from the epidermis with the aid of two forceps and discarded. The cell suspension was finally gently pipetted and washed three times in medium before subsequent operations.

To obtain spleen cell suspensions, mice were killed by cervical dislocation, and their spleens were removed aseptically and minced between two sterile microscope slides. The cell suspension was filtered, washed, and centrifuged. Buffered ammonium chloride (2 ml) was added, and the cells were suspended for 5 min at 37°C and finally washed twice before use. Cell viability was assessed by trypan blue exclusion and shown to be always greater than 95%. To eliminate macrophages from the cell suspension, the cells were incubated for 2 h in an incubator at 37°C and 5% CO_2, and the nonadherent cell population was gently decanted. To eliminate specific cell subpopulations, nonadherent splenocytes were incubated at 4°C for 30 min with anti-Ia monoclonal antibody (kindly provided by Eytan Yefenof, Department of Immunology, The Hebrew University Medical School), washed, and reincubated for 45 min at 37°C in the presence of fresh guinea pig serum, which was used as a source of complement. The serum was absorbed before the experiment on mouse cells to remove any nonspecific cytotoxic activity. To enrich the cell suspension for T cells, splenocyte suspensions were passed over a nylon-wool column, and the cells were eluted and plated on petri dishes for 2 h at 37°C and 5% CO_2 to eliminate residual adherent cells. The cells were finally washed twice before use and found to be 95% Thy. 1 positive by direct immunofluorescence.

HSV-1 presentation to T lymphocytes by epidermal cells. T lymphocytes (1.5×10^5 per well) from nonimmune animals (control) or immune animals (infected 7 weeks before the experiment) were cultured with epidermal cells (1.5×10^5 per well) in 96-well microtiter plates in RPMI 1640 supplemented with 10% heat-inactivated horse serum, 25 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) 5×10^{-5} M 2-mercaptoethanol, and antibiotics. To some wells, 50 μl of inactivated LP virus or 0.01 PFU of live virus (LP) per cell was added. Cells were cultured for 6 days. At 18 h before harvesting, 1 μCi of tritiated thymidine was added to each well, and the radioactivity incorporated into cellular DNA was determined as a measure of T-cell proliferation.

RESULTS

HSV-1 infection of mice in the footpad. The footpad route was used to study the pathogenicity of HSV-1 strains previously examined for their virulence by infection of mice via the eye route (13), intraperitoneally, and intracerebrally (3, 5). Paralysis and other clinical signs as well as mortality were monitored (Table 1). The Justin and KOS strains of HSV-1 were the most virulent. They caused a severe disease characterized by a high incidence of flaccid paralysis and death among the infected mice and the appearance of skin lesions. Mice died from severe encephalitis accompanied by neurological symptoms such as difficulty in locomotion and breathing. HSV-1 strains LP, F, HFEM, SP18, SP18 TK^-, SP11 TK^-, Justin TK^-, and R19 did not cause disease (Table 1).

C57BL/6 mice (Table 1) were resistant to all HSV-1 strains tested. Strain A mice were highly sensitive to infection with Justin and KOS strains and relatively insensitive to infection with LP and F strains (Table 1). C3H mice showed an intermediate sensitivity (Table 2).

Suckling mice (8 days old) were also inoculated by the footpad route and found to be sensitive to all HSV-1 strains tested. Sabra mice did not survive after having been inoculated in the footpad at 1 week of age with the LP, HFEM, F, SP11 TK^-, and Justin strains of HSV-1 (data not shown).

Effect of skin abrasion on Langerhans cell density and HSV-1 pathogenicity. The morphology of Langerhans cells in the footpad skin of Sabra mice, using ATPase staining, is shown in Fig. 1. The dendritic ATPase-positive cells occurred at a density of 1,750 cells per mm^2 (Fig. 1a). After injection of hypertonic saline and abrasion, ATPase-positive cell density decreased to 0 to 20 cells per mm^2 (Fig. 1b).

Several strains of mice were infected with different strains of HSV-1 after injection of hypertonic saline and abrasion of the skin, when the number of ATPase-positive cells in the skin had been markedly reduced (Fig. 1b). Injection of Sabra and A mice with Justin, KOS, LP, SP11, SP18, or F HSV-1 strains into the skin after depletion of the Langerhans cells

TABLE 1. Pathogenicity of different HSV-1 strains for Sabra, A, and C57BL/6 mice after infection of the right footpad with and without skin abrasion

Virus strain	Mouse strain	Pathogenicity							
		Survival[a] (%)		Paralysis[b] (%)		Double paralysis[c] (%)		Clinical observations	
		Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated
Justin	Sabra	18/29 (62)	0/20 (0)	14/29 (48)	20/20 (100)	5/29 (17)	11/20 (55)	Lesions	Severe lesions
	A	3/10 (30)	0/10 (0)	4/10 (40)	10/10 (100)	0/10 (0)	0/10 (0)	Very deep lesions + hair loss + necrosis	Extensive necrosis
	C57BL/6	10/10 (100)	3/20 (15)	0/10 (0)	0/10 (0)	0/10 (0)	0/10 (0)	None	Marked swelling, extensive lesions spread to the thighs, back, and genital regions
KOS	Sabra	7/10 (70)	2/10 (20)	4/10 (40)	10/10 (100)	3/10 (30)	6/10 (60)	Severe disease; recovery	Mild lesions
	A	17/22 (77)	0/9 (0)	4/22 (18)	9/9 (100)	2/22 (9)	8/9 (88)	Mice died suddenly	Severe disease
	C57BL/6	10/10 (100)	9/10 (90)	0/10 (0)	0/10 (0)	0/10 (0)	0/10 (0)	None	Lesions
LP	Sabra	53/56 (95)	5/38 (13)	3/56 (5)	37/38 (97)	0/56 (0)	29/38 (78)	Normal disease	Mild lesions
	A	9/9 (100)	0/10 (0)	0/9 (0)	10/10 (100)	0/9 (0)	0/10 (0)	None	Extensive necrosis
	C57BL/6	10/10 (100)	9/10 (90)	0/10 (0)	0/10 (0)	0/10 (0)	0/10 (0)	None	Swelling + lesions on one leg
SP18	Sabra	10/10 (100)	1/10 (10)	0/10 (0)	10/10 (100)	0/10 (0)	10/10 (100)	None	Normal course of disease
	A	6/6 (100)	ND[d]	0/6 (0)	ND	0/6 (0)	ND	None	ND
SP11	Sabra	5/5 (100)	0/10 (0)	0/5 (0)	10/10 (100)	0/5 (0)	10/10 (100)	None	None
	A	10/10 (100)	0/10 (0)	0/10 (0)	10/10 (100)	0/10 (0)	0/10 (0)	None	Necrosis
F	Sabra	12/12 (100)	4/10 (40)	0/12 (0)	6/10 (60)	0/12 (0)	6/10 (60)	None	Lesions + extensive necrosis
	A	15/17 (88)	0/19 (0)	2/17 (11)	19/19 (100)	2/17 (11)	15/19 (79)	None	
HFEM	Sabra	10/10 (100)	9/10 (90)	0/10 (0)	0/10 (0)	0/10 (0)	0/10 (0)	Marked swelling of footpad (7/10)	Severe skin lesions + hair loss + death
	A	10/10 (100)	2/8 (25)	0/10 (0)	0/8 (0)	0/10 (0)	0/8 (0)	Marked swelling of footpad (7/10)	Extensive skin lesions + death
R19	Sabra	10/10 (100)	8/8 (100)	0/10 (0)	0/8 (0)	0/10 (0)	0/8 (0)	None	Inflammation as with HFEM but no hair loss
	A	9/9 (100)	9/9 (100)	0/9 (0)	0/9 (0)	0/9 (0)	0/9 (0)	None	Some hair loss + necrosis
Justin TK$^-$	Sabra	10/10 (100)	7/7 (100)	0/10 (0)	0/7 (0)	0/10 (0)	0/7 (0)	None	None
SP18 TK$^-$	Sabra	4/4 (100)	7/10 (70)	0/4 (0)	7/10 (70)	0/4 (0)	0/10 (0)	None	None
SP11 TK$^-$	Sabra	5/5 (100)	10/10 (100)	0/5 (0)	1/10 (10)	0/5 (0)	0/10 (0)	None	None
	A	10/10 (100)	9/9 (100)	0/10 (0)	0/9 (0)	0/10 (0)	0/9 (0)	None	Hair loss + necrosis

[a] Number of surviving mice/number inoculated.
[b] Number of mice paralyzed in the right leg/number inoculated.
[c] Number of mice paralyzed in both legs/number inoculated.
[d] ND, Not determined.

TABLE 2. Comparison of HSV-1 virulence by the footpad and ear routes of inoculation

Mouse strain	Route	% Survival[a] after inoculation with virus strain:			
		Justin	KOS	LP	F
Sabra	Footpad	100 (9/9)	100 (10/10)	100 (7/7)	100 (5/5)
	Ear	78 (7/9)	60 (6/10)	70 (7/10)	87 (7/8)
A	Footpad	50 (5/10)	62.5 (5/8)	90 (9/10)	88 (15/17)
	Ear	55 (5/9)	33.3 (6/18)	60 (6/10)	63.6 (7/11)
C3H	Footpad	100 (6/6)	100 (8/8)	100 (10/10)	100 (10/10)
	Ear	62.5 (5/8)	25 (2/8)	87 (7/8)	100 (7/7)

[a] Percentage of survival; parentheses indicate number of surviving mice/,number inoculated.

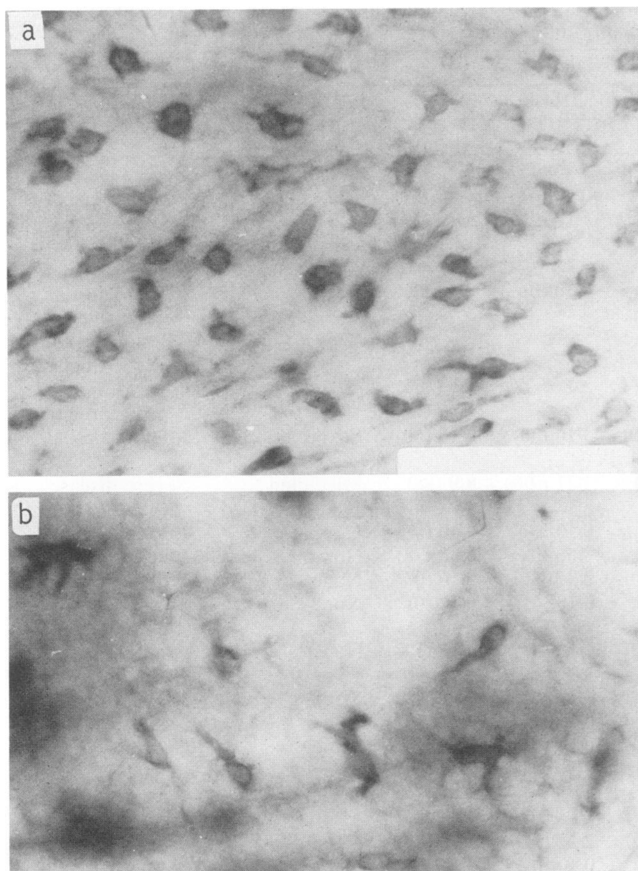


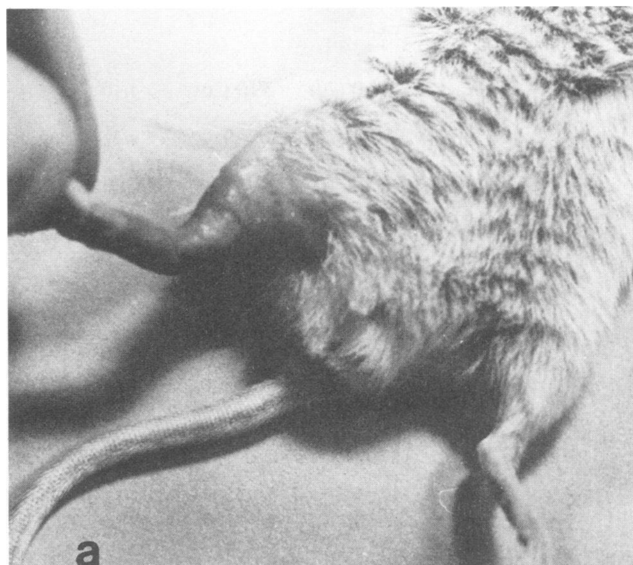
FIG. 1. Effect of abrasion on ATPase-positive cell density in the epidermis. Footpads of Sabra mice were treated by abrasion as described in the text, and the epidermis of each footpad was stained for ATPase activity. (a) Normal epidermis (ATPase staining, ×400) showing the dendritic network which covers the entire surface of the skin. (b) Epidermis 20 h after treatment (ATPase staining, ×400): only a few cells remain which have lost their dendritic appearance, and dark granular material seems dispersed over the epidermis. Identical pictures were observed after treatment of the footpad with steroids.

was characterized by a very marked increase in mouse mortality (Table 1), as compared to infection of untreated footpad skin (Table 1 and Fig. 1a).

The R19 and HFEM strains possess a deletion in the *Hpa*I P fragment of the HSV-1 genome which has been demonstrated as critical for the intraperitoneal virulence of HSV (3). R19 was characterized by a particularly low virulence. HSV-1 strain HFEM killed 75% of A mice, but virus was not recovered from the brain or liver of the diseased mice. In contrast, virus was recovered from the brains of Sabra and A mice infected with the four strains that were assayed: Justin, LP, KOS, and F. The HSV-1 TK^- mutants of strains Justin and SP11 were found to be avirulent as previously reported (5, 39). Despite its low TK activity, the TK^- mutant SP18 TK^- was pathogenic for treated mice (Table 1) as previously reported in another murine model (2).

The C57BL/6 mice, which are known for being naturally resistant to infection (24), died after infection with the HSV-1 Justin strain in the abraded footpad, but we could not recover virus from the liver, spleen, adrenals, brain, or blood of the infected mice. The reason for the mouse mortality has not been elucidated. C57BL/6 mice pretreated in the footpad and infected with HSV-1 strain Justin or LP developed extensive skin infection and inflammation, but after 2 months the swelling and necrosis had resolved in mice that were infected with the LP strain. On the other hand, C57BL/6 mice that were infected with the Justin strain were still showing signs of active inflammation in the hind leg and genitals 6 months after infection (Fig. 2a).

Three groups of A, C57BL/6, and Sabra mice were also treated by abrasion, and one drop of uninfected tissue culture sonic extract was placed on the abraded area with a Pasteur pipette, followed by four or five light strokes with



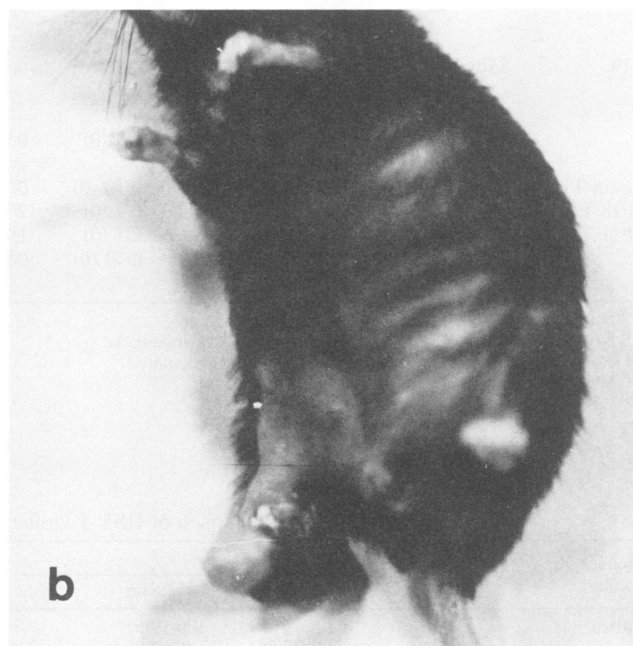


FIG. 2. Clinical signs in HSV-1-infected resistant and sensitive strains of mice. (a) Extensive loss of hair in a strain A mouse having survived infection in the footpad with HSV-1 F preceded by treatment. (b) Extensive necrosis, loss of hair, and inflammation in a C57BL/6 mouse (1 month postinfection) inoculated with the Justin strain of HSV-1 after abrasion as described in the text.

TABLE 3. Effect of prednisolone injection on Langerhans cell density in footpad skin[a]

Dose (μg)	Days postinjection	Cell density (per mm²) in[b]: RFP	LFP
312	1	1,800	1,530
	3	1,662	1,623
	6	537	1,573
	9	0–90	ND[c]
625	1	1,866	1,470
	3	1,360	1,725
	6	555	1,683
	9	0–80	ND
1,250	1	1,399	1,607
	3	1,163	1,597
	6	321	1,541
	9	0–80	1,600

[a] Sabra mice were injected with a suspension of prednisolone acetate (312, 625, or 1,250 μg in 0.05 ml of PBS). At 1, 3, 6, and 9 days postinjection, groups of three to five mice were sacrificed, and their footpad skin was stained for the ATPase activity of the Langerhans cells. Control counts were always between 1,500 and 1,900 cells per mm².

[b] RFP, Right footpad; LFP, left footpad.

[c] ND, Not done.

the point of a 19-gauge syringe needle. None of the 10 mice treated in each of the four groups died. In addition, mice treated by abrasion and infected with live viruses (e.g., R19) did not die. Thus, mortality was specifically due to HSV-1 infection and was strain specific.

Lesions developed externally to the site of infection (thigh, back, and sacrum ipsilaterally). The lesions were not particularly ulcerative but were characterized by extensive hair loss without large-scale damage to the skin, as opposed to the lesions developing in the footpad itself. These lesions appeared exclusively in mice that resisted the infection (Fig. 2b).

Effect of prednisolone on Langerhans cell density and on HSV-1 pathogenicity in the skin. Glucocorticoids have the ability to deplete the skin of Langhans cells (4, 26). Different amounts of prednisolone acetate were injected intradermally into the right footpad to obtain a local effect on Langerhans cell density. Table 3 shows a progressive decrease in Langerhans cell density in the right footpad from above 1,600 before treatment to 0 to 90 cells per mm² at 9 days after injection of a steroid dose of 312, 650, or 1,250 μg in 0.05 ml of PBS. Prednisolone did not affect the Langerhans cells systemically, as, after the steroid treatment, no significant change was observed in the Langerhans cell density in the uninjected left footpad.

At 9 days after injection of 1,250 μg of prednisolone acetate, groups of Sabra mice were infected with HSV-1 strains Justin, KOS, LP, HFEM, and F (Table 4). Prednisolone enhanced the virulence of all strains tested except the HSV-1 HFEM strain, which did not cause mouse death. The KOS strain killed 87% of the mice within 5 days, while the Justin strain killed 79% of the mice after 11 days. HSV-1 strains F and LP killed about 55% of the mice 11 days postinfection. These HSV-1 strains were unable to cause disease in the untreated mice (Table 4). Similar results were obtained for the A and Sabra strains (Table 4). The C57BL/6 mice succumbed to infection with HSV-1 strain Justin after pretreatment with prednisolone, but not to infection with the KOS strain. Lesions appeared in the footpad, especially in mice that were infected with the KOS strain.

To eliminate the possibility that the prednisolone affected HSV-1 pathogenicity due to systemic immunosuppression, 17 Sabra mice were treated in the right footpad with prednisolone and 9 days later were infected with the LP strain in the left, opposite footpad. All the infected mice survived. In contrast, mice that were treated with prednisolone in the right footpad and 9 days later were infected in the same footpad with the HSV-1 LP strain succumbed to the infection.

HSV-1 virulence when injected into the ear pinna skin, at a site naturally depleted of Langerhans cells. The Langerhans cell density in the ear was determined in three Sabra mice and was found to be 995, 1,098, and 1,055 cells per mm² as compared to 1,973, 1,860, and 1,733 cells per mm², respectively, in the footpad. The cells appeared to be rounder and darker in the ear. These results are in agreement with those obtained by Bergstresser et al. (6) for the A and C57BL/6 strains of mice.

We then compared the virulence of different HSV-1 strains in three strains of mice. In all but one (A mice infected with the Justin strain) of the combinations tested, mice were found to be more resistant to HSV-1 infection when the virus was inoculated in the footpad than when it was inoculated in the ear (Table 2). Similar experiments were also carried out with BALB/c and C57BL/6 mice, which were shown to be totally resistant to all HSV-1 strains tested (LP, Justin, KOS; 10 mice infected each time), whatever the route of inoculation used.

Murine Langerhans cells are able to present HSV-1 to lymphocytes. It is known that Langerhans cells are very potent antigen-presenting cells (1) and that the immune

TABLE 4. Effect of subcutaneous injection of prednisolone on HSV-1 virulence by the footpad route

Virus strain	Treatment[a]	Survival[b] (%) of mouse strain: Sabra	A	C57BL/6
Justin	Treated	4/19 (21)	0/8 (0)	1/8 (12)
	Untreated	17/17 (100)	6/10 (60)	10/10 (100)
KOS	Treated	2/15 (13)	1/10 (10)	7/7 (100)
	Untreated	20/20 (100)	2/10 (20)	10/10 (100)
LP	Treated	5/11 (45)	9/17 (52)	ND[c]
	Untreated	10/10 (100)	10/10 (100)	
F	Treated	7/15 (47)	2/8 (25)	ND
	Untreated	21/21 (100)	10/10 (100)	
HFEM	Treated	7/7 (100)	7/8 (87)	ND
	Untreated	10/10 (100)	10/10 (100)	

[a] Mice were inoculated with 1,250 μg of predisolone acetate in 0.05 ml of PBS (Treated).

[b] Number of survivors/total mice.

[c] ND, Not done.

TABLE 5. Ability of Langerhans cells to present HSV to T cells[a]

Expt	Responders	Stimulators	Antigen	cpm
1 and 2[b]	Immune T	EC	HSV inact.	24,421 ± 3,251
	Immune T	EC	HSV live	11,954 ± 1,898
	Nonimmune T	EC	HSV inact.	2,369 ± 97
	Nonimmune T	EC	HSV live	4,280 ± 460
	None	EC	HSV inact.	792 ± 151
	None	EC	HSV live	558 ± 84
	Immune T	None	HSV inact.	561 ± 138
	Immune T	None	HSV live	1,077 ± 373
	Nonimmune T	None	HSV inact.	579 ± 120
	Nonimmune T	None	HSV live	1,701 ± 608
	Immune T	None	None	1,596 ± 389
	Nonimmune T	None	None	1,077 ± 265
3	Immune T	EC	HSV inact.	19,714 ± 1,431
	Immune T	EC treated	HSV inact.	2,341 ± 454
	Immune T	EC control	HSV inact.	23,745 ± 2,345
	Immune T	None	HSV inact.	1,785 ± 786
	None	EC	HSV inact.	2,374 ± 140

[a] T cells and epidermal cells (EC), prepared as described in Materials and Methods, were cocultured in the presence or absence of live (HSV live) or UV-inactivated (HSV inact.) HSV-1. Proliferation was assessed after 6 days of culture. Results are presented as the mean counts per minute of four cultures. Immune T, Nonadherent spleen cells purified on nylon-wool columns. EC treated, Epidermal cells that were treated with anti-Ia monoclonal antibody and complement as described in Materials and Methods; EC control, epidermal cells that were treated with complement alone.
[b] Results are the mean of two separate experiments.

response in the skin is mainly cell mediated (see reference 28) and thus dependent on the presence of functional antigen-presenting cells. We found a correlation between the decrease in Langerhans cell density and increase in HSV-1 virulence. To test our hypothesis that this correlation can be explained by the absence of the normal antigen-presenting cell function in the skin, we investigated the capacity of Langerhans cells to present HSV-1 to T lymphocytes. Mice were immunized by intradermal injection of 10^7/PFU per mouse 7 weeks before the experiment. UV-inactivated and live virus (LP) induced a strong proliferative response of T lymphocytes derived from immune animals in the presence of epidermal cells (Table 5). Such a proliferation did not occur when nonprimed T cells were cultured with epidermal cells and HSV-1. T cells alone did not proliferate in the presence of the virus. Thus, the T-cell-enriched cell population was devoid of antigen-presenting cells. In addition, in a separate experiment, pretreatment of the cell suspension with an anti-Ia monoclonal antibody and complement, in contrast to treatment with complement alone, abolished the response of the T lymphocytes. The proliferation seen in the presence of the live virus was lower than that observed in the presence of the inactivated virus.

DISCUSSION

The present study extends our findings on the role of Langerhans cells in the control of murine skin herpetic infections (32). A correlation between the disappearance of ATPase-positive Langerhans cells from the skin after abrasion and enhancement of HSV-1 virulence was confirmed by using 11 HSV-1 strains and four mouse strains. Interestingly, the HFEM strain, which is known to lack intraperitoneal virulence in mice, caused extensive lesions in the skin of the infected mice as well as a high rate of mortality among A mice. Three types of virus strains could be distinguished: strains that were virulent with or without abrasion (Justin, KOS), strains that were virulent only when the footpad was pretreated by abrasion (LP, SP18, SP11, F, HFEM, SP18 TK$^-$), and strains that were avirulent in all situations (R19, Justin TK$^-$, SP11 TK$^-$).

Since abrasion of the skin can lead to nonspecific damage to all types of cells in the skin, we felt the need for a less drastic stimulus for depletion of Langerhans cells. Steroids have been shown to deplete the skin of Langerhans cells (4), as assessed by ATPase staining, and to impair the functional immune capacity of the epidermis (24). Application of steroids inhibits epidermal cell growth (12), as opposed to abrasion, which leads to an enhanced cell proliferation of both keratinocytes (29) and Langerhans cells (14). Abrasion leads to a characteristic inflammatory reaction accompanied by prostaglandin E_2 secretion (25), whereas steroids are known to inhibit prostaglandin synthesis (22). In contrast to their influence on other parameters in the skin, both stimuli commonly alter the state of the Langerhans cells by decreasing their density.

To deplete the skin of Langerhans cells locally by steroids, topical application of various commercial lotions has been used (4, 26). Not only do these methods make precise quantification impossible, but they are hardly applicable to the footpad model of HSV-1 skin infection. On the other hand, direct injection of steroids is very likely to induce systemic suppression. We, therefore, chose to use injection of a suspension of prednisolone crystals, to limit the diffusion of the steroids. No significant change was noticed in the untreated footpad. All Sabra mice inoculated with HSV-1 in the untreated footpad survived. On the other hand, when the virus was inoculated in the pretreated footpad, steroids induced a very strong enhancement of pathogenicity of all HSV-1 strains tested, except the defective HFEM strain (3). The rapidity of the disease correlated with the intrinsic virulence of the virus. Similar results were obtained with A mice. After pretreatment with steroids, the C57BL/6 mice showed resistance to the KOS strain but not to the Justin strain of HSV-1.

In view of the question of the specificity of the depleting stimuli, it was of interest to determine whether a site that normally possesses fewer Langerhans cells than the footpad would react differently to HSV-1 infection. Since the ear has a lower Langerhans cell density than the footpad, HSV-1 virulence was compared by these two routes. The virus was far more virulent by the ear route than by the footpad route.

It is true that the ear is located closer to the brain than the footpad, and thus the virus could have been able to spread to the brain at a higher concentration and produce more severe damage than when inoculated into the footpad. We consistently observed, however, that mice infected in the ear began to die 1 or 2 days later than when infected in the footpad. This would not have been expected if the proximity of the ear to the brain caused the higher rate of mortality observed by the ear route of infection. The results obtained are very consistent with the hypothesis that Langerhans cell density determines the degree of HSV virulence in the skin.

The Langerhans cells have been shown to belong to a large family of dendritic Ia-positive cells, particularly characterized by their outstanding antigen-presentation capacity (1). As shown by our results and other studies (9, 17) in various experimental systems, it was established that Langerhans cells are able to present HSV as an antigen to T cells very efficiently (Table 5). As it is known that the immune response to HSV-1 infection in the skin is mainly cellular (28) and thus dependent on antigen-presenting cells, it is understandable that elimination of the skin antigen presentation function could seriously impair the immune response to HSV-1 infection. Thus, our results strongly suggest that Langerhans cells, in analogy with the macrophages in the peritoneum (40), function as essential sentinels in the immune defense against HSV-1 skin infection.

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