VIROLOGY

Local and Systemic Functional Responses of Mouse Macrophages to Intravaginal Infection with Type 2 Herpes Simplex Virus and Vaccination

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Activity of cathepsin D and phagocytosis of macrophages from vaginal lavage fluid, peritoneal exudation, and spleen were studied in mice of sensitive (DBA/2) and resistant (BALB/c) lines after intravaginal infection with type 2 herpes simplex virus and vaccination. Activity of cathepsin D and intensity of phagocytosis (irrespective of the macrophage source) and their ratio in BALB/c mice in early terms after infection were close to the control levels taken as a unit. In DBA/2 mice, these parameters and their balance were shifted and changes in cathepsin D activity depended on the time after challenge. Activities of cellular and extracellular cathepsin D increased sharply on day 1 postinfection under conditions of local virus interaction with the vaginal mucosa and activation of the pathological process. Later, after generalization of the infection, activity of cathepsin D decreased, while phagocytosis increased in all the studied macrophage populations. Vaccination corrected the cathepsin D/phagocytosis imbalance and created conditions for rapid elimination of the virus.

Key Words: *macrophages; HSV-2; cathepsin D; intravaginal infection; vaccination*

The mucosa of the urogenital tract serves the first line defense from many infections, including sexually transmitted ones. For example, type 2 herpes simplex virus (HSV-2) infection is recognized by the congenital immunity system mainly through TLR-9 [4,9] and NOD-like (NLR) [7] receptors. Cathepsins play an important role in activation of these receptors. TLR-9 located in endolysosomes of macrophages and dendritic cells is activated upon cleavages with lysosomal proteases, in particular cathepsin D [11]. NLR are components of the cytosol multiprotein complexes,

inflammasomes [14]. NLRP3, one of the best studied inflammasomes, is activated in response to changes in lysosomal membrane permeability and release of proteases, *e.g.* cathepsins [6,8]. Inflammasomes are not activated without cathepsin D [12]. Inflammasomes activated by herpes virus induce secretion of inflammatory cytokines IL-1β and IL-18 [6,12,14]. However, many viruses use the mechanisms allowing them to prevent inflammasome activation or to hyperactivate then, which leads to immune pathology and death [10].

A specific feature of HSV-2 infection is that the cells infected with the virus produce IL-10 suppressing Th1-immune response [13]. We have previously detected imbalance of cathepsin D and cytokines in vaginal lavage fluid from women with HSV-2 infec-

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tion and predominance of Th2 response throughout the menstrual cycle [1,2]. These studies demonstrated the important role of cathepsins in immune response to viral infection. Analysis of the function of macrophages of various organs under conditions of experimental intravaginal infection is desirable for understanding of the defense mechanisms in HSV-2 infection.

We compare activities of cathepsin D and phagocytic activity of macrophages from vaginal lavage fluid (VM), peritoneal exudation (PEM), and spleen (SM) in mice of sensitive (DBA/2) and resistant (BALB/c) lines under conditions of intravaginal infection with HSV-2 and vaccination.

MATERIALS AND METHODS

The study was carried out on mice (14-16 g) of HSV-2-resistant (BALB/c) and sensitive (DBA/2) lines. The mice were vaccinated intravaginally 3 times at 1-week interval with Vitaherpavac herpetic cultural inactivated vaccine with 1% solution of high-molecular-weight hyaluronic acid. Two weeks after vaccination, the mice were intravaginally infected with HSV-2 (0.001 PFU/ml, 30 µl per mouse). Intact mice served as the control. Functional activity of adherent phagocytic cells (macrophages) from vaginal lavage fluid, peritoneal exudation, and spleen homogenate was studied in 1 and 3 days after infection. Vaginal lavage fluid was collected after repeated washing of the vagina with normal saline. Cell suspension was centrifuged at 2000 rpm for 10 min. Supernatant was collected for measuring the concentrations of extracellular cathepsin D. Cell precipitate was resuspended in medium 199. In order to prepare macrophage monolayer, peritoneal exudate cells were incubated in medium 199 in a concentration of 2×10^6/ml, suspension of splenic cells in medium 199 in a concentration of 10^7/ml, and vaginal lavage fluid in a concentration of 1.5×10^6/ml for 1 h in Petri dishes at 37ºC in atmosphere with 5% CO_2. Macrophage phagocytic activity was then evaluated by phagocytosis of ^{14}C typhoid vaccine and expressed in pulse/min/mg protein. Activity of cathepsin D was measured in lysates of cell monolayer obtained by 3-fold freezing/defrosting. Cathepsin D activity was expressed in µg tyrosine/mg protein [3]. Functional activity of macrophages was evaluated by the ratio (in %) of parameters measured in 1 or 3 days after infection to the level intact control. Cathepsin D/phagocytosis (CatD/phag) proportion was denoted as balance criterion (BC).

The results were statistically processed using Student's *t* test. The differences were considered significant at $p < 0.05$.

RESULTS

Functional activities of PEM, SM, and VM from BALB/c and DBA/2 mice were evaluated after intravaginal infection of animals with 100% lethal dose of HSV-2. In BALB/c mice resistant to HSV-2, phagocytosis intensity did not change in all the studied macrophage populations (Fig. 1, *a*, *b*). Activity of cathepsin D was reduced in SM on day 1 postinfection and did not change in PEM and VM.

In DBA/2 mice sensitive to HSV-2, changes in the function of the studied macrophage population depended on their location (Fig. 1, *c*, *d*). Activity of cathepsin D on day 1 postinfection increased significantly in VM, was below the normal in PEM, and remained unchanged in SM. In 3 days, cathepsin D activity dropped in VM and SM and increased in PEM reaching the control level (Fig. 1, *c*). Phagocytic activity of the studied macrophage populations on day 1 postinfection changed only in PEM and did not change in VM and SM. In 3 days, phagocytic activity increased in all studied macrophage populations (Fig. 1, *d*). Hence, the main differences in the systemic and local macrophageal response of DBA/2 mice evaluated in 1 day after intravaginal infection with HSV-2 consisted in a significant increase of cathepsin D activity. By day 3 postinfection, the response of PEM, SM, and VM to the infection manifested by a decrease of cathepsin D activity and an increase of phagocytosis.

These results indicate that changes in cathepsin D activity are an important characteristic of HSV-2 infection in mice. This enzyme is essential for inflammasome activation in response to stimulation with viral RNA; importantly, mature cathepsin D is secreted simultaneously with secretion of the central component of inflammasomes, ASC protein, and caspase-1 [8,12]. Recognition of viral RNA leads to inflammasome-mediated caspase-1-dependent cell death, known as pyroptosis. It is characterized by impairment (rupture) of the plasmatic membrane and release of cytoplasmatic contents to the extracellular space. For this reason, we carried out experiments with simultaneous measurements of cathepsin D activity in VM and in vaginal secretion after HSV-2 infection. The infection caused no shifts in enzyme activity in VM or vaginal secretion of BALB/c mice resistant to HSV-2 (Fig. 2). In DBA/2 mice sensitive to HSV-2, the virus caused a drastic increase of cathepsin D activity in VM and in vaginal secretion one day after challenge (Fig. 2), which could indicate labilization of the lysosomal and cellular membranes. By day 3 postinfection, activity of cathepsin D in cells and extracellular environment decreased.

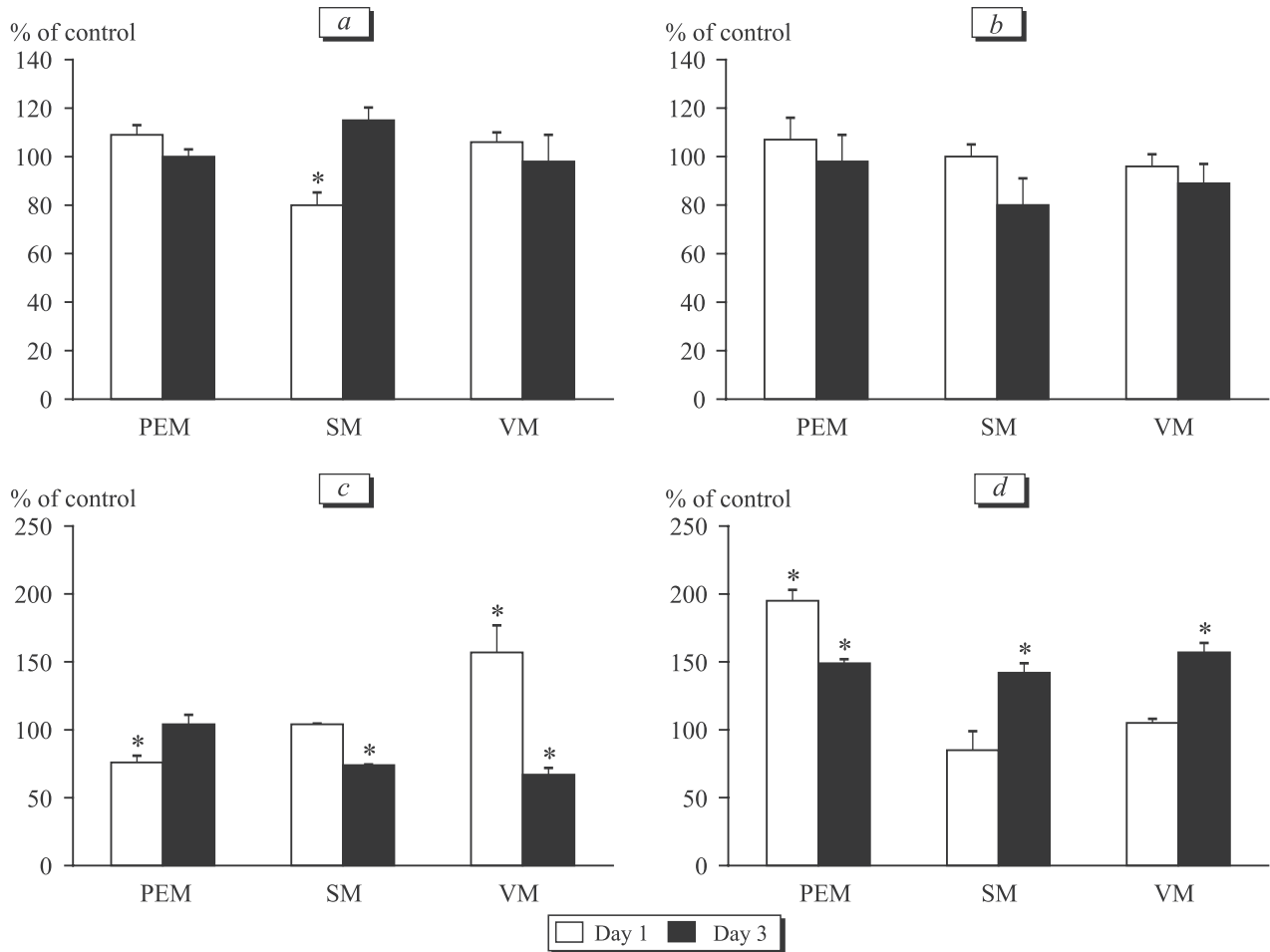Analysis of the data indicated differences in functional activities of macrophages of mice susceptible

Fig. 1. Functional status of PEM, SM, and VM of resistant (BALB/c) and susceptible (DBA/2) mice on days 1 and 3 after intravaginal infection with HSV-2. *a*) activity of cathepsin D in BALB/c mice; *b*) phagocytic activity in BALB/c mice; *c*) activity of cathepsin D in DBA/2 mice; *d*) phagocytic activity in DBA/2 mice. Here and in Figs. 2, 3: *$p<0.05$ in comparison with intact control.
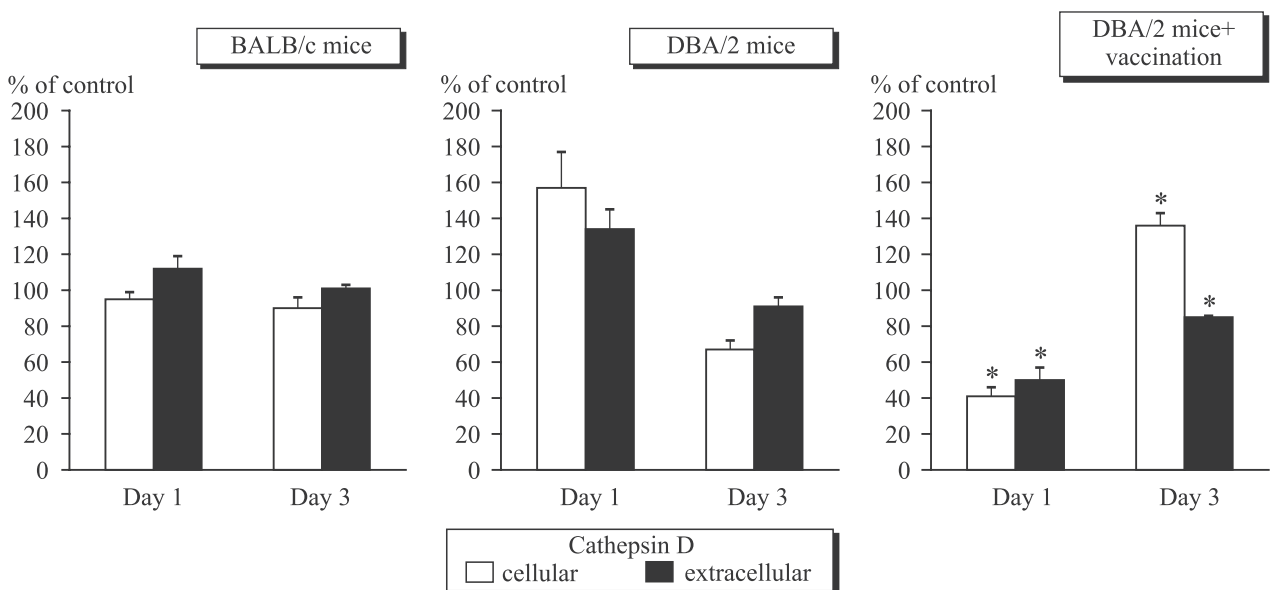

Fig. 2. Activities of cathepsin D in VM and vaginal secretion of BALB/c and DBA/2 mice in response to intravaginal infection with HSV-2 and vaccination by herpetic cultural inactivated vaccine on days 1 and 3 after infection
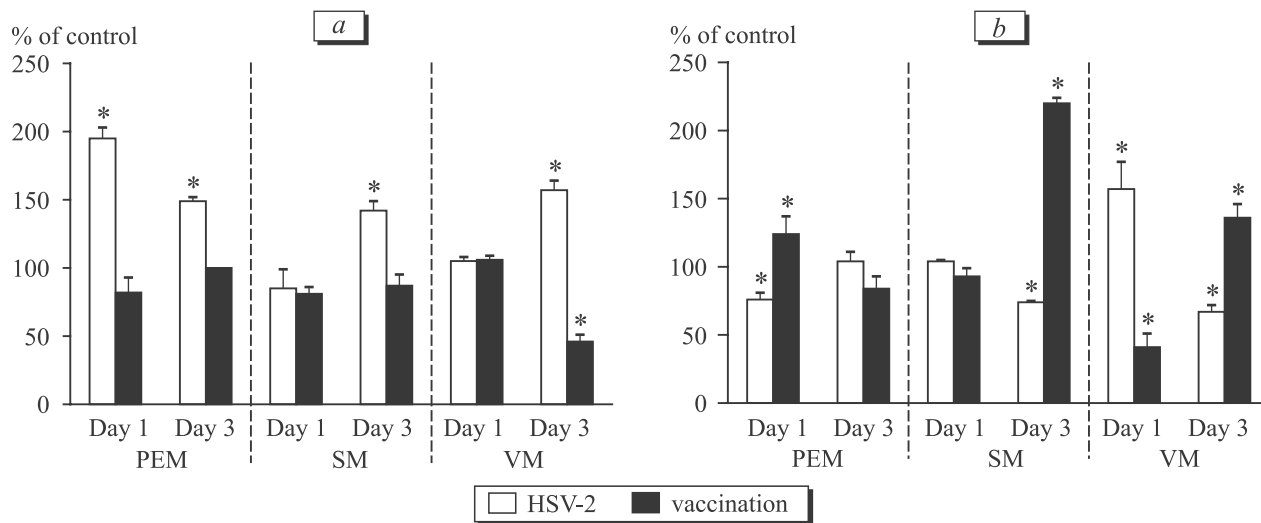
Fig. 3. Functional activities of PEM, SM, and VM on days 1 and 3 after intravaginal HSV-2 infection after 3-fold intravaginal vaccination of DBA/2 mice. *a*) Phagocytic activity; *b*) cathepsin D activity.

and resistant to HSV-2 infection. The challenge caused no appreciable changes in activity of cathepsin D, phagocytic activity, and cell membrane permeability in VM, PEM, and SM of the resistant mice. The CatD/Phag ratio in BALB/c mice was always close to one (BC), irrespective of the macrophage origin (Table 1). The only exclusion was SM on day 3 postinfection, when BC was 1.4. This value indicates active degradation of antigens with the involvement of proteolytic enzyme (cathepsin D) and virus elimination in HSV-2-resistant mice.

Virus infection of susceptible DBA/2 mice disturbed the balance of functional activity of macrophages (Table 1). Opposite shifts were observed for PEM and VM populations on day 1 postinfection. The parameter was significantly higher than in VM due to

high activity of cathepsin D. In PEM, the proportion was below one because of low activity of cathepsin D in the presence of active phagocytosis. By day 3, no differences in the reactions of local and systemic macrophages to HSV-2 infection were observed, the BC was always below one. CatD/Phag value was equal to 1.57 in VM of DBA/2 mice on day 1 postinfection. High activity of cathepsin D in cells was paralleled by its high extracellular activity, an important component of the unfolding pathological process in the vagina. Hence, effective defense from HSV-2 is realized at the early stages after infection and is associated with the maintenance of balance between cathepsin D activity and phagocytosis in vaginal mucosal cells, PEM, and SM.

Our data indicate that functional parameters of macrophages play an important role in viral pathol-

TABLE 1. Macrophage Response in BALB/c and DBA/2 Mice to Intravaginal HSV-2 Infection

Mouse strain		VM		PEM		SM	
		CatD/Phag*	BC	CatD/Phag*	BC	CatD/Phag*	BC
HSV-2 infection							
BALB/c	day 1	106/96	1.1	109/107	1.0	80/100	0.8
	day 3	98/89	1.1	100/98	1.0	115/80	1.4
DBA/2	day 1	157/105	1.57	76/195	0.39	104/85	1.2
	day 3	67/157	0.43	104/149	0.70	74/142	0.5
Vaccination+HSV-2							
DBA/2	day 1	41/106	0.39	124/82	1.5	93/81	1.1
	day 3	136/46	3.0	84/100	0.84	220/87	2.5

Note. *Percent of intact control.

ogy. Hence, factors limiting the infectious process can modulate these parameters. Three-fold mucosal vaccination of DBA/2 mice with herpetic vaccine with hyaluronic acid leading to 100% survival of mice infected by HSV-2 was associated with correction of functional parameters of macrophages. High phagocytic activity decreased by day 3 postinfection in VM as well as in PEM and SM (Fig. 3, *a*). Correction of cathepsin D activity (Fig. 3, *b*) depended on the source of macrophages. In VM, the vaccine inhibited cathepsin D production on day 1 (low CatD/Phag index) and stimulated it on day 3 postinfection (increase of CatD/Phag index; Table 1). This normalization of the parameters disturbed the program triggered by the virus for its propagation. High activity of cathepsin D in PEM on day 1 and in SM on day 3 could promote further elimination of the virus. Vaccination was associated with a decrease of extracellular cathepsin D activity in the vaginal secretion of DBA/2 mice (Fig. 2). This could serve as an indicator of cell membrane stabilization, which, in turn, could lead to attenuation of the pathological process in the vagina.

Hence, the reaction of macrophages to virus challenge can be characterized as two-staged. Stage 1 (day 1) is associated with local effect of the virus on the vaginal mucosa. Intense production of cathepsin D in vaginal macrophages and its release leads to damage to the vaginal mucosa, thus promoting further propagation of the virus. At stage 2 (day 3), the virus inhibits activity of cathepsin D and stimulates antigen capture in vaginal macrophages as well as in PEM and SM (Fig. 1). This creates conditions for active multiplication of the virus at the site of its introduction and in other organs and tissues and promotes generalization of the viral infection. Vaccination inhibits the production of cathepsin D in the cell and in extracellular environment on day 1 (Fig. 2). It is known that limited degradation of the antigen is associated with low expression of lysosomal proteases, including cathepsin D. Less digested forms of protein antigens are more immunogenic [5]. Reduction of cathepsin D activity in the extracellular environment decreases the probability of inflammatory and necrotic changes in mucosa and prevents the propagation of the virus. On day 3, the vaccination creates conditions for rapid elimination of the virus under conditions of unfolding activity of cathepsin D and decreasing phagocytosis in VM, PEM, and CM (Fig. 3).

Comparison of the local and systemic response of macrophages in mice of HSV-2 resistant and sensitive lines detected the cause-effect relationship between the function of VM and generalization of the infectious process. Important role of cathepsin D in the formation of natural resistance to infection at the early stages of HSV-2 infection is shown. Vaccination correcting the CatD/Phag imbalance in susceptible mice created conditions for more intense antiviral defense.

REFERENCES

1. Zaitseva LG, Sergeeva NS, Kireeva IV, Shaposhnikova GM, Bekhalo VA, Tischenko AL, Nagurskaya EV. Cathepsin D and anti-inflammatory cytokines are the indicators of balanced mucous immune response in vagina of uninfected and HSV-2 infected women. Allergol. Immunol. 2013;14(4):260-265. Russian.
2. Zaĭtseva LG, Sergeeva NS, Tishchenko AL, Shaposhnikova GM, Kireeva IV, Samoĭlenko II, Semenova EN, Nagurskaia EV. Cytokine levels in the vaginal secretions of healthy and herpes simplex virus-infected women in different phases of menstrual cycle. Klin. Dermatol. Venerol. 2012;10(5):26-30. Russian.
3. Nagurskaya EV, Zaitseva LG, Kobets NV, Kireeva IV, Alimbarova LM, Samoilenko II, Barinskii IF. Functional activity of peritoneal macrophages from sensitive and resistant mouse strains during intravaginal infection with herpes simplex virus type 2 and mucosal vaccination. Bull. Exp. Biol. Med. 2008;145(2):235-239.
4. Ashkar AA, Bauer S, Mitchell WJ, Vieira J, Rosenthal KL. Local delivery of CpG oligodeoxynucleotides induces rapid changes in the genital mucosa and inhibits replication, but not entry, of herpes simplex virus type 2. J. Virol. 2003;77(16):8948-8956.
5. Colbert JD, Matthews SP, Miller G, Watts C. Diverse regulatory roles for lysosomal proteases in the immune response. Eur. J. Immunol. 2009;39(11):2955-2965.
6. De Nardo D, De Nardo CM, Latz E. New insights into mechanisms controlling the NLRP3 inflammasome and its role in lung disease. Am. J. Pathol. 2014;184(1):42-54.
7. Idris A. Cellular responses to cytosolic double-stranded RNA — the role of the Inflammasome. Immunol. Immunogen. Insights. 2014;(6):7-11.
8. Latz E. The inflammasomes: mechanisms of activation and function. Curr. Opin. Immunol. 2010;22(1):28-33.
9. Lund J, Sato A, Akira S, Medzhitov R, Iwasaki A. Toll-like receptor 9-mediated recognition of Herpes simplex virus-2 by plasmacytoid dendritic cells. J. Exp. Med. 2003;198(3):513-520.
10. Lupfer C, Kanneganti TD. The expanding role of NLRs in antiviral immunity. Immunol. Rev. 2013;255(1):13-24.
11. Park B, Brinkmann MM, Spooner E, Lee CC, Kim YM, Ploegh HL. Proteolytic cleavage in an endolysosomal compartment is required for activation of Toll-like receptor 9. Nat. Immunol. 2008;9(12):1407-1414.
12. Rintahaka J, Lietzén N, Öhman T, Nyman TA, Matikainen S. Recognition of cytoplasmic RNA results in cathepsin-dependent inflammasome activation and apoptosis in human macrophages. J. Immunol. 2011;186(5):3085-3092.
13. Sloan DD, Jerome KR. Herpes simplex virus remodels T-cell receptor signaling, resulting in p38-dependent selective synthesis of interleukin-10. J. Virol. 2007;81(22):12,504-12,514.
14. Yamazaki T, Ichinohe T. Inflammasomes in antiviral immunity: clues for influenza vaccine development. Clin. Exp. Vaccine Res. 2014;3(1):5-11.