Functional Activity of Peritoneal Macrophages from Sensitive and Resistant Mouse Strains during Intravaginal Infection with Herpes Simplex Virus Type 2 and Mucosal Vaccination

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In the early period after intravaginal infection with herpes simplex virus type 2 (2 h), macrophages from sensitive DBA/2 mice were characterized by higher capacity to engulf the antigen, decreased function of the lysosomal apparatus, lower activity of cathepsin D, and reduced oxygen metabolism compared to cells from resistant BALB/c mice. Mucosal vaccination with herpes vaccine and hyaluronic acid promoted the increase in functional activity of macrophages and improved survival of sensitive mice (by 60%).

Key Words: *herpes simplex virus type 2; mucosal vaccination; hyaluronic acid; macrophages; phagocytosis*

The resistance to genital herpes depends on the function of the mucosal barrier and degree of the specific immune response to herpes simplex virus (HSV). Early inhibition of viral replication is of considerable importance in this respect. This process is determined by various components of the innate immune response [3,5]. Macrophages produce an antiviral effect on HSV within the first hours after infection [4]. It should be emphasized that HSV is an intracellular pathogen, and macrophages can phagocytize HSV. Hence, studying the phagocytic function of macrophages is an urgent problem. This approach suggests the evaluation of antigen-engulfing properties of macrophages, activity of the lysosomal apparatus and lysosomal enzymes (*e.g.*, cathepsin D), and oxygen metabolism. Only normal function of these components in the phagocytic process may provide suppression of viral replication.

Here we studied phagocytic activity of peritoneal macrophages from sensitive and resistant mouse strains that were subjected to intravaginal infection with HSV type 2 (HSV2) after single mucosal immunization by herpes vaccine and hyaluronic acid.

MATERIALS AND METHODS

Experiments were performed on resistant (BALB/c) and sensitive mouse strains (DBA/2). The mice weighting 14-60 g were obtained from the Central Nursery of Laboratory Animals (Kryukovo, Russian Academy of Medical Sciences). HSV2 (strain BH) was maintained by passage in Vero cell culture. These cells were grown in Eagle's medium (Paneko) containing 10% fetal bovine serum (Paneko), 2 mM glutamine, and 50 μg/ml gentamicin at 5% CO_2. Vaccination was performed with Vitagerpavak commercial vaccine (Vitafarma). The lyophilized

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vaccine preparation was diluted in 0.3 ml water for injection according to manufacturer's recommendations. The solution was diluted by 5 times with physiological saline. Equal volumes of the solution and 1% high-molecular-weight hyaluronic acid (molecular weight 10^6, CPN) were mixed. The test preparation (single volume 0.1 ml) was administered intravaginally through the tip of an automatic pipette. Nine days after vaccination, the mice were infected intravaginally with HSV2 in a dose of 2 $lgPFU_{50}$/ml (PFU, plaque-forming units). HSV2 infection (300 μl per mice) was induced through the tip of an automatic pipette. Functional activity of peritoneal macrophages and macrophage migration inhibition reaction (MMIR) were studied 2 h and 3 and 7 days after infection. Functional activity of peritoneal macrophages was estimated with the monolayer of adherent cells. The monolayer was obtained during incubation of peritoneal exudate cells in Petri dishes at 37°C and 5% CO_2 for 1 h. Phagocytic activity of peritoneal macrophages was evaluated from the ability to phagocytize ^{14}C-typhoid fever vaccine. Radioactivity and protein concentration were measured in the cell suspension. Phagocytosis of ^{14}C-typhoid fever vaccine was expressed in cpm/mg. Oxidative metabolism was studied using nitroblue tetrazolium. The total lysosomal activity was estimated by vital staining of cultured cells with acridine orange. The results were expressed in optical density units per mg protein. Cathepsin D activity (μg tyrosine per mg protein) and protein concentration (Lowry method) were measured in lysates of the cell monolayer to estimate lysosomal enzyme activity [1]. Lymphocyte from regional lymph nodes were washed 2 times and resuspended in the culture medium to perform MMIR. The tip of automatic pipette was filled with 5 μl cell suspension (5×10^6 cells/ml) and placed in a special stand. The stand was mounted in a 96-well plate prefilled with the culture medium (100 μl per well) [1]. Inactivated HSV2 in a concentration of 10 PFU was added to several wells as the antigen. The diameter of cell migration was estimated visually under a Leica inverted microscope with ocular micrometer. The migration inhibition index (MII) was calculated as follows:

$$MII=D_t^2/D_c^2-1\times100\%,$$

where D_t is the mean diameter of parallel treated microcultures; and D_c is the mean diameter of control microcultures.

The results were analyzed by Student's *t* test.

RESULTS

Preliminary studies showed that 100% DBA/2 mice die over 21 days after intravaginal infection with HSV2 in a dose of 2 $lgPFU_{50}$/ml. Under these conditions, the survival rate of BALB/c mice was 100%. Phagocytosis of ^{14}C-typhoid fever vaccine in HSV2-resistant mice slightly increased 2 h after infection (Fig. 1, *a*). Cathepsin D activity (Fig. 1, *b*), function of the lysosomal apparatus (Fig. 1, *c*), and oxidative metabolism (Fig. 1, *d*) in peritoneal exudate macrophages (PEM) from these animals did not differ from normal. On day 3, phagocytic activity was below normal, while other parameters remained unchanged. All parameters of functional activity of PEM decreased after 7 days. Hence, resistant mice were characterized by well-balanced function of the phagocytic system in PEM during intravaginal infection.

Phagocytosis of ^{14}C-typhoid fever vaccine in HSV2-sensitive mice sharply increased 2 h after infection (Fig. 1, *a*). These changes were accompanied by a significant decrease in cathepsin D activity (Fig. 1, *b*), function of the lysosomal apparatus (Fig. 1, *c*), and oxidative metabolism (Fig. 1, *d*). On days 3 and 7 after infection, functional activity of PEM approached normal (except for increased oxygen metabolism). These data show that sharp increase in virus-engulfing activity of macrophages from sensitive mice is accompanied by inhibition

TABLE 1. T-Cell Immune Response in Vaccinated Mice of Sensitive (DBA/2) and Resistant Strains (BALB/c) during Intravaginal Infection with HSV2

Period of study	Group of mice			
	BALB/c		DBA/2	
	intact	intravaginally infected	intact	intravaginally infected
2 h	15.03±2.03	-75.4±5.7*	-9.03±3.60	-27.7±2.7
3 days	5.03±1.03	-20.7±5.6	-9.03±3.6	-40.6±2.8*
7 days	10.03±1.53	-32.8±4.8	-9.03±2.4	-8.Гы1.4

Note. *$p<0.05$ compared to intact animals.

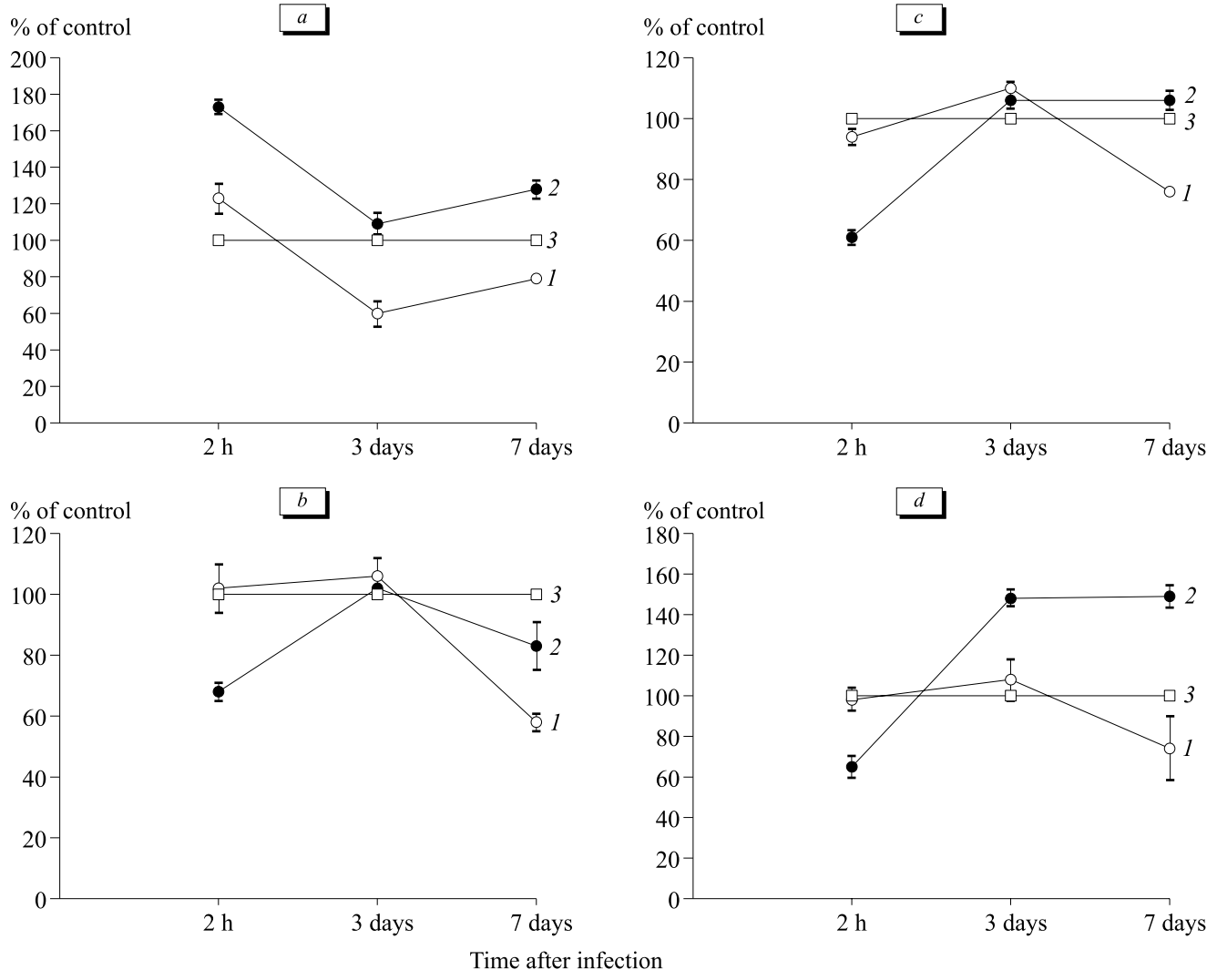

Fig. 1. Effect of HSV2 on functional activity of peritoneal macrophages from resistant (BALB/c) and sensitive mice (DBA/2). Ordinate: functional activity of peritoneal macrophages from HSV2-infected mice (compared to intact animals). Infected BALB/c mice (*1*); infected DBA/2 mice (*2*); and intact control (*3*). Here and in Fig. 2: phagocytic activity (*a*); cathepsin D activity (*b*); activity of the lysosomal apparatus (acridine orange test, *c*); and oxidative metabolism (nitroblue tetrazolium test, *d*).

of digestive and oxidative function of cells over the first hours after infection. These changes contribute to virus accumulation in the cell. Hence, HSV2-sensitive and resistant mice exhibit a correlation between the phagocytic apparatus of PEM and resistance to HSV2 in the initial phase of infection.

Phagocytic activity of PEM in sensitive mice was studied after intravaginal HSV2 infection against the background of single intravaginal (mucosal) immunization with herpes vaccine and hyaluronic acid (9 days before infection). Functional activity of macrophages from DBA/2 mice which were infected after vaccination (Fig. 2, *a-d*) differed from those in infected animals. Phagocytosis of the antigen decreased, while cathepsin D activity, function of the lysosomal apparatus, and oxygen metabolism increased in vaccinated mice 2 h after infection. These parameters in pre-vaccinated mice returned to normal in the late stage after infection (days 3 and 7). The exception was high activity of the lysosomal apparatus. These data are consistent with 60% survival rate of sensitive mice after pre-vaccination and intravaginal infection. It may be suggested that pre-vaccination normalizes phagocytic activity of PEM in sensitive mice, which modifies the outcome of infection.

Apart from macrophage response, changes in the T-cell response during infection and vaccination were studied in MMIR. The specific T-cell response in sensitive and resistant mice was formed in a normal period after intravaginal infection with HSV2 (7 days postinfection, Fig. 3). The animals infected after vaccination were characterized by the secondary specific T-cell response (Table 2).

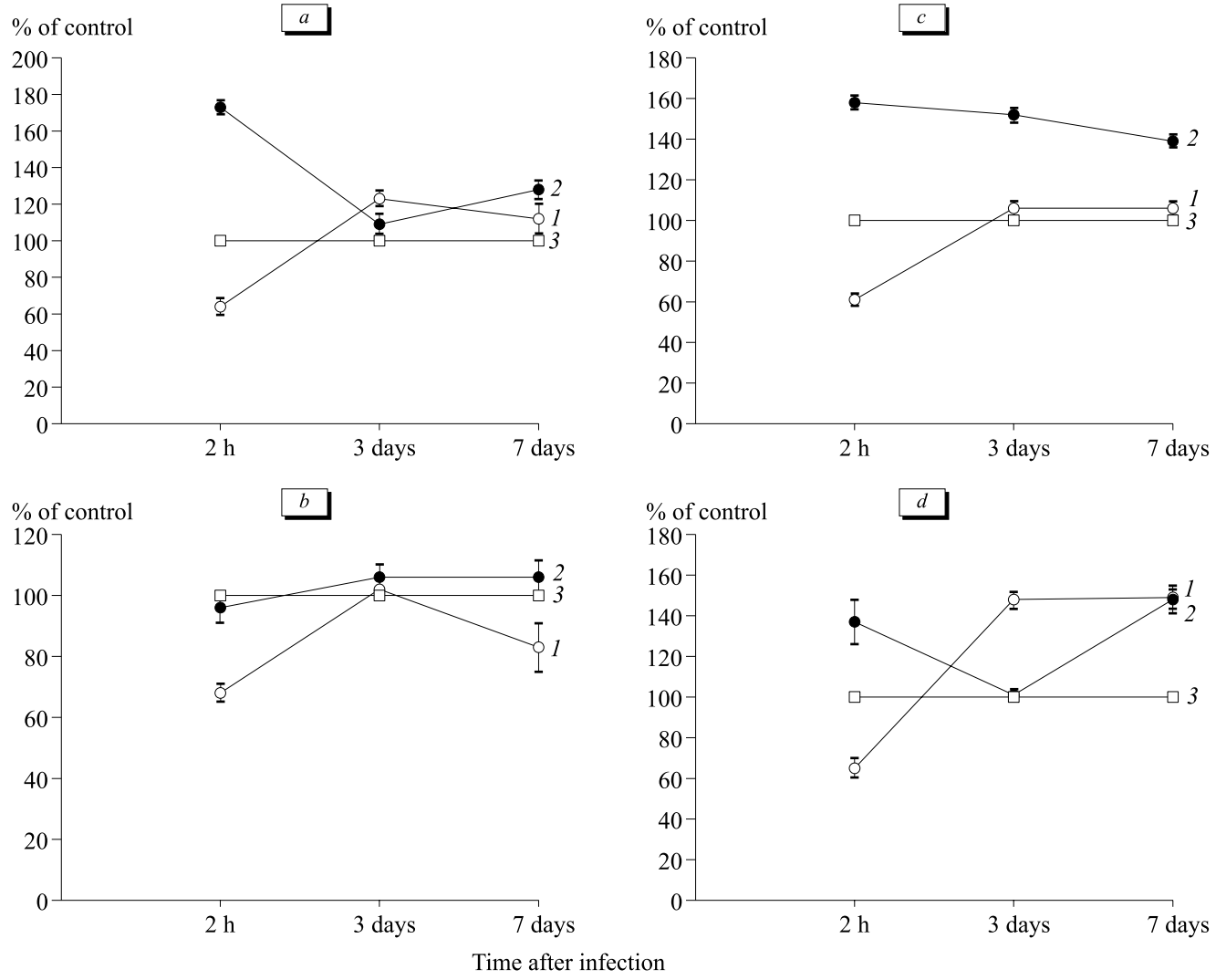

Fig. 2. Effect of vaccination and HSV2 infection on functional activity of peritoneal macrophages from sensitive mice. HSV2 infection (*1*); HSV2 on day 9 after immunization with herpes vaccine and hyaluronic acid (*2*); and intact control (*3*). Ordinate: functional activity of peritoneal macrophages from mice vaccinated and infected with HSV2 (as compared to intact animals).

Comparative study indicates that the sensitivity of DBA/2 and BALB/c mice correlates with macrophage activity in the early stage after intravaginal infection (phagocytic function, cathepsin D activity, and state of the lysosomal apparatus). Mucosal prevaccination with herpes vaccine and hyaluronic acid and intravaginal HSV2 infection improve the resistance of DBA/2 mice and are accompanied by an increase in the function of the lysosomal apparatus, cathepsin D activity, and oxygen metabolism. This treatment also normalizes phagocytosis of the antigen. Our results suggest that the increase in the resistance of vaccinated mice is associated with not only the nonspecific effect of hyaluronic acid (modulator of cell phagocytosis and chemotaxis [6,7]), but also the specific T-cell immune response to HSV.

The observed functional differences between mouse peritoneal macrophages indicate that well-balanced response of phagocytic components to HSV2 over the first hours after infection determines the resistance to herpes infection. A special attention is paid to the lysosomal apparatus and associated systems, which play a role in the early nonspecific defense response. Hence, one of the approaches to normalize macrophage function during HSV infection is the use of lysosome-activating drugs. Published data show that interferon-α (IFN-α) is also involved in the early nonspecific defense response to herpes infection [8]. As compared to sensitive mice, resistant animals are characterized by earlier activation of IFN-α (after 2 h) and autocrine IFN-α/β-regulated respiratory burst [4]. It should be emphasized that the increase in IFN-α/β activity depends on activation of the intracellular receptor TLR9. Function of this receptor depends on the presence of mature lysosomal apparatus and lysosomal enzymes (*e.g.*, cathepsin D) [2].

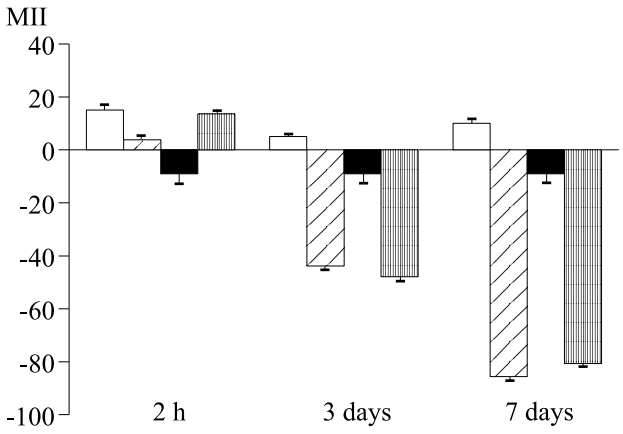

Fig. 3. T-cell immune response to HSV2 in intravaginally infected mice of sensitive (DBA/2) and resistant strain (BALB/c). Light bars, intact BALB/c mice; vertical shading, infected BALB/c mice; dark bars, intact DBA/2 mice; horizontal shading, infected DBA/2 mice.

It may be suggested that activity of the proteolytic enzyme cathepsin D and lysosomal apparatus at early terms after HSV2 infection is an important parameter, which correlates with the sensitivity of mice to HSV2. Study of this enzyme holds promise for evaluation of the effectiveness of vaccine preparations.

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