IMMUNOLOGY AND MICROBIOLOGY

Comparative Study of Macrophage Response in Mice after DNA Immunization and Infection with Herpes Simplex Virus Type 1

E. V. Nagurskaya, L. G. Zaitseva, N. V. Kobets, I. V. Kireeva, V. A. Bekhalo, A. Yu. Kozlov*, R. R. Klimova*, S. V. Gur'yanova, T. M. Andronova**, L. N. Shingarova**, E. F. Boldyreva**, and O. V. Nekrasova****

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 140, No. 12, pp. 670-673, December, 2005
Original article submitted February 15, 2005

Functional activity of macrophages and intensity of T cell immune response in mice were studied after intravaginal and intraperitoneal infection with herpes simplex virus type 1 and DNA vaccination in combination with adjuvant treatment (recombinant granulocyte-macrophage colony-stimulating factor and glucosaminylmuramyl dipeptide). DNA vaccination induced a virus-specific T cell immune response with no macrophagic inflammatory reaction. Infection with herpes simplex virus type 1 was accompanied by sustained inflammation, but not by the T cell immune response.

Key Words: *herpes simplex virus type 1; vaccination; inflammation; macrophages; T cells*

Macrophages mediating inflammatory response to herpes simplex virus (HSV) infection play a special role in initiation of inflammatory processes, their resolution, and differentiation of regulatory T cells [10,11]. Activated virus-specific T cells increase activity of macrophages by modulating their antiviral potency [7].

Macrophages are an important component of antivial immunity; adequate modulation of this component determines the effectiveness of vaccination [9]. Macrophages are involved in immune reactions, inactivate viruses [13], eliminate cells damaged by the virus, stimulate production of various cytokines (IFN-α, IFN-β, interleukin-1, tumor necrosis factor-α, *etc.*), initiate protective or pathological immune reactions [8], play a role in processing and presentation of the antigen [14], and induce specific T cell immune response. However, little in known about functional activity of macrophages at various stages of the anti-HSV immune response remains unknown.

Here we studied functional activity of macrophages in mice under conditions of experimental intravaginal infection with HSV type 1 (HSV1) or DNA immunization with a plasmid expressing the HSV gD gene (pDNAgD). DNA construct containing granulocyte-macrophage colony-stimulating factor (pDNAGM-CSF) gene or synthetic glucosaminylmuramyl dipeptide (GMDP) served as the adjuvant.

N. F. Gamaleya Institute of Epidemiology and Microbiology, Russian Academy of Medical Sciences; *D. I. Ivanovskii Institute of Virology, Russian Academy of Medical Sciences; **M. M. Shemyakin and Yu. A. Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow. ***Address for correspondence:*** nkobets2000@yahoo.com. N. V. Kobets

0007-4888/05/1406-0716

MATERIALS AND METHODS

Experiments were performed on male BALB/c mice weighing 16-18 g and obtained from the central nursery of laboratory animals (Kryukovo, Russian Academy of Medical Sciences). HSV1 reference strain F was kindly provided by Prof. L. Pereira (USA) and maintained by passage on cultured Vero cells. 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. The pDNAgD plasmid alone or in combination with adjuvants pDNAGM-CSF and GMDP served as a specific immunogen [4]. The mice were immunized intramuscularly with purified pDNAgD in a single dose of 100 μg. pDNAgD adjuvants were applied in combination with pDNAGM-CSF (100 μg) or GMDP (300 μg). Functional activity of peritoneal macrophages and reaction of macrophage migration inhibition were studied 2 weeks after infection. Intravaginal infection included treatment with HSV1 (8 LD) in 50 μl physiological saline and caused death of 50% animals (LD_{50}) [12]. The mice received intraperitoneally HSV1 (160 LD_{50}) in 1 ml physiological saline.

Functional activity of peritoneal macrophages (phagocytic activity) and macrophage migration inhibition were studied 2 weeks after infection or immunization. The monolayer of adherent cells was obtained during incubation of peritoneal exudate cells in Petri dishes at 37°C for 1 h (CO_2 atmosphere) using a thermostat.

Phagocytic activity of peritoneal cells was determined by the capacity for absorbing ^{14}C-labeled typhoid vaccine [2]. We measured radioactivity and protein content in the cell suspension. Absorption of ^{14}C-labeled typhoid vaccine was expressed in cpm/mg protein. The intensity of oxidative metabolism was estimated using nitro blue tetrazolium [3]. Total lysosomal activity was assayed by vital staining of cultured cells with acridine orange [3]. The results were expressed in optical density units per mg protein. Activity of lysosomal enzymes was measured using lysates of the cell monolayer. We estimated activities of cathepsin D (μg tyrosine/mg protein) and acid phosphatase (μg inorganic phosphorus/mg protein) and concentration of protein (method of Lowry) [3]. To study macrophage migration inhibition, lymphocytes were isolated from regional lymph nodes, washed 2 times, and resuspended in the culture medium. The cell suspension (5 μl, 5×10^6 cells/ml) was collected using a tip of an automatic pipette and put in a special holder. The holder was placed in a 96-well plate prefilled with the culture medium [6]. Inactivated HSV1 in a concentration corresponding to 10 plague-forming units served as the antigen and was added to some wells. The diameter of cell migration was estimated visually under a Leica inverted microscope with an ocular micrometer. The index of migration inhibition was calculated as follows:

$$IM=D_0^2/D_k^2-1\times100\%,$$

where D_0 is the mean diameter of parallel experimental microcultures; and D_k is the mean diameter of control microcultures.

Stimulation of T lymphocyte proliferation was determined by the number of blast cells. Changes in morphological characteristics of cells was studied *in vitro* in the reaction of blast transformation [4]. The results were expressed as the index for stimulation of proliferation. It was calculated as the ratio between the numbers of blast cells in the population of splenocytes from immunized and non-immunized animals.

The results were analyzed by Student's *t* test. The means and standard errors were calculated using GraphPAD InStat 1.12a software.

RESULTS

Functional activity of peritoneal macrophages in mice was studied after infection with HSV and administration of DNA for the viral gD gene (Fig. 1).

Intravaginal and intraperitoneal infection of BALB/c mice with HSV1 stimulated the initial stage of phagocytosis (absorption of ^{14}C-labeled typhoid vaccine) and increased lysosomal activity (vital staining of cells with acridine orange). Activity of marker lysosomal enzyme cathepsin D significantly increased under conditions of intravaginal and intraperitoneal infection. The increase in acid phosphatase activity was observed only after intravaginal infection.

These changes reflect the inflammatory response persisting for a long time during HSV-infection (day 14 after infection) [15].

The macrophage response remained practically unchanged after intramuscular injection of purified pDNAgD in a single dose of 100 μg (Fig. 1). The exception was cathepsin D activity, which decreased under these conditions. Functional activity of peritoneal macrophages underwent significant changes 14 days after immunization with pDNAgD and adjuvants. Phagocytic activity decreased, while cathepsin D activity increased in mice immunized with DNA and adjuvants. Acid phosphatase activity was lower compared to the control.

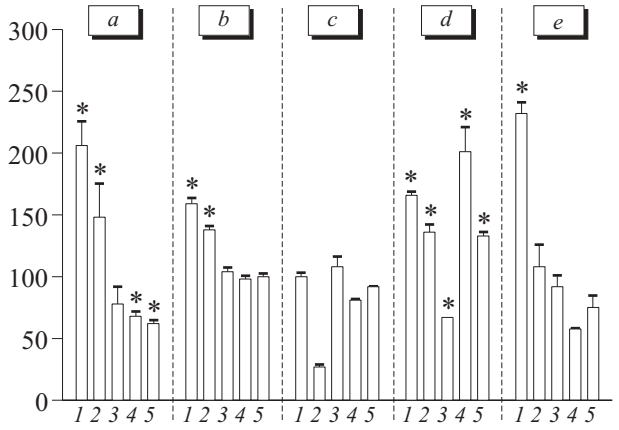

Fig. 1. Functional activity of macrophages in mice infected with herpes simplex virus type 1 (HSV1) or immunized with DNA containing the HSV1 gD gene and adjuvants. Phagocytosis (*a*); test with acridine orange (*b*); test with nitro blue tetrazolium (*c*); cathepsin D (*d*); acid phosphatase (*e*). Ordinate: change in functional activity of peritoneal macrophages (% of intact control animals). HSV1 intravaginally (*1*); HSV1 intraperitoneally (*2*); pDNAgD (*3*); pDNAgD and glucosaminylmuramyl dipeptide (GMDP, *4*); pDNAgD and pDNAGM-CSF (*5*). Here and in Fig. 2: **p*<0.05 compared to intact control animals (100%).

For evaluation of the intensity of T cell immune response we used the reaction of macrophage migration inhibition. Migration of macrophages in response to antigen treatment was much lower in animals preimmunized with pDNAgD alone or in combination with adjuvants, which attests to the formation of specific T cell response (Fig. 2). However, migration of macrophages increased in mice intraperitoneally or intravaginally infected with HSV1. This suggest that complete T cell immune reaction was not formed after HSV-infection. The absence of the specific T cell response was accompanied by persistent activation of macrophages (Fig. 1). The study of T lymphocyte proliferation in the reaction of blast transformation provided support for our conclusion about the T cell response. Specific activation of T cell proliferation was not observed in mice intraperitoneally receiving 50 μg inactivated HSV1 (Fig. 3). The index for stimulation of proliferation was much higher after DNA immunization using pDNAgD alone or in combination with adjuvants GMDP and pDNAGM-CSF (7.9, 10.8, and 12.4, respectively).

Published data show that activated T lymphocytes modulate activity of macrophages and shorten the inflammatory response [10]. The development of inflammation is associated with activity of cytokines secreted by phagocytic cells and HSV-specific memory T cells. One of these cytokines is IFN-γ. Previous studies showed that proteases (*e.g.*, cathepsin D) modify the molecular structure of IFN-γ [1]. Therefore, adjuvants maintain high level of cathepsin D in macrophages. Adjuvants and pDNAgD play a role in the regulatory mechanisms that attenuate inflammation at the late stage of the immune response.

Single administration of pDNAgD alone or in combination with adjuvants GMDP and pDNAGM-CSF induced virus-specific T cell response (reaction of macrophage migration inhibition; and blast transformation). DNA immunization was not accompanied by persistent inflammation due to activation of macrophages. Tests with macrophages and phagocytes showed that intravaginal and intraperitoneal infection with HSV1 is followed by the development of persistent inflammation. However,

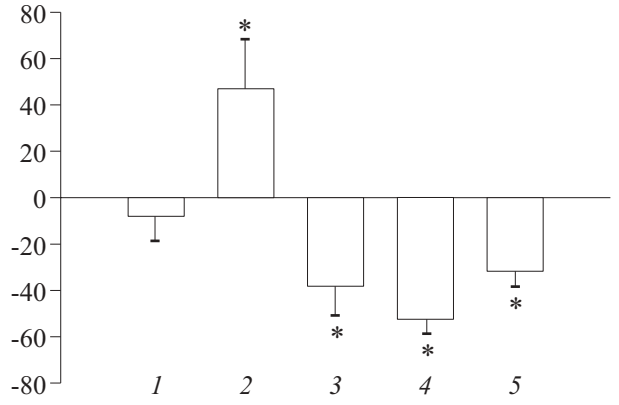

Fig. 2. Inhibition of macrophage migration in mice infected with HSV1 and immunized with DNA containing the HSV gD gene in combination with various adjuvants. Ordinate: index of macrophage migration inhibition. Intact control animals (*1*); HSV1 intravaginally (*2*); pDNAgD (*3*); pDNAgD and GMDP (*4*); pDNAgD and pDNAGM-CSF (*5*).

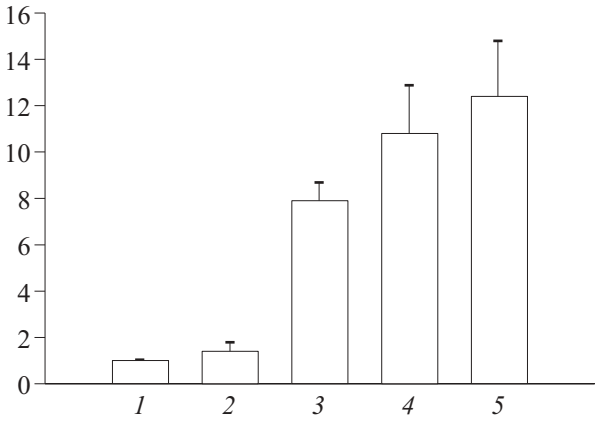

Fig. 3. Proliferative response of mouse T lymphocytes to immunization with antigens. Ordinate: index for stimulation of proliferation in the reaction of blast transformation. Control nonimmunized animals (*1*); HSV1 intraperitoneally (*2*); pDNAgD (*3*); pDNAgD and GMDP (*4*); pDNAgD and pDNAGM-CSF (*5*).

the T cell response to HSV did not increase under these conditions.

This model of DNA immunization with the viral gD gene and adjuvants allowed us to avoid uncontrolled inflammatory reactions during induction of the specific T cell immune response. It may be suggested that these characteristics determine the protective effect of DNA immunization against HSV1. Single immunization using the HSV gD gene in combination with the peptide adjuvant GMDP or genetic adjuvant pDNAGM-CSF provides 96-100% protection of animals from HSV1 in LD [5].

REFERENCES

1. F. I. Ershov, *Interferon System under Normal and Pathological Conditions* [in Russian], Moscow (1996), p. 121.
2. L. G. Zaitseva, M. Yu. Vaisburg, G. M. Shaposhnikova, and E. B. Mysyakin, *Byull. Eksp. Biol. Med.*, **130**, No. 9, 309-312 (2000).
3. L. G. Zaitseva, E. I. Vasil'eva, and E. M. Gorskaya, *Zh. Mikrobiol.*, No. 8, 68-71 (1991).
4. R. R. Klimova, A. Yu. Kozlov, L. N. Shingarova, *et al.*, *Mol. Biol.*, **38**, No. 2, 333-342 (2004).
5. A. Yu. Kozlov, R. R. Klimova, L. N. Shingarova, *et al.*, *Ibid.*, **39**, No. 3, 504-512 (2005).
6. A. M. Shuster, A. P. Suslov, and E. K. Onufrieva, *Vestn. Otorinolaringol.*, No. 2, 40-44 (1991).
7. M. T. Heise and H. W. Virgin 4th, *J. Virol.*, **69**, No. 2, 904-909 (1995).
8. H. Irie, A. Kiyoshi, and A. H. Koyama, *Int. Rev. Immunol.*, **23**, Nos. 1-2, 173-185 (2004).
9. D. M. Koelle and L. Corey, *Clin. Microbiol. Rev.*, **16**, No. 1, 96-113 (2003).
10. M. M. Kosiewicz and P. Alard, *Immunol. Res.*, **30**, No. 2, 155-170 (2004).
11. T. H. Mogensen, J. Melchjorsen, P. Hollsberg, and S. R. Paludan, *J. Immunol.*, **170**, No. 12, 6224-6233 (2000).
12. K. A. Soderberg, M. M. Linehan, N. H. Ruddle, and A. Iwasaki, *Ibid.*, **173**, No. 3, 1908-1913 (2004).
13. E. W. Son, D. K. Rhee, and S. Pyo, *Arch. Pharm. Res.*, **26**, No. 11, 960-966 (2000).
14. A. T. Stock, S. N. Mueller, A. L. van Lint, *et al.*, *J. Immunol.*, **173**, 2241-2244 (2004).
15. R. Yaraee, M. Ebtekar, A. Ahmadiani, and F. Sabahi, *Int. Immunopharmacol.*, **3**, No. 13-14, 1883-1887 (2003).