GENETICS

Expression of TLR9 and BD-2 Protein Genes in Corneal Cells of Mice of Different Strains with Herpetic Keratitis

V. A. Chereshnev[2], L. V. Gankovskaya[1], L. V. Koval'chuk[1], M. V. Chereshneva[2], O. A. Gankovskaya[3], and T. V. Gavrilova[4]

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 153, No. 2, pp. 203-206, February, 2012
Original article submitted September 13, 2010

The dynamics of gene expression of two proteins, TLR9 (one of the key receptors recognizing CpG repeats of herpes virus DNA) and β-defensin 2 (antibacterial peptide), was studied on the model of herpetic keratitis in C57Bl/6 and BALB/c mice. New data on differences in TLR9 gene expression in mice of the two strains infected with the virus were obtained. Reduced TLR9 gene expression in the cornea of C57Bl/6 mice was associated with their high sensitivity to infection caused by herpes simplex 1 virus.

Key Words: *innate immunity; herpetic infection; keratitis; Toll-like receptors; β-defensin 2*

The innate immunity system plays the key role in the protection of eye tissues from bacterial and viral pathogens [4,8]. The main receptors of the innate immunity system are Toll-like receptors (TLRs) recognizing conservative molecular structures common for various microorganisms. Stimulation of these receptors triggers a cascade of adapter molecules and leads to the production of pro- and anti-inflammatory cytokines, chemokines, IFN-1, antibacterial peptides, with the action directed at elimination of pathogens [4,8,9].

Herpetic keratites are the most prevalent corneal diseases [3,7]. Type 1 herpes simplex virus (HSV-1) is assumed to be the main cause of corneal diseases and one of the causes of blindness developing as a result of viral keratitis [5,6].

[1]Russian State Medical University, Ministry of Health and Social Development of the Russian Federation, Moscow; [2]Institute of Immunology and Physiology, Ural Division of the Russian Academy of Sciences, Ekaterinburg; [3]I. I. Metchnikov Institute of Vaccines and Sera, Russian Academy of Medical Sciences, Moscow; [4]Institute of Ecology and Genetics of Microorganisms, Ural Division of the Russian Academy of Sciences, Perm, Russia. ***Address for correspondence:*** lvgan@yandex.ru. L. V. Gankovskaya

Studies of TLRs and antibacterial peptides in eye tissues in viral keratitis are scanty [10,11]. Study of gene expression of TLR9 (one of the key receptors recognizing unmethylated CpG repeats of viral DNA) and β-defensin 2 (BD-2) effector molecule with a direct antiviral effect will provide new data on the role of innate immunity mechanisms in the pathogenesis of herpetic keratitis.

We studied the time course of TLR9 and BD-2 gene expression by the corneal epithelial cells in health and herpetic keratitis in C57Bl/6 and BALB/c mice.

MATERIALS AND METHODS

The study was carried out on male C57Bl/6 and BALB/c mice (18-19 g). A previously described model [11] served as the basis for our study.

The animals of each strain were divided into 3 groups, 10 per group. In group 1 animals, 3 μl virus-containing fluid (HSV-1 titer was 10^5 cytopathic doses causing death of 50% cells; this dose was contained in 0.1 ml) was instilled into the conjunctival sac after scarification of the cornea with a sterile needle. Group

0007-4888/12/1532-0236

2 mice received 3 μl RPMI-1640 into the conjunctival sac after a similar trauma. Group 3 animals were intact. The animals were observed over 7 days. The development of infection was verified by HSV-1 detection by real-time nested PCR. The level of TLR9 and BD-2 gene expression was evaluated by real-time PCR in corneal epithelial cells collected on days 1, 3, and 7 after scarification.

RNA was isolated from corneal cells using RNeasy Mini Kit Qiagen and RIBO-sorb (ILS) kits in strict accordance with the instructions. The resultant RNA was stored at -70°C. Reverse transcription (RT) was carried out using Qiagen OneStep RT-PCR Kit and reverse transcriptase (SibEnzyme). Primers and probes for RT-PCR were selected for mRNA sequences (TLR9, BD-2) using Vector NTI 8.0 software and synthesized by Syntol company. PCR was carried out with the reaction mixture prepared from reagents of a kit for RT-PCR with SYBR Green I intercalating dye (Syntol). After the reaction mixtures were prepared, the tubes were placed into an ANK-32 RT-PCR amplifier (Institute of Analytical Engineering, Russian Academy of Sciences). The reaction was carried out by the following protocol: 5 min at 50°C, 5 min at 95°C, and 40 cycles of 50 sec at 64°C and 20 sec at 95°C. The data were calculated using software supplied with ANK-32 amplifier. The data on genes expression are presented in decimal logarithms (*vs.* 1 million of β-actin gene copies) [1].

The statistical significance of differences in gene expression in the studied groups was evaluated by nonparametric Mann–Whitney test [2].

RESULTS

Significant differences in the course of viral process in the corneal epithelial cells of mice of the two strains were revealed in the viral keratitis model. In C57Bl/6 mice, the virus was detected in 33% animals 1 day after infection, in 90% after 3 days, while on day 7 the number of infected animals started to decrease (Fig. 1). In BALB/c mice, HSV-1 was detected in corneal cells in 50% animals mainly on day 3. Hence, C57Bl/6 mice proved to be more sensitive to the infection caused by HSV-1 than BALB/c mice.

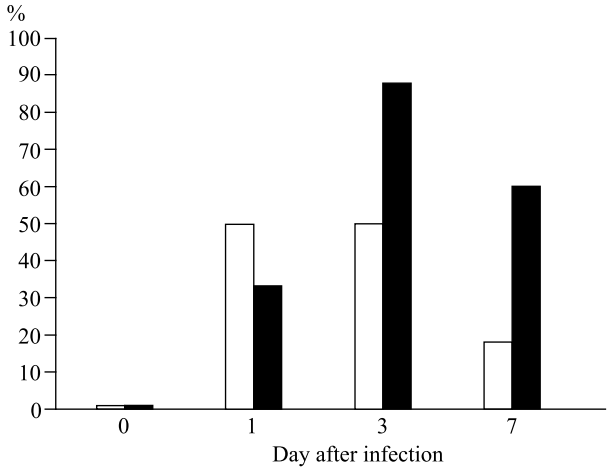

Fig. 1. Dynamics of HSV-1 detection in corneal cells of BALB/c (light bars) and C57Bl/6 mice (dark bars) after infection.

TLR9 is the main signal receptor recognizing unmethylated CpG repeats of HSV-1 DNA, and hence, we evaluated gene expression of TLR9 and BD-2 antibacterial peptide in the cornea of mice of the two strains.

The expression of TLR9 gene in the cornea was significantly reduced in C57Bl/6 mice infected by HSV-1 at all terms of the study in comparison with mice of other groups (Fig. 2, *a*). In BALB/c mice infected with the virus, the studied parameter virtu-

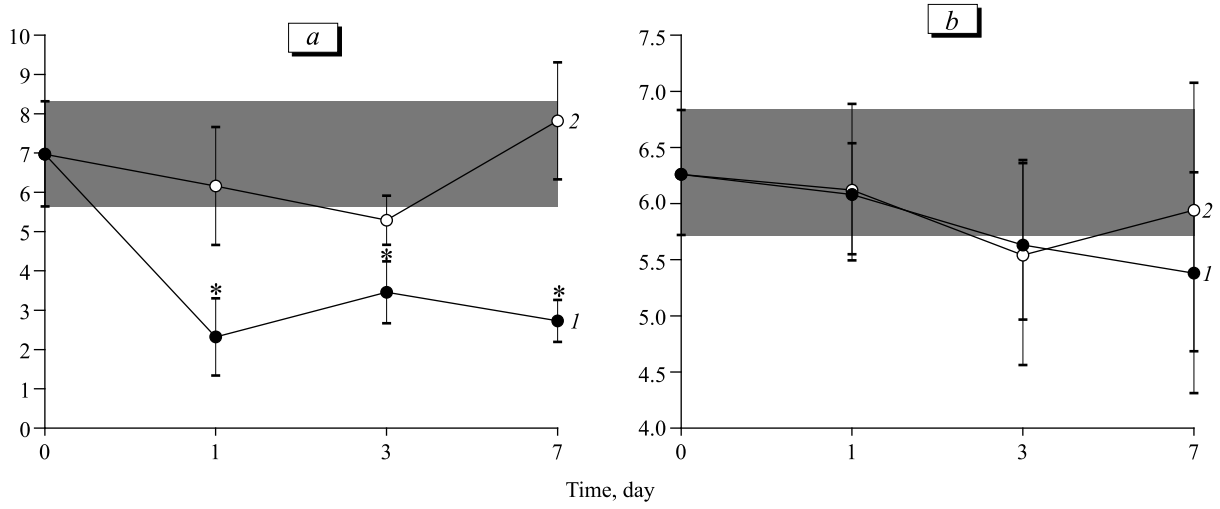

Fig. 2. Changes in TLR9 gene expression in corneal epithelial cells of C57Bl/6 (*a*) and BALB/c mice (*b*). Ordinates: lg (number of TLR9 gene mRNA copies) in comparison with the number of mouse β-actin copies. *1*) group 1; *2*) group 2; dark area: group 3. *$p<0.05$ in comparison with group 2.

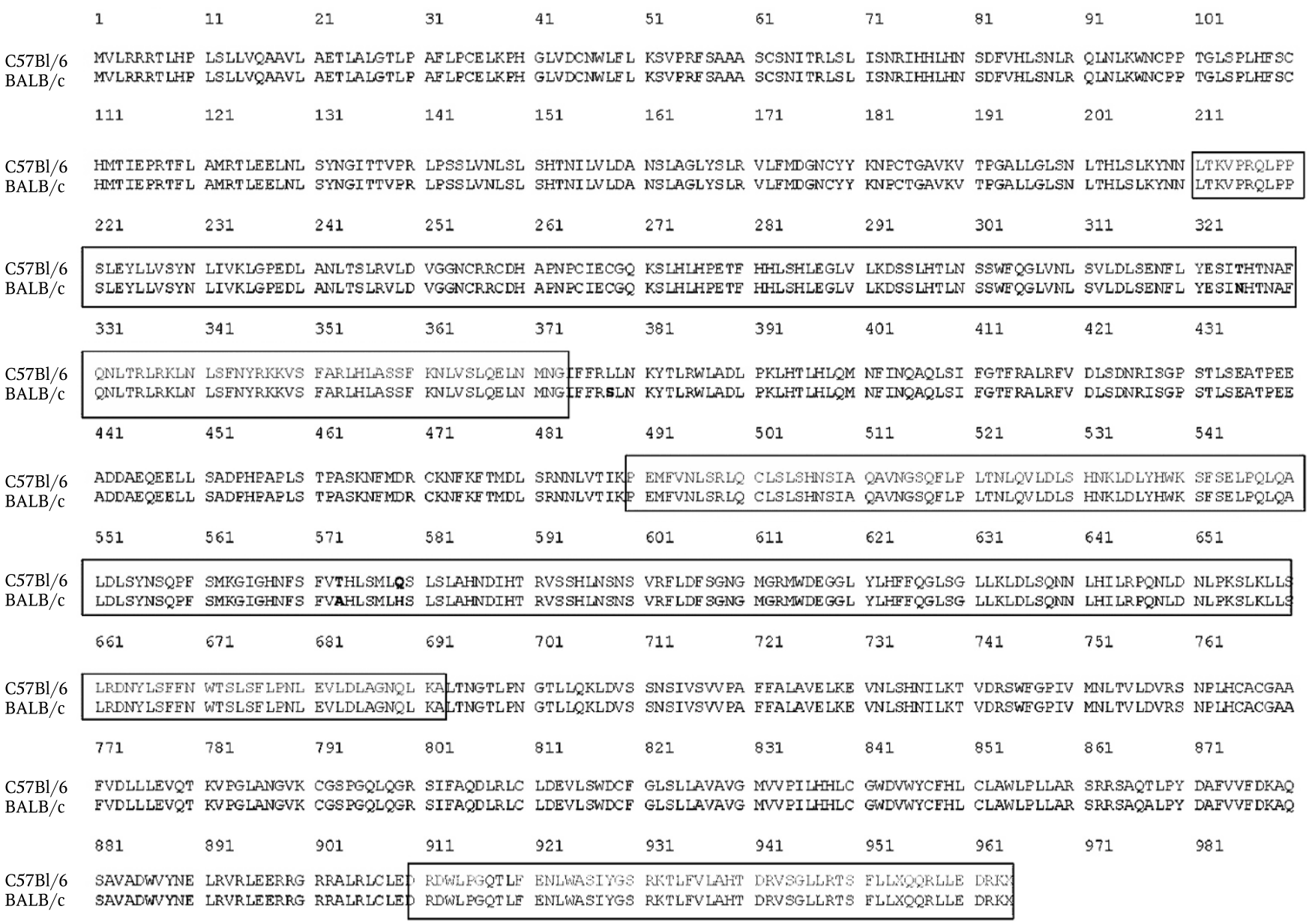

Fig. 3. Amino acid residues of TLR9 in C57Bl/6 and BALB/c mice. Amino acid substitutions are shown with bold letters. The frames show encoding domains of TLR9 gene.

ally did not differ from the values in intact (Fig. 2, *b*). What factors could be responsible for the differences in TLR-9 expression in the two mouse strains? Analysis of TLR9 gene nucleotide sequences in C57Bl/6 and BALB/c mice using the GeneBank database showed the presence of solitary nucleotide sustitutions leading to modification of the TLR9 domain amino acid sequences (Fig. 3). The detected differences were significant for DNA recognition by HSV receptor, which could be essential for the course of HSV infection.

The virus can reduce the expression of TLR9 recognizing receptor, which explains high susceptibility of C57Bl/6 mice to viral keratitis. In addition to this mechanism of immune surveillance avoidance, HSV can inhibit the Toll-mediated inflammatory response [10]. According to this mechanism, ICP0 protein playing an important role in HSV-1 transition from latent to reactivation status blocks the NF-κB nuclear transcription factor through TRAF6 adaptor molecule of the TLR9 signal pathway. This reduction, consequently, leads to suppression of production of proinflammatory cytokines, specifically, TNF-α blocking HSV-1 replication [11].

The levels of expression of BD-2 antibacterial peptide in the corneas of mice of the two strains did not change. Only on day 3 we recorded its significant elevation in infected C57Bl/6 mice (*BD-2* gene expression was 5.25±1.58 in group 1 and 2.984±0.57 in group 2; $p<0.05$). No differences in BD-2 gene expression in C57Bl/6 and BALB/c mice were detected. The data on the different course of herpetic infection in mice of the two strains were largely explained by differences in the expression of TLR9 signal receptor, but not of BD-2 antibacterial peptide molecule.

Our results suggest new approaches to evaluation of the role of innate immunity of ocular tissues in herpetic infection. The model of viral keratitis and evaluation of the expression of TLR9 recognizing receptor and effector molecules can be used for evaluation of potentialities of immunotropic drugs in combined therapy of ophthalmic herpes.

REFERENCES

1. O. A. Gankovskaya, L. V. Koval'chuk, L. V. Gankovskaya, *et al.*, *Zh. Mikrobiol. Epidemiol. Immunol.*, No. 1, 46-50 (2008).
2. S. Glantz, *Biomedical Statistics* [in Russian], Moscow (1999).
3. A. A. Kasparov, *Gerpes*, No. 1, 13-19 (2006).
4. L. V. Koval'chuk, L. V. Gankovskaya, N. I. Martirosova, *et al.*, *Refraktsion. Khir.*, No. 1, 44-48 (2008).
5. Yu. F. Maichuk, *An Unknown Epidemic: Herpes* [in Russian], Smolensk (1997), pp. 62-74.
6. A. G. Rakhmanova, V. K. Prigozhin, and V. A. Neverov, *Infectious Diseases: Manual for Physicians of Common Profile* [in Russian], Moscow, St. Petersburg (1995).
7. M. V. Chereshneva, Yu. I. Shilov, V. A. Chereshnev, *et al.*, *Immunocorrection in Combined Therapy of Patients with Inflammatory Diseases of the Corneal and Vascular Ocular Membranes* [in Russian], Ekaterinburg (2004).
8. R. J. Haynes, J. E. McElveen, H. S. Dua, *et al.*, *Invest. Ophthalmol. Vis. Sc.*, **41**, No. 10, 3026-3031 (2000).
9. J. Li, J. Shen, and R. W. Beuerman, *Mol. Vis.*, **13**, 813-822 (2007).
10. K. Liu, K. Fitzgerald, E. Kurt-Jones, *et al.*, *Proc. Natl. Acad. Sci. USA*, **105**, No. 32, 11,335-11,339 (2008).
11. T. Wuest, B. A. Austin, S. Uematsu, *et al.*, *J. Neuroimmunol.*, **179**, Nos. 1-2, 46-52 (2006).