

Analysis of Immunogenicity of Intracellular CTAR Fragments of Epstein—Barr Virus Latent Phase Protein LMP1

Ya. A. Lomakin^{1,2}, A. A. Shmidt¹, T. V. Bobik^{1,2}, A. S. Chernov³,
A. Yu. Pyrkov¹, N. M. Aleksandrova², D. O. Okunola², M. I. Vaskina¹,
N. A. Ponomarenko¹, G. B. Telegin³, M. V. Dubina⁴, and A. A. Belogurov Jr^{1,2}

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 163, No. 6, pp. 732-737, June, 2017
Original article submitted November 1, 2016

Intracellular fragments of latent phase protein LMP1 of Epstein—Barr virus, denoted as CTAR1/2/3, can trigger a variety of cell cascades and contribute to the transforming potential of the virus. Generation of recombinant proteins CTAR1/2/3 is expected to yield more ample data on functional and immunogenic characteristics of LMP1. We created genetic constructs for prokaryotic expression of LMP1 CTAR fragments and selected optimal conditions for their production and purification. Using a new library of LMP1 CTAR fragments, we carried out epitope mapping of a diagnostic anti-LMP1 antibody S12. Analysis of polyclonal serum antibodies from mice immunized with full-length LMP1 confirmed immunogenicity of CTAR elements comparable with that of full-length protein

Key Words: *S12 monoclonal antibody; latent membrane protein 1 (LMP1); epitope detection; prokaryotic expression*

Latent membrane protein 1 (LMP1) is one of the most intensely studied and mysterious proteins of Epstein—Barr virus (EBV). According to various estimations, EBV infects 80-95% adult population of the planet and the infection is asymptomatic in the majority of cases. However, disturbed balance of lytic (productive) and latent (nonproductive) phases in viral life cycle can lead to grave diseases with serious complications. EBV is associated with many autoimmune diseases, such as multiple sclerosis [4,6], rheumatoid arthritis [3], and systemic lupus erythematosus [9]. The relationship between the virus and lymphoid and epithelial carcinomas, such as Burkitt's lymphoma, Hodgkin's lymphoma, nasopharyngeal carcinoma, and EBV-associated gastric carcinoma, is even more evident [8].

The virus infects mainly B cells and to a lesser extent T cells, epithelial, and NK cells. A specific feature of B and T cell infection is cell immortalization followed by their reactivation. In most cases immune system controls infection, and virus remains in latent form in the infected cells throughout all human life. The main reservoir for EBV are the long-living B-memory cells that passed stage of somatic hypermutation [12]. During the latent phase, infected cells produce a set of EBV genes, including 6 nuclear proteins (EBNA-1/2/3A/3C/LP), minor RNA (EBER-1/2), and membrane proteins (LMP-1/2A/2B). These proteins control cell cycle, allowing the infected cells to escape apoptosis.

The main transducer protein of EBV, LMP1, acts as a classical oncogene on model rat fibroblasts and is essential for EBV-dependent B-cell transformation *in vitro* [15]. LMP1 functions as a constantly activated TNF receptor in ligand-independent manner. Functionally, LMP1 mimics CD40 and can even partially substitute it *in vivo*, providing growth and differential signals to B cells [13]. LMP1 is a polypeptide consisting of 386 amino acids with a molecular weight

¹M. M. Shemyakin and Yu. A. Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow; ²Kazan (Volga Region) Federal University, Kazan, Republic of Tatarstan, Russia; ³Affiliated Department of M. M. Shemyakin and Yu. A. Ovchinnikov Institute of Bioorganic Chemistry, the Russian Academy of Sciences, Pushchino, Russia; ⁴St. Petersburg Academic University, Russian Academy of Sciences, St. Petersburg, Russia. **Address for correspondence:** belogurov@mx.ibch.ru. A. A. Belogurov Jr