

# IMMUNIZATION WITH FC-BASED RECOMBINANT EPSTEIN-BARR VIRUS GP350 ELICITS POTENT NEUTRALIZING HUMORAL IMMUNE RESPONSE IN A BALB/C MICE MODEL

Bingchun Zhao, State Key Laboratory of Oncology in South China, Collaborative Innovation Center for Cancer Medicine, Department of Experimental Research, Sun Yat-sen University Cancer Center, Guangzhou, China  
zhaobch@sysucc.org.cn

Xiao Zhang, Ling Gao, Haojiong Zhang, Miao Xu, Lin Feng, Musheng Zeng, Yixin Zeng, State Key Laboratory of Oncology in South China, Collaborative Innovation Center for Cancer Medicine, Department of Experimental Research, Sun Yat-sen University Cancer Center, Guangzhou, China

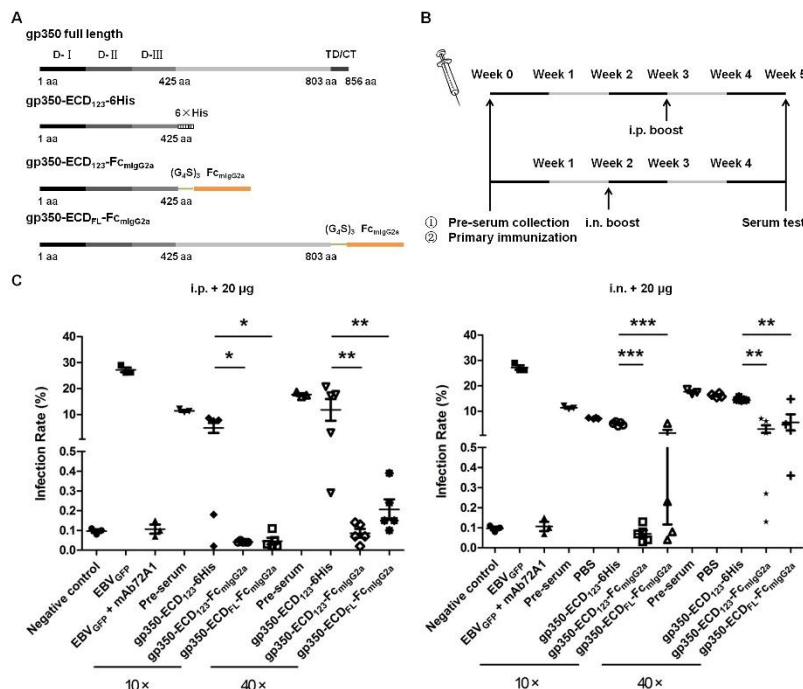
Claude Krummenacher, Departments of Biological Sciences, and Molecular and Cellular Biosciences, Rowan University, Glassboro NJ, USA

Shuo Song, State Key Laboratory of Molecular Vaccinology and Molecular Diagnostics, National Institute of Diagnostics and Vaccine Development in Infectious Diseases, School of Life Sciences, School of Public Health, Xiamen University, Xiamen, China

Yuting Xu, Guiyang City National High School, Guiyang, China

**Key Words:** Epstein-Barr virus, envelope protein, gp350, Fc-based vaccine, neutralizing antibody.

Epstein-Barr virus (EBV) was the first human virus proved to be closely associated with tumor development, such as lymphoma, nasopharyngeal carcinoma (NPC) and EBV-associated gastric carcinoma. Despite many efforts to develop prophylactic vaccines against EBV infection and diseases, no candidates have succeeded in effectively blocking EBV infection in clinical trials. Previous investigations showed that EBV gp350 plays a pivotal role in the infection of B lymphocytes. Nevertheless, using monomeric gp350 proteins as antigens has not been effective in preventing infection. Multimeric forms of the antigen are more potently immunogenic than monomers, however the multimerization elements used in previous constructs are not approved for human clinical trials. To prepare a much-needed EBV prophylactic vaccine that is potent, safe and applicable, we constructed an Fc-based form of gp350 to serve as a dimeric antigen. Here we show that the Fc-based gp350 antigen exhibits dramatically enhanced immunogenicity compared to wild-type gp350 protein. The complete or partial gp350 ectodomain was fused with the mouse IgG2a Fc domain. Fusion with the Fc domain did not impair gp350 folding, binding to a conformation-dependent neutralizing antibody and binding to its receptor by ELISA and SPR. Specific antibody titers against gp350 were notably enhanced by immunization with gp350-Fc dimers compared to gp350 monomers. Furthermore, immunization with gp350-Fc fusion proteins elicited potent neutralizing antibodies against EBV. Our data strongly suggest that an EBV gp350 vaccine based on Fc fusion proteins may be an efficient candidate to prevent EBV infection in clinical applications.



**Figure 1. Immunization with Fc fusion antigens potently induced production of neutralizing antibodies. (A) Diagrams for design of Fc-based recombinant gp350 proteins. (B) Diagram of the immunization protocols. Mice were boosted on week 2 for i.n. group or week 3 for i.p. group. (C) Neutralization of EBV<sub>GFP</sub> infection by sera collected at week 5 post-immunization. Recombinant EBV<sub>GFP</sub> was preincubated with 10x or 40x dilutions of sera from mice immunized with the indicated antigens. Virus was added to Akata cells and GFP fluorescence was recorded as a measure of infection.**