P-11

# Preventing Human CD8$^+$ Cytotoxic T Lymphocyte(CTL)-mediated Cytotoxicity against Swine Fetal Fibroblast Cells by Overexpression of Human Cytomegalovirus Glycoprotein US2

K. W Park[1]. J. Y. Yoo[1], K. M. Choi[1], S. P. Hong[1], G. S. Han[1], E. J. Kim[1], S. H. Kim[1], T. S. Kim[1], S. Y. Park[1], B. S. Yang[2], G. S. Im[2], K. S. Jun[1], K. N. Heo[1] and J. G. Seol[1]

*[1]Mgen, Inc., [2]NLRI(National Livestock Research Institute), RDA*

For a number of reasons, the pig has been identified as the most likely animal to provide donor organs on a large scale for transplantation into human. However, multiple immune barriers preclude its clinical application. Especially, the initial type of rejection in xenotransplantation is acute cellular rejection by host CD8$^+$ cytotoxic T lymphocyte(CTL) cells that react to donor class I MHC.

The Human Cytomegalovirus(HCMV) is a $\beta$-herpesvirus characterized by its restricted host range, production of nuclear as well as cytoplasmic inclusion, and its long life cycle. Its glycoprotein US2 specifically targets class I MHC heavy chains for dislocation from endoplasmic reticulum(ER) membrane to the cytosol, where they are degraded by proteasome.

In this study, we transfected the US2 gene into mini-pig fetal fibroblast, resulting in five US2 clonal cell lines. The integration of US2 in transgenic mini-pig cell was confirmed by PCR and Southern blot assay. The reduction of SLA(Swine Leukocyte Antigen)-I by US2 was also detected by Flow cytometry assay. FACS analysis of US2 clonal cell lines demonstrated substantial reduction in SLA surface expression. The decrease in the level of SLA-I expression for US2 clonal cell lines ranged from 22% to 34% relative to non-transfected control. The expression US2 significantly suppressed CD8$^+$-mediated cell lysis, and the rate of its cell cytotoxicity was reduced as compared to the control

group(US2: 23.8%±15.1%, control : 59.8%±8.4%, $p<.05$).

In conclusion, US2 can directly protect $CD8^+$-mediated cell lysis. These results indicate that the expression of US2 in the pig cells may provide a new approach to overcome CTL- mediated immunity to xenotransplantation.

This study was supported by a grant(Code No. NLRI 2006-0101 from "Live-stock & Biotechnology Research Program of National Livestock Research Institute", RDA, Republic of Korea.