NF-KB INDUCES OVEREXPRESSION OF BOVINE FCRN: A NOVEL MECHANISM THAT FURTHER CONTRIBUTES TO THE ENHANCED IMMUNE RESPONSE IN GENETICALLY MODIFIED ANIMALS CARRYING EXTRA COPIES OF FCRN

Judit Cervenak[1]

M. Doleschall[2], B. Bender[1], B. Mayer[3], Z. Schneider[4], Z. Doleschall[5], Y. Zhao[6], Zs. Bősze[7], L. Hammarström[8], W. Oster[1], I. Kacskovics[1,4]

[1]ImmunoGenes Kft, Budakeszi, Hungary; [2]Mol. Med. Res. Group, Hungarian Academy of Sciences and Semmelweis Univ., Budapest, Hungary; [3]Dep. of Dermatology, Venereology and Dermatooncology, Semmelweis Univ., Budapest, Hungary; [4]Dep. of Immunology, Eötvös Loránd Univ., Budapest, Hungary; [5]Dep. of Pathogenetics, Nat. Inst. of Oncology, Budapest, Hungary; [6]State Key Lab. of Agrobiotech., China Agricultural Univ., Beijing, China; [7]Agricult. Biotech. Center, Gödöllő, Hungary; [8]Div. of Clin. Immunology, Karolinska Inst., Stockholm, Sweden

Among many functions of the neonatal Fc receptor (FcRn) for IgG, it binds to IgG-opsonized antigen (Ag) complexes and propagates their traffic into lysosomes where Ag processing occurs. We previously reported that transgenic (Tg) mice that express multiple copies of bovine (b)FcRn have augmented humoral immune response. Among the mechanisms that contribute to the boosted immune response, the augmented Ag-IgG immune complex presentation via professional Ag presenting cells (APCs) such as dendritic cells that express bFcRn seems to be especially important. Nuclear Factor-kappa B (NF-κB) is a critical molecule in the signaling cascade in the immune response. NF-κB induces human FcRn expression and our previous *in silico* analysis suggested NF-κB binding sites in the promoter region (PR) of the bFcRn α-chain gene (FCGRT).

This study was undertaken to analyze additional mechanisms that contribute to the immune capabilities observed in the bFcRn Tg mice. We investigated NF-κB binding sites in the PR of bFCGRT using luciferase reporter gene technology and electromobility shift assays. NF-κB mediated bFcRn regulation was studied in lipopolysaccharide (LPS)-treated primary bovine endothelial cells (BAECs) by quantitative PCR; in the spleen of LPS-injected bFcRn Tg mice by Northern blot analysis; and at protein level in macrophages isolated from the bFcRn Tg mice using flow cytometry.

We identified three functional NF-κB binding sites in the PR of bFCGRT. Stimulation of BAECs with LPS, which mediates its effect via NF-κB, resulted in rapid upregulation of the bFcRn expression, which was also observed in the spleen of bFcRn Tg mice treated with LPS. NF-κB mediated bFcRn upregulation was confirmed in macrophages isolated from the bFcRn Tg mice with a newly developed FcRn specific monoclonal antibody that does not cross-react with the mouse FcRn.

We concluded that NF-κB regulates bFcRn expression and thus optimizes its functions, e.g., in the professional APCs, and contributes to the much augmented humoral immune response in the bFcRn Tg mice.

Supported by the grants OTKA T049015, K 101364 and OM-00117-119/2008.