

BAC transgenic mouse model to analyze the role of the bovine FcRn in IgG metabolism

Balazs Bender¹, Lilla Bodrogi¹, Balazs Mayer², Yaofeng Zhao³, Lennart Hammarstrom³, Zsuzsanna Bosze¹, Imre Kacs Kovics²

¹ Department of Animal Biology, Agricultural Biotechnology Center, Godollo, Hungary

² Faculty of Veterinary Science, Szent Istvan University, Budapest, Hungary

³ Division of Clinical Immunology, Karolinska Institute, Huddinge, Sweden

IgG has the longest survival time in the circulation of the immunoglobulin classes and the lowest fractional catabolic rate. The neonatal Fc receptor (FcRn) which is composed of the FcRn heavy chain and the beta2-microglobulin plays an important role in regulating these processes. Recently, we have cloned the bovine FcRn (bFcRn) alpha chain and detected its expression in various epithelial cells which are mediating IgG secretion. In ruminants maternal immunity is exclusively mediated by colostral immunoglobulins. The presence of the FcRn in the acinar epithelial cells of the mammary gland and the obvious change in its distribution before and after parturition indicate that FcRn plays an important role in the IgG transport during colostrum formation in ruminants. We have also detected FcRn in the small intestine and lower respiratory tract where IgG secretion is clearly predominant over IgA, in ruminants. We have also demonstrated that FcRn is expressed in endothelial cells and shown that IgG with enhanced binding to FcRn has increased serum persistence in cattle, and thus FcRn can effectively protect IgG from degradation.

In order to study the regulation of the bovine FcRn heavy chain gene and analyze its role in IgG metabolism we have generated BAC transgenic mice overexpressing the bovine FcRn heavy chain. A 102 kb BAC clone was isolated from bovine BAC library harboring the bovine FcRn alpha chain gene and its 44 kb and 50 kb 5' and 3' flanking regions. The BAC clone was microinjected into mouse zygotes and three independent transgenic lines were generated. Two of them showed Mendelian transgene inheritance (line #14, #19).

Transgene copy numbers were determined by real-time PCR and found to be 2 and 5 in lines #14 and #19, respectively. Bovine FcRn alpha chain specific mRNA was detected by RT-PCR and Northern analysis in the liver and intestine in all transgenic lines and in lactating mammary gland of line #14. The bovine FcRn BAC transgenic mouse lines having showed copy number dependent but integration site independent expression.

To reveal whether bovine FcRn alpha chain and mouse beta2-microglobulin is able to form a functional receptor, 10 and 20 mg/BWkg IgG was injected i.v. into transgenic (homozygous #14 line) and control mice. Analysis of the injected IgG concentration of the animals in the first 10 days was done by fitting the data to the two-compartmental model using WinNonLin Professional, version 4.1 (Pharsight, Mountain View, CA). Pharmacokinetic studies showed that the IgG had around 180 hours serum half-life in transgenic mice, which is almost two times longer than its half-life in normal mice. This data indicates that bovine FcRn heavy chain is indeed expressed in the mouse endothelial cells and formed a functional receptor.

Future studies should be aimed at testing the different lines (#14 and #19) if they differ in IgG catabolism, and secretion. Furthermore, we intend to study factors that may alter the expression of this gene, in vivo.

Supported by grants of COST B20 Action, GAK-CALVES05, and the Swedish Research Council