Themes of thesis

Effects of bFcRn overexpression on humoral immune response

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Department of Immunology, Institute of Biology
Eotvos Loránd University, Budapest, Hungary
2012.

Introduction

In response to antigen, plasma cells differentiate from antigen-specific B-lymphocytes and these cells produce antigen-specific immunoglobulins. Maintenance of increased antigen-specific antibody level requires continuous immunoglobulin secretion of plasma cells as well as protection of these valuable antibodies from rapid degradation. Frequent immunizations of animals involved in polyclonal antibody production are required to achieve the highest possible level of antigen-specific antibody. During the hyperimmunization process, the most valuable antigen-specific immunoglobulin G (IgG) level may exceed the physiological IgG serum concentration resulting in decreased IgG protection and accelerated IgG degradation. Therefore continuous and frequent immunizations are needed to maintain high level antigen-specific IgG. Monoclonal antibodies have replaced polyclonal immunoglobulins in several areas in research, diagnostics and therapy. As a general rule, the more viable and antigen-specific hybridomas are generated from a fusion, the greater the chance of finding an ideal candidate of them to produce the appropriate monoclonal antibody. From all the immunoglobulin isotypes, IgG molecules have the longest half-life due to the protective effect of the neonatal Fc receptor

(FcRn). This receptor also transports IgG molecules through epithelial barriers, mediates maternal IgG transport, regulates albumin homeostasis. It is also involved in phagocytosis and antigen presentation of antigen-IgG immune complexes in professional antigen presenting cells.

Aims

Dr. Imre Kacskovics and his colleagues have been studying the role of the bovine neonatal Fc receptor (bFcRn) and showed its critical role in IgG homeostasis in cattle. Their *in vitro* experiments showed that bFcRn binds human IgG more efficiently than it binds to bovine IgG. This result was proven *in vivo* as the half-life of injected human IgG was prolonged compared to endogen bovine IgG in normal and human IgG producing transchromosomal cattle. Based on the request to get more insight of the regulation of the bFcRn expression they have created transgenic (Tg) mouse models that overexpressed the bFcRn together with Dr. Zsuzsanna Bősze and collegaues (Agricultural Biotechnology Center). In these animals the receptor showed tissue and copy-number related expression at DNA and mRNA level, so thus proved to be appropriate for further characterizing the bFcRn gene expression and the immune-phenotypes of these Tg mice.

Our aims were:

- to study the mouse and human IgG catabolism (determining the half-life of these antibodies injected into bloodstream of mice) in the bFcRn Tg mice to analyze the functionality of this heterodimer receptor that is composed of the bFcRn α-chain and mouse β2-microglobulin and also the effect of the FcRn overexpression to the IgG clearance rate.
- to analyze several factors of the immune phenotype of these bFcRn Tg mice, such as:
  - the antigen-specific and total IgG level after immunization with T-dependent antigens
  - the serum IgG and albumin levels
  - the antigen-specific antibody titer, spleen cell number and antigen-specific antibody secreting spleen cell numbers after immunization
  - the number, ratio and specificity of hybridomas and hybridoma microcultures after fusion
  - the level of autoantibodies in elderly mice
- to develop and characterize bFcRn specific antibodies for Western blot and immunohistochemical applications

Methods

- ELISA
- ELISPOT
- hybridoma production
- antibody profiling microarray
- Western blot
- flow cytometry
- fluorescent immunohistochemistry

Results

- The half-lives of mouse and human IgGs were prolonged in bFcRn Tg mice compared to wt controls.
- Antigen-specific and total IgG levels were 2.5 – 3 fold higher in bFcRn Tg mice than in wt controls when immunized them with ovalbumin.
- Total serum IgG and albumin levels were significantly higher in bFcRn Tg mice than that of wt controls.
- Hapten- and carrier-specific IgG titer; size of the spleen, number of spleen cells and antigen-specific spleen cells of bFcRn Tg mice were elevated compared to wt controls after immunization.
- Fusion of these splenocytes resulted in significantly higher number of antigen-specific hybridoma microcultures than in wt controls.
- Fusion of spleen cells of bFcRn Tg mice resulted not only in elevated number, but also in higher ratio of hybridomas and antigen-specific hybridoma microcultures, thus hybridization frequency of the fusion was higher than in wt counterparts.
- Autoantibody profiles of wt and Tg mice were similar, and there was no detectable presence of anti-nuclear antibodies in the circulation of the animals.
- Peritoneal macrophages and bone marrow dendritic cells of bFcRn Tg mice showed high level bFcRn expression with a recently developed bFcRn-specific mouse monoclonal antibody.
- Non-immunized bFcRn Tg mice showed similar spleen structure as wt controls, and we could detect several cells, including MARCO+ marginal zone macrophages, that express considerable amount of FcRn, as demonstrated with a bFcRn-specific polyclonal chicken antibody.

Discussion

The better IgG rescue results in higher level of antigen-specific IgG in immunized bFcRn Tg animals which leads to the formation of more antigen-IgG immune complexes (ICs). Dendritic cells that overexpress bFcRn phagocytose and present antigens more efficiently to T helper cells when loaded with antigen-IgG ICs. The higher number of DCs in transgenic FcRn animals compared to the wild-type controls after immunization suggests that these cells are more abundant and active in spleen of bFcRn Tg mice and very likely contribute to the augmented immune response observed. The role of FcRn in the secondary lymphoid organs is also supported by the fact that we could demonstrate strong FcRn expression in the white pulp of the spleen (e.g. in MARCO positive marginal zone macrophages). Based on these observations, we suggest that the overexpression of the FcRn does more than protect antigen-specific IgG from degradation. It also enhances the priming of naïve B cells, the expansion of antigen-specific memory B cells and plasma cells in the secondary lymphoid organs.

The higher number of antigen-specific hybridomas produced from splenocytes of immunized bFcRn Tg mice as well as elevated hybridization frequency of the fusion makes these animals especially suitable for monoclonal antibody production as they provide a wider spectrum of antigen-specific clones. In contrast to other transgenic mice tested earlier to increase the efficiency of monoclonal antibody production, the use of bFcRn Tg mice is not limited due to the presence of autoimmune antibodies.

FcRn transgenesis thus confers a number of practical benefits, including faster antibody production, higher antibody yields and improved generation of hybridomas for monoclonal antibody production.

Publications related to the thesis

Bender, B., Bodrogi, L., Mayer, B., Schneider, Z., Zhao, Y., Hammarstrom, L., Eggen, A., Kacskovics, I., Bosze, Z.: **Position independent and copy-number-related expression of the bovine neonatal Fc receptor alpha-chain in transgenic mice carrying a 102 kb BAC genomic fragment.** Transgenic Research 2007. 16, 613-627.

Cervenak, J., Bender, B., Schneider, Z., Magna, M., Carstea, B.V., Liliom, K., Erdei, A., Bosze, Z., Kacskovics, I.: **Neonatal FcR overexpression boosts humoral immune response in transgenic mice.** Journal of Immunology 2011. 186(2), 959-968.

Schneider, Z., Cervenak, J., Baranyi, M., Papp, K., Prechl, J., Laszlo, G., Erdei, A., Kacskovics, I.: **Transgenic expression of bovine neonatal Fc receptor in mice boosts immune response and improves hybridoma production efficiency without any sign of autoimmunity.** Immunology Letters 2011. 137(1-2), 62-69.

Vegh A, Farkas A, Kovesdi D, Papp K, Cervenak J, Schneider Z, Bender B, Hiripi L, Laszlo G, Prechl J, Matko J, Kacskovics I (2012) **FcRn Overexpression in Transgenic Mice Results in Augmented APC Activity and Robust Immune Response with Increased Diversity of Induced Antibodies.** PLoS One 7 (4):e36286. doi:10.1371/journal.pone.0036286