# Regulation of humoral immune response: Nature and differantial expression of GL7 epitope and modulation of immune cells by estrogen

Ph.D.thesis

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## INTRODUCTION

During maturation and activation, immune cells continually alter their cell surface molecular pattern that allow them a dynamic interaction with the environment. Activation and differentiation of lymphocytes are associated with rearrangement of the cell surface glycoproteins, glycolipids glycan chains by glycosyltransferases (e.g. sialyltransferases, fucosyltransferases) and glycosidases (e.g. sialydases/neuraminidases), alteration of glycosilation. An increasing number of studies show the importance of glycosylation in pathogen recognition, in controlling homeostasis and inflammation or in the maturation and activation of adaptive immune cells. Abnormalities in glycosilation leads to development of autoimmun diseases and cancer. Some steroid hormons (e.g estrogen) are involved in controlling activity and expression of glycosyltransferases. *In vitro* and *in vivo* data also support, that estrogen can modulate immune response, influence the cytokine and antibody production. It is well known that females shown an increased susceptibility to autoimmune diseases than males. However, the exact molecular/cellular mechanism behind this difference is still largely unknown.

The GL7 epitope expressed in mouse and widely used as a marker of germinal center, it is shown regulated differential expression during B and T cell maturation and activation. Recentely, the antibody was shown to recognize the Neu5Ac carbohydrate epitope. The expression of GL7 epitope in other species, especially in humans, where the supposed enzyme, CMAH, that mainly controls appearance of the epitope, is absent, has not

yet been sufficiently characterized. The function of GL7 epitope is still not known.

Therefore we propose to study the nature and the expression of GL7 epitope-bearing molecule(s) in humans. Furthermore we intend to create a single chain molecule (scFv) using in experiment reveal the functional role of GL7 epitope. In addition we want to study the effect of estrogen on humoral immune response *in vivo* and *in vitro*.

**OBJECTIVES**

GL7 epitope originally described on mouse splenocytes after *in vitro* activation. The epitope-bearing cell surface molecule has not yet characterized, though the differencial expression pattern during cell maturation and activation suggest its role in adhesion and/or activation, the exact function is still waiting to be found. The antibody, recognizing the GL7 epitope, has an IgM isotype, therefore it cannot be efficiently used in many methods.

Therefore our aims were to investigate:

- The chemical nature of the GL7 epitope in mouse and human lymphoid cells.
- The expression of GL7 epitope on rat spleen cells.
- The expression of GL7 epitope on human primary immunocytes.



- The alteration of GL7 epitope expression upon *in vitro* activation in human lymphocytes.
- To create a single chain construction contain only the variable domains of antibody recognize GL7 epitope.

It is well known that estrogen influences the differentiation, maturation, and emigration of lymphocytes, which are all essential for an adequate immune response. Since the effect of estrogen on humoral immune response has been investigated mainly *in vitro*, therefore, we set the following goal:

- We examined the estrogen effect on immune response to T cell dependent and T cell independent antigen *in vivo*
- Existence of membrane and intracellular receptors for estrogen was previously reported on T and B cells by our laboratory so we examined the nongenomic estrogen effect on NFκB activation and IFNγ gene activation

## METHODS APPLIED

- immunization of ovariectomized or sham-operated C57Bl/6 mice
- separation and in vitro activation of mice and human B and T cell
- ELISA (Enzyme-Linked Immunosorbent Assay)
- ELISPOT (Enzyme-Linked ImmunoSPOT)
- SDS-PAGE and Western blot
- PCR, cloning and sequence analysis
- expression and purification of recombinant scFv construction
- flow cytometry
- confocal laser scanning microscopy

## RESULTS

- Our results show that the GL7 epitope is fully neuraminidase- and partially papain-sensitive in human and mouse lymphocytes. Binding of GL7 Ab and α2-6-sialic acid-specific SNA lectin to 6 different human T and B cell lines and primary lymphocytes showed strong positive correlation, whereas MAA II lectin (α2-3 sialci acid specific) and GL7 Ab displayed a strong negative correlation in staining of cells. The PNA lectin lacking sialic acid specificity showed no correlation at all with GL7 Ab staining.

- Expression of GL7 epitope is activation-dependent on rat lymphocytes, similarly to the earlier finding in mouse. In human, the appearance of the epitope is also lymphocyte-restricted, but is constitutively expressed. Its expression bidirectionally changes upon *in vitro* activation.



- We succesfully created a single chain antibody from GL7 monoclonal rat IgM producing hybridoma cells.

- We have shown, that estrogen enhances the B cells from C57Bl/6 mice immune response to TD antigen, since elevated number of hapten-specific antibody producing B cells and increased antibody response could be detected. Response to TI-2 antigen did not change, indicating that estrogen mainly influences T cell-dependent immune response.

- Estrogen treatment induced a rapid activation and nuclear translocation of p65 and p52 NFκB, while had no effect on cRel.

- Agreement with previous in vivo data we could show that estrogen treatment of spleen cell *in vitro* enhances IFNγ synthesis in ConA stimulated cell culture. Together with the data showing estrogen triggered NFκB activation, these results indicate that estrogen may enhance B cell response to TD antigen via inducing NFκB and IFNγ gene transcription.

## CONCLUSIONS

In conclusion, our result show that estrogen has a direct impact on B and T cells by inducing rapid nongenomic effects *via* both intracellular ER (in B cells) and membrane ER (mainly in T cells). These effects result in activation of p65 NFκB in both B and T cells and a chance for enhanced survival of B cells. Altogether these effects may result in an improved collaboration between B and T cells during the TD immune response. Consistent with this, our in vivo studies first demonstrate that estrogen indeed positively modulates the T cell-dependent but not the T-independent humoral immune response.

It was discussed by many papers, that steroid hormones can control the expression of certain glycosyltransferases. Estorgen specifically down-regulates and up-regulates activity of 2,6- and 2,3- sialyltransferases, respectively. Furthermore, it was also shown that estrogen modulates the expression of molecules, regulating B cell survival (e.g. directly upregulates CD22 expression). This raises the question, how estrogen affects the accessibility of CD22 ligand and, in this context, the cell surface appearance of GL7 epitope. The epitope on both mouse and human cells was found full neuraminidase-, and partial papain-sensitive suggesting the GL7 carbohydrate epitope can be linked to either one or more glycoproteins or glycolipids as well, depending on the cell type. The GL7 epitope cell type, cycling stage of cell maturation and activation-dependent selective expression pattern allow us to suggest a functional role for GL7 epitope during lymphocyte maturation and activation. The successfully generated scFv construction will hopefully be a useful tool to study *in vivo* function of GL7 epitope-bearing molecules and their supposed counterparts.

**Publications connected to the thesis:**

1. **A closer look into the GL7 antigen: Its spatio-temporally selective expression and localization in lymphoid cells and organs in human.**
   *Andrea Balogh*, Mónika Ádori*, Katalin Török, János Matkó and Glória László*
   **Immunology Letters.** Epub ahead:doi:10.1016/j.imlet.2009.12.008
   Impact factor: 2,858

2. **Estrogen augments the T cell-dependent but not the T-independent immune response**
   *Mónika Ádor*i, Endre Kiss*, Zsuzsanna Barad, Klaudia Barabás, Edda Kiszely, Andrea Schneider, Erna Sziksz, István M. Ábrahám, János Matkó and Gabriella Sármay*
   **Cellular and Molecular Life Sciences.** Epub ahead: DOI 10.1007/s00018-010-0270-5
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