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Intestinal IgA Synthesis:
Localization and Requirements for IgA Class Switch Recombination

AKADEMISK AVHANDLING

Som för avläggande av medicine doktorsexamen vid Sahlgrenska akademin, Göteborgs Universitet, kommer att offentligen försvaras i hörsal Tor Bjurström, Medicinaregatan 3b, Göteborg

Torsdagen den 24 september 2009 klockan 13.00

av

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Avhandlingen baseras på följande delarbeten:

I. **Gut IgA Class Switch Recombination in the Absence of CD40 Does Not Occur in the Lamina Propria and Is Independent of Germinal Centers.**
Bergqvist, P., Gardby, E., Stensson, A., Bemark, M. & Lycke, N.Y.
J Immunol **177**, 7772-7783 (2006).

II. **T Cell-independent IgA Class Switch Recombination is Restricted to the GALT and Occurs Prior to Manifest Germinal Center Formation.**
Bergqvist, P., Stensson, A., Lycke, N.Y. & Bemark, M.
Submitted to J Immunol

III. **Germ Free Mice Express High IgA Class Switch Recombination Activity But Develop Few IgA Producing Plasma Cells.**
Bergqvist, P., Stensson, A., Bemark, M., & Lycke, N.Y.
Manuscript

IV. **The T-dependent specific gut anti-NP ((4-hydroxy-3-nitrophenyl)acetyl) IgA response is oligoclonal and is affinity matured in gut associated lymphoid tissue.**
Bergqvist, P., Bemark, M., Stensson, A., Holmberg, A. & Lycke, N.Y.
Manuscript

GÖTEBORGS UNIVERSITET

Intestinal IgA Synthesis:
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Abstract

Production of IgA at mucosal surfaces is one of the most striking features of the mucosal immune system. Despite that IgA was first discovered in the 1950's and secretory IgA described in gut secretions and breast milk in the mid 1960's we still have limited information about the sites and exact requirements for IgA class switch recombination. The aim of this thesis work was to investigate potential locations for induction of T-independent IgA responses using CD40 deficient mice as a model. Furthermore, as germ free mice have very poor IgA levels in the gut lamina propria (LP) we investigated whether this is because of a lack of IgA CSR at the inductive sites or whether the commensal flora is involved in maintaining IgA plasma cells at the effector site in the LP itself. Finally we used new ways of assessing the development of T-dependent IgA responses during oral immunizations using NP-hapten-conjugated cholera toxin as our oral immunogen.

$CD40^{-/-}$ mice have very low levels of serum IgG, are unable to form GC and as a consequence, cannot respond to TD antigens. However, we found that $CD40^{-/-}$ mice hosted near normal levels of IgA plasma cells in the gut LP, indicating that IgA CSR was intact and could occur in the absence of GC-formations and CD40-signalling. The ongoing controversy between researchers claiming evidence for two types of IgA CSR processes in the gut; one TD in the organized gut associated lymphoid system (GALT), and another pathway dependent on the commensal flora and ongoing in the non-organized LP itself, prompted us to investigate these theories in more detail using $CD40^{-/-}$ mice and molecular markers for IgA CSR. We found no evidence for IgA CSR in the gut LP and that IgA CSR was restricted to the GALT and the Peyer's patches (PP), in particular. In support of this notion, we observed clonally related Ig heavy chain variable sequences in widely separated segments of small intestinal biopsies, suggesting a common source rather than a disseminated process in the non-organized gut tissue. In addition, analyzing the $GL7^{int}$ cells for molecular markers of IgA CSR clearly showed that the cells could undergo IgA CSR despite not being derived from histologically detectable GCs. Therefore, we believe that the main pathway for CD40-independent IgA CSR is via the PPs, as in WT mice, and that the IgA CSR precedes the GC-stage where somatic hypermutations are introduced.

Furthermore, studies in germ free mice revealed that GCs were present and IgA CSR was ongoing in the PPs, despite the lack of commensal gut microflora. Therefore, we hypothesize that the effector site, the lamina propria, is deficient in supporting IgA responses.

Finally, we studied TD IgA responses at a molecular level during oral immunizations using NP-CT conjugates as antigen. We found that repeated oral immunization generated affinity matured and clonally selected IgA responses originating from the GALT. Three immunizations generated 15% antigen specific IgA plasma cells in the LP, distributed evenly thoughout the intestine.

In conclusion, we have provided evidence that TI IgA CSR occurs exclusively in the GALT prior to SHM in GCs. IgA CSR activity was never found in the non-organized LP, and peritoneal cavity B-cells do not significantly contribute to LP IgA plasma cells. Additionally, we show that the induction of IgA CSR is intact in GF mice, but subsequent IgA plasma cell development appears to be impaired, resulting in a 90% reduction in gut IgA plasma cells in the small and large intestine. Finally we show that TD IgA responses are efficiently generated in the GALT and that the responses early on undergo mutational selection events that result in high affinity IgA plasma cells seeding the gut LP.

Keywords: IgA, Intestine, Gut Associated Lymphoid Tissue, Class Switch Recombination, CD40, Germ Free
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