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Regulation of gut IgA induction by helper T cells

Akademisk avhandling

Som för avläggande av medicine doktorsexamen vid Sahlgrenska akademin, Göteborgs universitet kommer att offentligen försvaras i hörsal Arvid Carlsson, Academicum, Medicinaregatan 3

Torsdagen den 5 december 2019, klockan 9:00

av **Inta Gribonika**

Fakultetsopponent:
Professor Andrew Macpherson
University of Bern, Bern, Switzerland

Avhandlingen baseras på följande delarbeten

I. **Class-switch recombination to IgA in the Peyer's patches requires natural thymus-derived Tregs and appears to be antigen independent.** Gribonika I, Eliasson DG, Chandode RK, Schön K, Strömberg A, Bemark M, Lycke NY. Mucosal Immunol. 2019 Nov; 12 (6): 1268-1279.

II. **Oral cholera toxin adjuvant blocks pTreg-differentiation which allows for strong gut IgA responses**. Gribonika I, Eliasson DG, Schön K, Lycke NY. Manuscript

III. **Antigen-specific CD4 T cell responses in PP following oral immunizations with cholera toxin are dominated by Tfh cells and independent of Th17 cell differentiation**.Gribonika I, Strömberg A, Lebrero-Fernandez C, Moon J, Bemark M, Lycke NY. Manuscript

SAHLGRENSKA AKADEMIN
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Regulation of gut IgA induction by helper T cells

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The gut is the largest lymphoid organ in the body. Due to intense and constant exposure to the outside world, it also functions as the most important portal of entry for many pathogens. T cell-dependent secretory immunoglobulin A (IgA) prevents pathogens from spreading to systemic tissues and, hence, oral immunization represents the most effective route for vaccination against these pathogens. The detailed mechanism of oral vaccination-induced protective IgA immunity is not fully understood. The main aim of this thesis was to investigate the role of the gut CD4 T subsets for the induction of IgA responses. By using Ovalbumin-specific TCR-Tg CD4 T cells in an adoptive transfer system and mucosal immunization with or without cholera toxin (CT) adjuvant I show that IgA induction in the Peyer's patch (PP) is regulated in a distinct two-step process, where T follicular helper cells (TFH) and thymus-derived T regulatory cells (tTreg) orchestrate the IgA induction. Effective B cell help in the germinal center (GC) is maintained by antigen-specific TFH cells, while IgA class-switch recombination (CSR) is promoted by tTregs independently of the immunizing antigen.

It should be emphasized that the default response pathway activated by oral antigen administration is oral tolerance. In this doctoral thesis, I demonstrate that the suppressive pathway is regulated by IL-10. Thus, CD4 T cells upon exposer to cognate antigen in the presence of IL-10 differentiate into peripherally induced Tregs (pTreg). In the absence of IL-10 or after addition of CT adjuvant TFH differentiation is enhanced, resulting in a strong gut IgA response. CT has been reported to be the most potent oral adjuvant. Some reports suggest that CT preferentially exerts the adjuvant function via Th17 cells. The immuno-dominant part of CT is its B subunit, therefore, I used CTB-specific tetramer to monitor if CT induced T cell response is dominated by Th17 cells. Surprisingly, the CTB-specific T cell repertoire was nearly absent of Th17 lineage, however that did not prevent adjuvant's ability to induce a strong gut IgA response. Instead, CT induced CD4 T cells were overrepresented by TFH lineage that did not derive from Th17 cells as shown by using IL-17 fate reporter mice. These observations were confirmed using single-cell RNAseq technology. Gene signature of sorted CTB-specific CD4 T cells showed an almost complete dominance of the TFH phenotype with virtually no Th17 signature. Besides, the adoptive transfer of Th17 deficient CD4 T cells ($Rorc^{-/-}$) into nude host allowed for a robust gut IgA induction after oral immunization with CT. These findings argue strongly against the observations that upon CT immunization gut IgA B cell responses are driven by Th17 cells that exhibit great plasticity towards the TFH lineage. Interestingly, obtained data suggest that TFH cells in the PP do not share clonal relatedness with Th17, Th1 or Treg cells which have been a long-standing controversy in this field. Together, these findings provide a new paradigm for how gut IgA responses are regulated and which two types of CD4 T cell subsets are needed; tTregs for IgA CSR and TFH for GC formation and B cell maturation.

Keywords: Immunoglobulin A, oral immunization, helper T cells, Peyer's patch, ovalbumin, cholera toxin, interleukin 10, transforming growth factor β

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