

# Gut bacteria, regulatory T cells and allergic sensitization in early childhood

## Akademisk avhandling

som för avläggande av medicine doktorexamen vid Sahlgrenska Akademien vid Göteborgs universitet  
kommer att offentlig försvaras i föreläsningssalen, våning 3, Guldhedsgatan 10A, Göteborg

**Torsdagen den 30 januari 2014 kl 9:00**

av

**Hardis Rabe**

**Fakultetsopponent: Prof. Lucy S.K. Walker**

Institute of Immunity & Transplantation,

University College London

Avhandlingen baseras på följande arbeten:

- I. Hardis Rabe, Inger Nordström, Kerstin Andersson, Anna-Carin Lundell and Anna Rudin  
***Staphylococcus aureus* convert neonatal conventional CD4<sup>+</sup> T cells into FOXP3<sup>+</sup>CD25<sup>+</sup>CD127<sup>low</sup> T cells via the PD-1/PD-L1 axis**  
*Immunology*<sup>1</sup>, 2013, in press.
- II. Hardis Rabe, Anna Strömbeck, Annicka Ljung, Anna-Carin Lundell, Kerstin Andersson, Agnes E. Wold, Ingegerd Adlerberth and Anna Rudin  
**The infantile gut bacterial colonization pattern is associated with higher cytokine responses but not to the proportions of putative regulatory T cells in childhood**  
*Manuscript*.
- III. Hardis Rabe, Anna-Carin Lundell, Kerstin Andersson, Ingegerd Adlerberth, Agnes E. Wold and Anna Rudin  
**Higher proportions of circulating FOXP3<sup>+</sup> and CTLA-4<sup>+</sup> regulatory T cells are associated with lower fractions of memory CD4<sup>+</sup> T cells in infants**  
*Journal of Leukocyte Biology*<sup>2</sup>, 2011; 90:1133-40
- IV. Anna Strömbeck, Hardis Rabe, Anna-Carin Lundell, Kerstin Andersson, Susanne Johansen, Ingegerd Adlerberth, Agnes E. Wold, Bill Hesselmar and Anna Rudin  
**High proportions of FOXP3<sup>+</sup>CD25<sup>high</sup> T cells in neonates are positively associated with allergic sensitization later in childhood**  
*Revised version resubmitted to Clinical and Experimental Allergy*

<sup>1</sup> Reprinted with permission from John Wiley & Sons, Inc

<sup>2</sup> Reprinted with permission from Journal of Leukocyte Biology

# Gut bacteria, regulatory T cells and allergic sensitization in early childhood

Hardis Rabe

Department of Rheumatology and Inflammation research, Institution of Medicine, Sahlgrenska Academy  
University of Gothenburg, Gothenburg, Sweden, 2014

The hygiene hypothesis postulates that reduced or altered microbial exposure early in life may lead to impaired immune maturation and, as consequence of this, development of allergic disorders. Thus, we examined if the infantile gut microbiota was related to the postnatal T cell development *in vivo* and if certain commensal gut bacteria were able to induce regulatory T cells (Tregs) *in vitro*. We also investigated if the proportion of Tregs was associated with allergic sensitization and allergic disease in the first 3 years of life.

We showed that the gut commensal *Staphylococcus aureus* (*S. aureus*) could convert neonatal CD4<sup>+</sup> T cells into FOXP3<sup>+</sup>CD25<sup>+</sup>CD127<sup>low</sup> Tregs *in vitro* and that certain culture conditions were required for this conversion. Depletion of pre-existing Tregs before stimulation with *S. aureus* resulted in activated CD25<sup>+</sup>CD127<sup>low</sup> T cells that increased proliferation of CD4<sup>+</sup> responder T cells. In contrast, naive CD4<sup>+</sup> T cells stimulated in the presence of pre-existing Tregs induced suppressive FOXP3<sup>+</sup>CD25<sup>+</sup>CD127<sup>low</sup> Tregs. Finally, blocking programmed cell death ligand-1 (PD-L1) expressed on antigen presenting cells during stimulation with *S. aureus*, reduced or completely inhibited the induction of FOXP3<sup>+</sup>CD25<sup>+</sup>CD127<sup>low</sup> T cells.

In the prospective FARMFLORA birth-cohort study, we found that children with an early gut microbiota including bifidobacteria and *Escherichia coli* (*E. coli*) had mononuclear cells with higher capacity to produce proinflammatory and Th2-related cytokines in response to phytohaemagglutinin (PHA) than children not colonized by these bacteria. In contrast, early colonization by *S. aureus* and enterococci was inversely related with the PHA-induced cytokine responses. The early bacterial gut colonization pattern was not associated with the proportion of putative FOXP3<sup>+</sup>CD25<sup>high</sup> Tregs within the circulating CD4<sup>+</sup> T cell population during early childhood. However, high proportions of FOXP3<sup>+</sup>CD25<sup>high</sup> cells of the CD4<sup>+</sup> T cell population in early infancy were inversely related to the capacity of mononuclear cells to produce cytokines in response to PHA as well as to the proportions of CD45RO<sup>+</sup> of CD4<sup>+</sup> T cells later in childhood. Moreover, children who were sensitized at 18 and 36 months of age had higher proportions of putative FOXP3<sup>+</sup>CD25<sup>high</sup> Tregs at birth and 3 days of life than children who remained non-sensitized. Allergic disease, on the other hand was not associated with the proportion of putative FOXP3<sup>+</sup>CD25<sup>high</sup> Tregs.

In conclusion, these results indicate that *S. aureus* has an ability to convert naive neonatal CD4<sup>+</sup> T cells into FOXP3<sup>+</sup>CD25<sup>+</sup>CD127<sup>low</sup> regulatory T cells *in vitro*, a process which is dependent on the presence of both thymic derived Tregs and of APCs that express PD-L1. However, the early bacterial gut colonization pattern was not related to the proportion of putative FOXP3<sup>+</sup>CD25<sup>high</sup> Tregs within the circulating CD4<sup>+</sup> T cell population in children during the first 3 years of life. Furthermore, as infants who were sensitized had higher proportion of FOXP3<sup>+</sup>CD25<sup>high</sup> within the CD4<sup>+</sup> T cell population early in life compared to healthy children, higher proportions of Tregs early in life do not seem to be protective against atopic disorders. Thus, it is possible that high proportions of putative FOXP3<sup>+</sup>CD25<sup>high</sup> Tregs within the CD4<sup>+</sup> T cell population early in infancy may modulate the effector T cell development in a way that could predispose to allergic sensitization. However, early gut colonization with a gut microbiota including bifidobacteria and *E. coli* might instead enhance the effector T cell development.

**Keywords:** Regulatory T cells, T cell development, bacterial colonization of the gut, allergy, allergic sensitization, cohort study, children

ISBN 978-91-628-8876-3