Interplay between Pathogenic and Immune Regulatory Mechanisms in Gαi2 deficient colitis

Akademisk avhandling

Som för avläggande av medicine doktorsexamen vid Sahlgrenska akademin vid Göteborgs Universitet kommer att offentligen försvaras i hörsal Ragnar Sandberg, Medicinaregatan 7A, Göteborg.

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av

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Avhandlingen baseras på följande delarbeten:

I. **Interplay between Th1 and Th17 effector T cell pathways in the pathogenesis of spontaneous colitis in the Gαi2-deficient mouse.**
Yu-Yuan Götlind*, Maria Fritsch Fredin*, Ashok Kumar Kumawat, Rogen Willén, Hilja Strid, Ignacio Rangel, Paul W. Bland, Elisabeth H. Hörnquist
*Submitted manuscript, * both authors contributed equally*

II. **$CD4^+FoxP3^+$ regulatory T Cells from $G\alpha i2^{-/-}$ mice are functionally active in vitro, but do not prevent colitis.**
Yu-Yuan C. Götlind, Sukanya Raghavan, Paul W. Bland, Elisabeth H. Hörnquist
PLoS One. 2011, *6:*e25073.

III. **The application and relevance of ex vivo culture systems for assessment of IBD treatment in murine models of colitis**.
Fritsch Fredin M, Vidal A, Utkovic H, Götlind Yu-Yuan, Willén R, Jansson L, Hultgren Hörnquist E, Melgar S.
Pharmacol Res. 2008, *58:*222-231.

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ABSTRACT

The two major forms of inflammatory bowel disease (IBD), Crohn's disease (CD) and ulcerative colitis (UC) are gastrointestinal disorders characterized by chronic and relapsing inflammation. Mice deficient in the inhibitory G protein subunit Gαi2 spontaneously develop chronic colitis and have been used as a model for IBD, with particular similarities to UC. They have been used in this thesis to study the changes in immune pathology during disease progression; to investigate the role of regulatory T cells (Tregs) in chronic intestinal inflammation; and as a model for testing anti-inflammatory agents.

During the progression of the colitis, continuing increases in colon weight/cm and spleen weight, and a gradual decrease in thymus weight were observed. The proteins levels of proinflammatory cytokines/chemokines IL-1β, IL-6, IL-12p40, IL-17, TNF-α, CCL2 and CXCL1 increased during colitis progression and were all significantly increased in mice with moderate and severe colitis in colonic, but not small intestinal tissues. In colon, IFN-γ mRNA and IL-27 mRNA were gradually elevated during the course of the colitis, whereas IL-21 mRNA expression was significantly enhanced in mice with severe colitis. Thus, the lack of Gαi2 elicits an expansion of gut Th17 responses, possibly as a result of a $G\alpha i2^{-/-}$-driven epithelial barrier defect; this drives the production of neutrophil-attractant chemokines, resulting in the influx of neutrophils; in turn promoting an adaptive Th1 response.

Numbers of lamina propria $CD4^{+}FoxP3^{+}$ cells were significantly increased in colitis and were dispersed in the tissue, in contrast to non-inflamed colon in which they concentrated within organized lymphoid structures. *In vitro* data showed that both $G\alpha i2^{-/-}$ and wild-type (WT) splenic Tregs were able to suppress wild type (WT) effector T cells (Teffs) , but that pathogenetic $G\alpha i2^{-/-}$ Teffs could not be suppressed by either type of Tregs. Adoptive transfer experiments in the colitis model showed that neither type of Tregs could prevent disease induced by co-transfer of $G\alpha i2^{-/-}$ Teffs. It is possible that Treg regulatory function is suppressed in the inflamed colonic milieu, and/or that they are unable to overcome the heightened activity of $G\alpha i2^{-/-}$ Teffs.

Acute colitis induced by dextran sodium sulphate (DSS) and spontaneous $G\alpha i2^{-/-}$ chronic colitis were used to assess the efficacy of *ex vivo* anti-inflammatory treatment. The gene profiles reflected the different mechanisms underpinning these two types of colitis. Thus, genes related to pro-inflammatory innate cytokines, chemokines and chemokine receptors were up-regulated in DSS-induced colitis, whereas genes related to T cell markers were preferentially elevated in $G\alpha i2^{-/-}$ colitic tissues. In general, the same panel of genes displayed increased transcription in the *in vivo* and *ex vivo* cultured tissues in DSS model. The well-characterised corticosteroid methyl-prednisolone and the proteasome inhibitor MG132, were used to compare the efficacy between *in vivo* treatment and *ex vivo* cultures of colon obtained from DSS-induced and $G\alpha i2^{-/-}$ colitic mice. After steroid treatment, IL-1β, IL-6 and iNOS were suppressed in both models, both in *in vivo* and *ex vivo*. The anti-inflammatory function of methyl-prednisolone was mostly at the innate level, as shown in DSS-colitis, but MG132 acted effectively on the chronically activated adaptive immune response in the $G\alpha i2^{-/-}$ colitis model. Thus the changes in inflammatory gene expression in the murine *ex vivo* culture system reflected the *in vivo* response in the inflamed colonic tissue, suggesting that the murine culture system can be used for validation of future IBD therapies.

We conclude that the genetic Gαi2 deficiency leads to amplification of gut Th17 responses, possibly mediated by a gut barrier defect, and this leads to a mixed Th17/Th1 effector phenotype in late-stage colitis. The hyper-reactive $G\alpha i2^{-/-}$ effector T cells further amplify the inflammation and increased numbers of memory effector T cells are generated as disease progresses. The lack of appropriate regulation by Tregs further exacerbes the $G\alpha i2^{-/-}$ colitis.

Key words: IBD, $G\alpha i2^{-/-}$ colitis, Tregs, Teffs, Th17/Th1, pathogenesis/immunoregulation