Intracellular regulation of TLR signalling
Basic mechanisms and importance for intestinal inflammation

Akademisk avhandling

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

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av

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Avhandlingen baseras på följande delarbeten:

I. **Serum interleukin-1 receptor antagonist is an early indicator of colitis onset in Gαi2-deficient mice.**
OH Hultgren, M Berglund, M Bjursten, E Hultgren Hörnquist.
World J Gastroenterol. 2006 Jan 28;12(4):621-4.

II. **Toll-like Receptor Cross-hyporesponsiveness is Functional in Interleukin 1-receptor-associated Kinase-1 (IRAK-1)-deficient Macrophages: Differential Role Played by IRAK-1 in Regulation of Tumour Necrosis Factor and Interleukin-10 Production.**
M. Berglund, J. A. Thomas, E. H. Hörnquist, O. H. Hultgren.
Scandinavian J Immunol. 2008 May; 67(5):473-9.

III. **Gender dependent importance of IRAK-1 in dextran sulfate sodium induced colitis.**
Berglund M, Thomas JA, Fredin MF, Melgar S, Hörnquist EH, Hultgren OH.
Cell Immunol. 2009 May; 259(1):27-32.

IV. **IL-1 Receptor-associated Kinase M Downregulates DSS-induced Colitis.**
Berglund M, Melgar S, Kobayashi KS, Flavell RA, Hörnquist EH, Hultgren OH.
Inflamm Bowel Dis. 2010 Oct; 16(10):1778-86.

Abstract

Toll-like receptors (TLRs) recognize conserved structures on/in microorganisms. The intracellular signalling pathways of TLRs are shared with IL-1R and IL-18R and their activation leads to transcription of pro-inflammatory cytokines and type-I interferons. Signalling downstream of these receptors is strictly regulated via diverse mechanisms including downregulation of proteins important for signalling transduction and upregulation of proteins that negatively regulates signalling transduction. The intestinal lumen is populated with an enormous number of bacteria separated from the immune system with only a single layer of intestinal epithelial cells (IECs). Interestingly, IECs and immune cells in the lamina propria (LP) have a restricted expression of TLRs and an increased expression of negative regulators contributing to intestinal homeostasis. Mutations in several TLRs have been associated with inflammatory bowel disease (IBD) whereas less is known about the importance of intracellular signalling components. The aim with this thesis was to investigate the regulation of TLR signalling during homeostasis and intestinal inflammation.

First, we tried to identify serum markers for early detection of intestinal inflammation in Gαi2$^{-/-}$ mice that spontaneously develop intestinal inflammation 12-25 weeks after birth. Serum concentrations of IL-18 was upregulated in ongoing colitis whereas IL-1Ra was upregulated in ongoing and in early coilits. Furthermore, splenocytes from Gαi2$^{-/-}$ mice had increased production of pro-inflammatory cytokines in response to TLR stimulation and Gαi2$^{-/-}$ peritoneal macrophages had an intact TLR cross-tolerance.

To investigate the mechanisms involved in TLR signalling and cross-tolerance, IRAK-1$^{-/-}$ peritoneal macrophages were stimulated with LPS and/or LTA. IRAK-1$^{-/-}$ peritoneal macrophages had a reduced production of TNF and IL-10 in response to low concentrations of LTA whereas high concentrations of LPS resulted in decreased IL-10, but not TNF, production. Interestingly, increased concentration of LTA restored TNF production and reduced concentrations of LPS impaired TNF production from IRAK-1$^{-/-}$ peritoneal macrophages. With regard to TNF production, cross-tolerance was intact in IRAK-1$^{-/-}$ peritoneal macrophages after pre-stimulation with LPS followed by LTA stimulation whereas pre-stimulation with LTA followed by LPS stimulation induced a hyporesponsive trend. With regard to IL-10 production, cross-tolerance was not induced in IRAK-1$^{-/-}$ peritoneal macrophages after pre-stimulation with LPS followed by LTA stimulation whereas pre-stimulation with LTA followed by LPS stimulation, unexpectedly, resulted in increased IL-10 production.

Next, we investigated the importance of IRAK-1 for intestinal inflammation by treating IRAK-1$^{-/-}$ mice with dextran sulfate sodium (DSS). IRAK-1$^{-/-}$ mice had reduced body- and spleen weight at sacrifice. However, only male IRAK-1$^{-/-}$ mice were protected from intestinal inflammation as judged by colon inflammation score and thymic weights, indicating that the importance of IRAK-1 might be gender dependent.

IRAK-M is a negative regulator that inhibits IRAK-1 signalling transduction in response to TLR stimulation. DSS treatment of IRAK-M$^{-/-}$ mice resulted in increased intestinal inflammation and reduced body- and thymic weight. Furthermore, mRNA expression of pro-inflammatory cytokines was up-regulated in distal colon tissue and in plasma. These results suggest that IRAK-M has an important role in intestinal homeostasis.

In conclusion, results presented in this thesis highlight the delicate regulation of TLR/IL-1R signalling involved in homeostasis and intestinal inflammation. We identify IL-1Ra as a candidate serum marker for early detection of colitis in Gαi2$^{-/-}$ mice, we demonstrate that IRAK-1 is of importance for TLR2 and TLR4 signalling regulation and that IRAK-1 and IRAK-M regulates the immune response during intestinal inflammation induced in mice.

Keywords: TLR/IL-1R signalling, Gαi2$^{-/-}$ mice, IRAK, peritoneal macrophages, cross-tolerance, dextran sulfate sodium, IBD

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