Regulatory CD4\(^+\)FOXP3\(^+\) T cells in *Helicobacter pylori*-induced disease

Akademisk Avhandling

som för avläggande av medicine doktorsexamen vid Sahlgrenska akademin kommer att försvaras offentligt i föreläsningsalen Ragnar Sandberg Medicinaregatan 7, Göteborgs Universitet, Göteborg

Tisdagen den 26 maj, 2009 klockan 13.00

av

Bert Kindlund

Fakultetsopponent Docent Vivianne Malmström, Karolinska Institutet, Enheten för Reumatologi, Institutionen för Medicin, Stockholm

Avhandlingen baseras på följande delarbeten


FOXP3-expressing CD4\(^+\) T-cell numbers increase in areas of duodenal gastric metaplasia and are associated to CD4\(^+\) T-cell aggregates in the duodenum of *Helicobacter pylori*-infected duodenal ulcer patients. *Helicobacter, In press*

II. Karin Enarsson, Anna Lundgren, Bert Kindlund, Mikael Herrmansson, Giovanna Roncador, Alison H Banham, B. Samuel Lundin and Marianne Quiding-Järbrink

Function and recruitment of mucosal regulatory T cells in human chronic *Helicobacter pylori* infection and gastric adenocarcinoma. *Clin Immunol* 2006 Dec; 121 (3): 358-68

III. B. Samuel Lundin, Karin Enarsson, Bert Kindlund, Anna Lundgren, Erik Johnsson, Marianne Quiding-Järbrink and Ann-Mari Svennerholm

The local and systemic T-cell response to *Helicobacter pylori* in gastric cancer patients is characterised by production of interleukin-10. *Clin Immunol* 2007 Nov; 125 (2) 205-13

IV. Bert Kindlund, Åsa Sjöling, Jenni Adamsson, Anders Janzon, Lars-Erik Hansson, Mikael Herrmansson and B. Samuel Lundin

Increased proliferation of CD4\(^+\)FOXP3\(^+\) T cells in gastric cancer mucosa contribute to higher local numbers of regulatory T cells. *Submitted*
**ABSTRACT**

*Helicobacter pylori* colonize the gastric or duodenal mucosa of approximately half of the world’s population. Although most individuals are asymptomatic, *H. pylori* infection cause peptic ulcers or gastric cancer in 10-15 % and 1-2 %, respectively, of the infected individuals. It has previously been suggested by us and others that the life-long infection caused by *H. pylori* may be due to dysregulation of the immune response and that the host often fails to eradicate the infection due to the presence of regulatory T cells that down-regulate the proper immune response. In this thesis we have further analyzed the suppressive capacity, frequency, location and proliferation of regulatory T cells both locally at the site of infection and systemically in the blood in individuals with duodenal ulcer and gastric cancer. We could show by immunohistochemistry that FOXP3-expressing CD4+ T-cell numbers increase in areas of duodenal gastric metaplasia compared to normal duodenal mucosa and are associated to CD4+ T-cell aggregates in the duodenum of *Helicobacter pylori*-infected duodenal ulcer patients. The increase of FOXP3-expressing T cells in the antrum of infected individuals was dependent on the presence of *Helicobacter pylori*, since eradication therapy resulted in 4-fold lower levels of FOXP3 and IL-10 mRNA in the antrum. Higher numbers of CD4+FOXP3+ T cells were found in duodenal ulcer patients than in asymptomatic *H. pylori* infected individuals. These results show that CD4+FOXP3+ expressing T cells are increased at the site of infection and decrease when the bacteria are eradicated.

When analysing patients with gastric cancer we found increased numbers of CD4+FOXP3+ T cells in the tumor compared to tumor-free gastric mucosa. We could determine that gastric CD4+CD25high T cells expressed FOXP3 and were able to suppress *H. pylori*-induced T-cell proliferation and IFN-γ production. These regulatory T cells (Treg) expressed increased levels of the homing receptor L-selectin and CCR4, which indicated that the increased levels of CD4+FOXP3+ Tregs in tumors are due to active recruitment to the tumor mucosa.

Suppressive activities of Tregs have been proposed to be exerted by the anti-inflammatory cytokines IL-10 or TGF-β. To evaluate the immune response in individuals that developed gastric cancer compared to asymptomatic individuals we stimulated T cells from both peripheral blood and gastric mucosa of *H. pylori*-infected gastric cancer patients with *H. pylori* antigens. All T cells from gastric cancer patients produced high amounts of IL-10, while the IL-10 production from blood T cells of *H. pylori*-infected asymptomatic subjects was low. Furthermore, the mRNA levels of IL-10 were increased in the gastric mucosa of GC patients and the frequency of activated CD8+ T cells was markedly reduced compared to asymptomatic individuals.

Finally, we analysed CD4+FOXP3+ T cells from gastric cancer patients by flow cytometry. We found that CD4+FOXP3+ T cells from gastric cancer patients have significantly higher levels of proliferation than CD4+FOXP3+ cells within the tumor, and that CD4+FOXP3+ cells within tumors proliferate significantly more than CD4+FOXP3+ cells in tumor free mucosa and in blood. When CD4+ T cells were isolated directly from the tumor and tumor free mucosa and sorted into CD25high and CD25low populations we found that CD4+CD25low T cells express higher transcription levels of both IFN-γ and TGF-β compared to CD4+CD25high T cells, but CD4+CD25high have a higher IL-10 / IFN-γ ratio, which indicates a suppressive function. Furthermore, the tumor mucosa had a higher expression of the pro-inflammatory cytokines IFN-γ and IL-8 compared to tumor-free mucosa.

In conclusion, we show increased numbers of CD4+FOXP3+ T cells both in the mucosa of duodenal ulcer patients and in gastric tumor tissue. This increase is associated to the presence of *H. pylori*, local inflammation or cancer, and is mediated by both increased recruitment into the mucosa and increased proliferation of resident FOXP3+ cells. Furthermore, the local CD4+FOXP3+ T cells are associated to IL-10 expression but lack of IFN-γ, while TGF-β is produced to a larger extent by other T cells. We believe that these findings contribute to increased understanding of the immunoregulatory processes related to *H. pylori*-induced diseases.

**Keywords:** Regulatory T cells, *Helicobacter pylori*, duodenal ulcer, gastric cancer, FOXP3

ISBN: 978-91-628-7748-4