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 föreläsningssalen Ragnar Sandberg Medicinaregatan 7, Göteborgs Universitet, Göteborg

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av

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Avhandlingen baseras på följande delarbeten

I. Bert Kindlund, Åsa Sjöling, Malin Hansson, Anders Edebo, Lars-Erik Hansson, Henrik Sjövall, Ann-Mari Svennerholm and B. Samuel Lundin

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 Hermansson, 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 Hermansson 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*

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ABSTRACT

Helicobacter pylori colonize the gastric or duodenal mucosa of approximately half of the worlds' 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 $CD4^+CD25^{high}$ 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 activites 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 $CD4^+FOXP3^-$ T 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 $CD25^{high}$ and $CD25^{low/-}$ populations we found that $CD4^+CD25^{low/-}$ T cells express higher transcription levels of both IFN-γ and TGF-β compared to $CD4^+CD25^{high}$ T cells, but $CD4^+CD25^{high}$ 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

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