

Recruitment of regulatory and conventional T cells to colon adenocarcinomas

Akademisk avhandling

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av

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Avhandlingen baseras på följande arbeten:

- I. Svensson H, Olofsson V, Lundin S, Yakkala C, Björck S, Börjesson L, Gustavsson B, Quiding-Järbrink M.
Accumulation of CCR4⁺CTLA-4⁺FOXP3⁺CD25^{hi} regulatory T cells in colon adenocarcinomas correlate to reduced activation of conventional T cells. *PLoS One.* 2012;7(2):e30695.
- II. Langenes V, Svensson H, Börjesson L, Gustavsson B, Bemark M, Sjöling Å, Quiding-Järbrink M. *First author*
Mucosal expression of the chemokine decoy receptor D6 is decreased in colon adenocarcinomas.
Submitted
- III. Sundström P, Stenstad H, Langenes V, Theander L, Gordon Ndah T, Fredin K, Börjesson L, Gustavsson B, Quiding-Järbrink M
Regulatory T cells from colon cancer patients express CD39 and inhibit transendothelial effector T cell migration by an adenosin-dependent mechanism.
In manuscript
- IV. Langenes V, Fasth P, von Mentzer A, Ragahavan S, Quiding-Järbrink M
Depletion of regulatory T cells promote infiltration of conventional T cells in gastrointestinal tumors in APC^{Min/+} mice.
In manuscript



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Recruitment of regulatory and conventional T cells to colon adenocarcinomas

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Colorectal cancer is one of the most common malignant diseases, with an annual incidence of over one million cases worldwide. Although survival depends strongly on tumor stage at diagnosis, lymphocyte infiltration has been clearly correlated to a favourable prognosis in several studies. The aim of this thesis was to determine the mechanisms for lymphocyte infiltration in colon adenocarcinomas, with emphasis on the effect of regulatory T (Treg) cells on the recruitment of conventional T cells.

First, we characterized the lymphocyte infiltrate in human colon adenocarcinomas compared to surrounding unaffected tissue. In tumors, we detected substantial accumulation of CD4⁺ FOXP3⁺CTLA4⁺CCR4⁺ CD39⁺ Tregs with potential to suppress anti-tumor immunity. Also, the frequencies of activated intratumoral, Th1 like T cells, important for anti-tumor immune responses, were decreased. The accumulation of CCR4⁺ Tregs may be due to increased production of the ligand CCL22 in the tumor. Furthermore, MAdCAM-1 expression, an adhesion molecule used by lymphocytes to migrate to the gut, was decreased in tumor tissue, potentially contributing to shaping the repertoire of tumor infiltrating lymphocytes. Since directed lymphocyte migration is controlled by chemokines and chemokine receptors, we decided to investigate alternative mechanisms for lymphocyte recruitment to tumors by examining the mRNA levels of chemokine decoy receptors D6, DARC and CCX-CKR. By using real time RT-PCR, we detected strongly decreased levels of the chemokine decoy receptor D6, with affinity for inflammatory chemokines, in human colon tumors compared to unaffected mucosa, whereas there was no change in expression of DARC and CCX-CKR.

Further, we observed that Treg isolated from colon cancer patients inhibited transendothelial migration of conventional T cells *in vitro*, while Tregs from healthy control subjects had no such effect. Also, we detected elevated levels of the adenosine-generating enzyme CD39 on circulating Tregs from cancer patients. Adenosine suppress lymphocyte functions and indeed, exogenous adenosine resulted in inhibition of conventional T cell migration in our system, while blocking of adenosine receptors restored the migration of T cells from cancer patients.

To directly assess the function of Tregs in colon cancer, we crossed APC^{Min/+} mice, a model of intestinal cancer, with DEREK mice that allow selective depletion of Tregs. The tumor tissue in these mice presents a similar distribution of T cells as human colon tumors, since there is decreased infiltration of activated T cells and accumulation of Tregs in intestinal tumor tissues. When depleting Tregs for 10 days, we detected improved CD4⁺ and CD8⁺ lymphocyte infiltration to tumors, indicating that accumulation of Treg impairs migration of conventional T cells into tumors.

Taken together, results from this thesis show differential lymphocyte composition in colon tumors compared to surrounding unaffected mucosa, possibly induced by accumulated Tregs and modulated chemokine decoy receptor and homing molecule expression in the local environment.

Keywords: colon adenocarcinoma, T cell, Regulatory T cell, chemokine receptor, D6, adhesion molecule, lymphocyte trafficking, APC^{Min}, DEREK,