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The frequency of T regulatory cells is increased in peripheral blood of patients with colon-rectal cancer

D. Saverino[1]*, C. Stabilini[2], R. Simone[1-3], E. Gianetta[2], F. Milintenda-Floriani[1]

[1] Department of Experimental Medicine, Section of Human Anatomy, University of Genova, Via De Toni 14, 16132 Genova, Italy
[2] Department of Specialist and Surgical Sciences, University of Genova, Largo R. Benzi 10,16132 Genova, Italy
[3] New address: Departments of Medicine and Cell Biology, North Shore University Hospital, 300 Community Dr., Manhasset, NY, USA
* daniele.saverino@unige.it

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Abstract

Immune system can recognise the tumor-associated antigens present in peripheral blood of cancer patients. Recent studies suggest that $CD4^+CD25^+$ regulatory T cells (Treg) might hamper effective immune-surveillance of cancer cells and impede effective immune responses to established tumours. The aim of this study is to demonstrate the presence of Treg in the peripheral blood of colon-rectal cancer (CRC) patients. Results indicate an increase frequency of Treg in the peripheral blood of CRC patients. In addition, Treg is able to regulate the *in vitro* ***proliferative response to CEA. Thus, these cells could regulate the immunosuppression and, eventually, the neoplastic progression in CRC patients.***

Introduction

Colorectal cancer (CRC) is the fourth most commonly diagnosed malignant disease worldwide and the second leading cause of death in Western countries. Interestingly, improved clinical outcome is associated with presence of tumor-infiltrating lymphocytes [1,2]. This suggests that anti-tumor immunoresponses can bearing on the growth of primary tumor, and controlling metastatic disease. In addition, regulatory T-cells are found in the tumor context and anti-tumor immunity could be enhanced by removing these cells [3]. In the present study, we verified the presence of $CD4^+$ $CD25^{high}$ Treg in peripheral blood of CRC patients. In addition, we tested the hypothesis that Treg developed in CRC patients are able to regulate anti-tumor specific immunoresponses.

Materials and methods

Patient selection

CRC patients were identified from multi-disciplinary team meetings with the first presentation of an adenocarcinoma and no reported distant metastases.

Flow cytometric analysis

Antibodies used were: CD4 (clone RPA-T4), CD25 (clone M-A251), CD45RO (clone UCHL1) and CTLA4 (clone BNI3) (BD Pharmingen) and FOXP3 (eBioscience).

In vitro immunosuppression assay

Purified PBMC were enriched for $CD4^+$ cells and then separated into $CD4^+CD25^{high}$ and $CD4^+CD25^-$ fractions using magnetic beads (Miltenyi). A proliferation assay was set up using $2x10^5$ $CD4^+CD25^-$ cells, activated by irradiated SW403 cell line (colon adenocarcinoma line expressing CEA) alone or in the presence of $CD4^+CD25^{high}$.

Statistical analysis

Statistical analysis was performed using GraphPad Prism (GraphPad Software Inc., CA, USA).

Results

Defining the human Treg population. Using the strict gating parameters reported [4], Treg were identified as $CD4^+$ cells with brigh CD25 staining (in Figure 1 results from two representative subjects). The CD25high population was further analyzed by expression of FOXP3 (data not shown).
CRC patients have increased numbers *of Treg in peripheral blood.* Figure 1 shows the frequencies of Treg in the PBMCs of CRC patients (n =12, age 51–70y) compared with healthy controls (n= 11, age 43–77y). The frequency of Treg was less than 4% of $CD4^+$ for CRC patients, and less than 2% for healthy donors. However, the mean frequency in

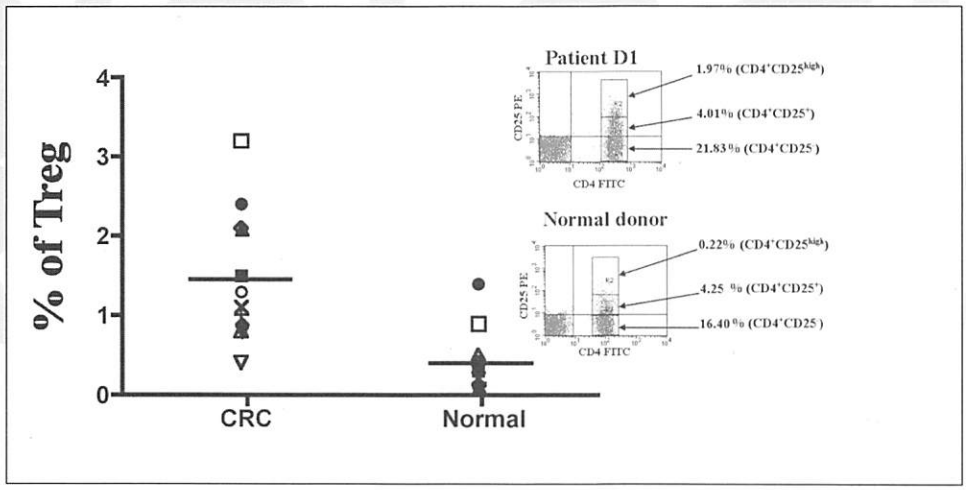

Figure 1. CRC patients have increased numbers of Treg cells. Freshly isolated PBMC from 12 CRC patients, and 11 healthy age-matched controls were stained with anti-CD4 and anti-CD25 mAb. The percentage of Treg was evaluated using the gating outlined in the right part of the figure. T cell populations were characterized as $CD4^+CD25^-$, $CD4^+CD25^+$ intermediate and $CD4^+CD25^{+high}$ as shown. $CD4^+CD25^{high}$ Treg were also positive for FOXP3 expression (data not shown). The differences between the two groups of patients were determined by non-parametric Mann-Whitney rank sum test ($p<0.01$).

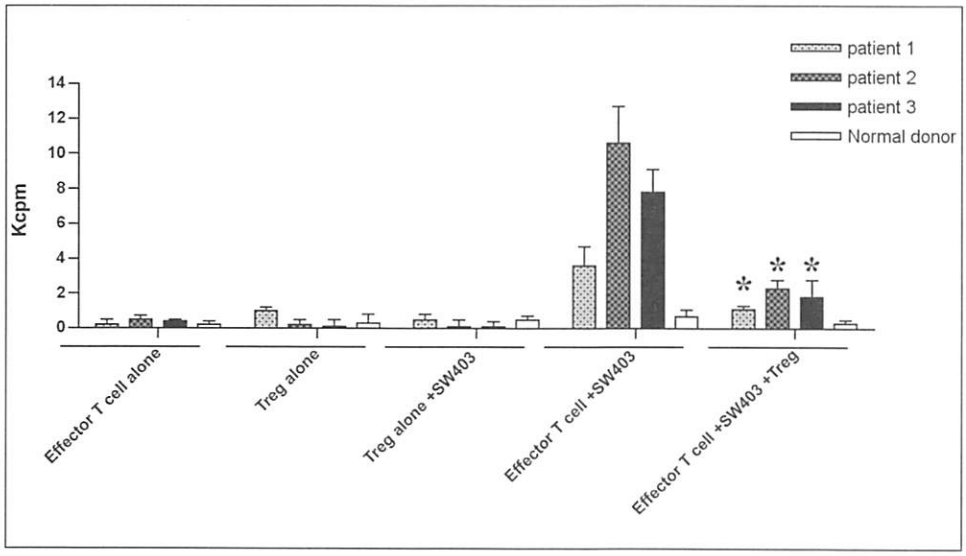

Figure 2. Treg isolated from the peripheral blood of CRC patients can suppress autologous $CD4^+CD25^-$ T cell proliferation. Freshly sorted Treg cells were able to suppress antigen-specific $CD4^+CD25^-$ T cells proliferation in response to CEA-expressing SW403 cell line. Data were analyzed with one-way ANOVA and samples significantly different are indicated with an asterisk. Of note, Treg were anergic.

CRC patients (1.43%) was significantly increased compared with the control groups (0.46%, $p<0.01$).

Treg from CRC patients can suppress autologous $CD4^+CD25^-$ T cell proliferation. To determine the suppressive capacity of Treg CRC, we sorted $CD4^+CD25^{hgh}$ T cells from CRC peripheral blood. From the same subjects a proliferative assay was performed. The adding SW403 CEA-expressing irradiated cells induced proliferation of $CD4^+CD25^-$ T cells (Figure 2). Of note, autologous Treg cells were able to suppress $CD4^+CD25^-$ T cell proliferation. Finally, Treg were anergic, contrary to $CD4^+CD25^-$ T cells.

Discussion

Immune cells infiltrating colorectal tumours appear to have a major role in tumour control, and their activity correlates with a good prognosis. However, tumours can become "invisible" or unreachable to cytotoxic effector cells. Three main mechanisms can be hypothesised underlying tumour immune escape: (1) expression of molecules such as B7 inducing T cell anergy and/or apoptosis; (2) production of immunosuppressant molecules (i.e. TGFβ, IL10, and VEGF),; and (3) presence of $CD4^+CD25^+$ regulatory T cells.

There is accumulating evidence that $CD4^+CD25^{high}$ Treg cells are recruited to human carcinomas and their abundance may predict for reduced survival [5-8]. Here, we present results from a study demonstrating that the frequency of $CD4^+CD25^{high}$ Treg cells is clearly increased in peripheral blood of CRC patients. These cells show suppressive functions, playing a role in modulation of effector T cell responses against colon tumors.
New therapeutic strategies aimed at inhibiting Tregs, such as the IL-2 diphtheria toxin conjugate recently shown to enhance vaccine-mediated immunity in renal cancer patients [9], may also improve tumor-specific immunotherapy in CRC patients.

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