Radiotherapy and Oncology 104 (2012) 131–138

Contents lists available at SciVerse ScienceDirect

Radiotherapy and Oncology

journal homepage: www.thegreenjournal.com

Circulating regulatory T cells

Circulating regulatory T cells of cancer patients receiving radiochemotherapy may be useful to individualize cancer treatment

Manuel A. Schmidt [a,1], Claudia Förtsch [a,1], Manfred Schmidt [a], Tilman T. Rau [b], Rainer Fietkau [a], Luitpold V. Distel [a,*]

[a] Department of Radiation Oncology, Friedrich-Alexander-University Erlangen-Nürnberg, Universitätsstraße 27, Erlangen, Germany; [b] Institute of Pathology, Friedrich-Alexander-University Erlangen-Nürnberg, Universitätsstr. 22, Erlangen, Germany

ARTICLE INFO

Article history:
Received 28 November 2011
Received in revised form 2 May 2012
Accepted 14 May 2012
Available online 9 June 2012

Keywords:
Regulatory T cells
Radiochemotherapy
Tumor stage
Colorectal cancer
Blood
Dendritic cells
Breast cancer

ABSTRACT

Background and purpose: Dendritic cells (DCs) and regulatory T cells (Treg) play a major role in anti-tumor immune response of cancer patients. We investigated the effect of radiochemotherapy on patients' blood immune cells and their predictive value for tumor response.

Materials and methods: DCs and Treg of colorectal cancer (CRC) or breast cancer (BC) patients were examined through multicolor flow cytometry before the beginning and after the first week of radiochemotherapy (RCT). DCs were stained for BDCA1 and BDCA2, Treg were stained for CD4, CD25, CD127 and FoxP3. IL-2, IL-10 and TNF-α plasma levels of CRC patients were also determined. We examined the interrelationship between immune cell count alterations, applied dose values, cytokine plasma levels as well as histopathological parameters.

Results: DCs were increased in BC and CRC patients compared to healthy control individuals (HC). CRC patients had higher levels of Treg (59.0%) compared to BC patients (31.3%) and HC (27.0%). Treg of CRC (58.7% vs. 41.3% $p < 0.001$) but not BC patients (31.3% vs. 38.8%, $p = 0.164$) decreased distinctly after the first week of radiation therapy. Applied dose values and decrease of Treg correlated positively ($r = 0.216$, $p = 0.054$). We also found a positive correlation of IL-10 plasma levels and Treg levels ($r = 0.748$, $p = 0.021$). CRC patients with favorable tumor stage (<ypT3a) have higher levels of Treg after 5 days of RCT (49.4% vs. 34.0%, $p = 0.043$).

Conclusion: Higher Treg levels are associated with favorable tumor stage. We hypothesize that a dramatic decrease of Treg after in vivo irradiation may be a good indicator for necessary dose adjustments in radiation therapy of CRC patients.

 Radiotherapy and Oncology 104 (2012) 131–138

Dendritic cells (DC) and regulatory T cells (Treg) play an important role in host anti-tumor immune response. DCs as professional antigen presenting cells (APCs) have successfully been used in cancer immunotherapy and recent data suggest that elevated levels of tumor infiltrating and circulating DCs in cancer patients are associated with favorable prognosis [1,2].

The existing data for Treg in cancer patients are rather controversial. High levels of Treg in peripheral blood of patients with hepatocellular carcinoma have been described to be associated with higher tumor stage and reduced survival [3]. However, Haas et al. showed that high numbers of FoxP3$^+$ lymphocytes infiltrating the peritumoral stroma are associated with better survival in gastric cancer patients [4]. Similar results were found in colorectal cancer patients [5,6]. In colorectal carcinoma and in various other types of cancer including medullary thyroid carcinoma, squamous cell carcinoma of the head and neck and transitional cell carcinoma, increased levels of circulating Treg can be detected [7–10].

Despite all these findings there are only few data on the impact of systemic anti-cancer therapy on peripheral immune cells [11,12]. This is of major interest, because investigation of the effects of RCT on peripheral immune cells, which are easy to acquire during RCT, may contribute to develop individualized cancer treatment strategies [13]. New methods have been developed to predict the tumor response to radio(-chemo)therapy and led to significant progress in this field [14,15]. An individualized cancer therapy for every patient is the ultimate ambition of the European Society of Therapeutic Radiology and Oncology [16]. In this prospective study, we wanted to further investigate the effects of radiochemotherapy on peripheral immune cells. Our aim was to evaluate the ability of therapeutic ionizing radiation to influence the amounts of DCs and Treg and to estimate their predictive value for the tumor response.

* Corresponding author.
E-mail address: Luitpold.Distel@uk-erlangen.de (L.V. Distel).
[1] These authors contributed equally to this work.

0167-8140/$ - see front matter © 2012 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.radonc.2012.05.003

Materials and methods

Study group

Our study group comprised a total of 110 individuals. We included 44 CRC patients who received neoadjuvant and 39 BC patients who received adjuvant radiochemotherapy. We also included 27 healthy age matched control individuals (HC). With this study design, it was possible to analyze data of cancer patients undergoing radiochemotherapy as well as data of patients who have no existing tumor mass but receive adjuvant RCT. Thus, we were also able to compare a wide range of applied doses during radiotherapy. Patients' blood samples were taken prior to and after 5 days of RCT and an interval of 3 days (Fig. 1A). For a possible later clinical usage this approach seems to be appropriate, because it leaves the possibility to adjust treatment plans.

Informed consent from all patients was obtained. Patients' characteristics are summarized in Table 1.

Immunostaining of blood cells

Blood was drawn into heparinized tubes and promptly processed. To identify DCs, 300 µl of whole blood was stained for CD1c (anti-BDCA1-PE) as a marker for myeloid DC and for CD303 (anti-BDCA2-FITC) as marker for plasmacytoid DC. Blocking reagent was added and samples were incubated at 4 °C for 10 min. To detect Treg, peripheral blood mononuclear cells (PBMC) were separated from 1 ml of whole blood using Ficoll hypaque density gradient centrifugation. Cells were resuspended in RPMI-1640. Freshly isolated PBMC were stained with surface markers anti-CD4-FITC, anti-CD25-APC and anti-CD127-PE-Cy7 at 4 °C for 10 min, followed by washing with a buffer containing PBS, 0.5% bovine serum albumin (BSA) and 2 mM EDTA. PBMC of 13 of our CRC patients were also stained with anti-CD8-V450 accordingly. For intracellular FoxP3 staining, cells were permeabilized using fix/perm buffer for 60 min at 4 °C. After washing twice, cells were blocked and then incubated with anti-FoxP3-PE at 4 °C for

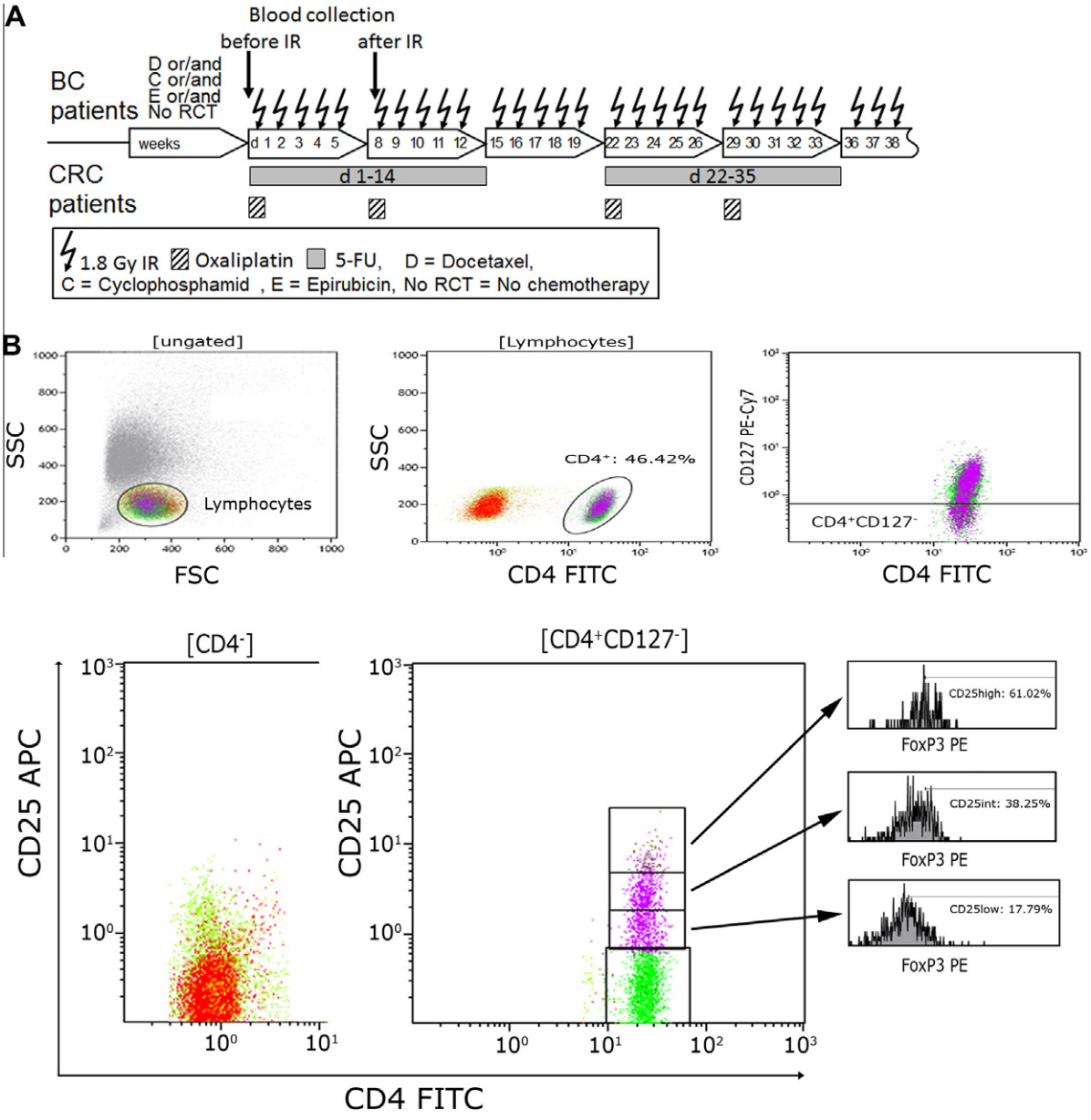

Fig. 1. (A) Appointed time of blood withdrawal during CRC and BC patients treatment. (B) Lymphocytes were gated according to their forward scatter (FSC) and side scatter (SSC) properties. $CD4^+CD127^-CD25^+$ cells were divided into $CD25^{high}$, $CD25^{int}$ and $CD25^{low}$ subgroups according to the CD25 staining pattern of $CD4^-$ cells. $CD25^{high}$ have brighter CD25 staining than $CD4^-CD25^+$ cells. FoxP3 expression is depicted in CD25 subgroups.