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European Association of Urology

Brief Correspondence

Immunoregulation of Dendritic Cell Subsets by Inhibitory Receptors in Urothelial Cancer

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Article info

Article history:
Accepted October 8, 2016

Associate Editor:
James Catto

Keywords:
Urothelial cancer
Inhibitory receptors
TIM-3
BTLA
Dendritic cells

Abstract

Blockade of inhibitory receptors (IRs) overexpressed by T cells can activate antitumor immune responses, resulting in the most promising therapeutic approaches, particularly in bladder cancer, currently able to extend patient survival. Thanks to their ability to cross-present antigens to T cells, dendritic cells (DCs) are an immune cell population that plays a central role in the generation of effective antitumor T-cell responses. While IR function and expression have been investigated in T cells, very few data are available for DCs. Therefore, we analyzed whether DCs express IRs that can decrease their functions. To this end, we investigated several IRs (PD-1, CTLA-4, BTLA, TIM-3, and CD160) in circulating $CD1c^+$ DCs, $CD141^+$ DCs, and plasmacytoid DCs from healthy donors and patients with urothelial cancer (UCa). Different DC subsets expressed BTLA and TIM-3 but not other IRs. More importantly, BTLA and TIM-3 were significantly upregulated in DCs from blood of UCa patients. Locally, bladder tumor–infiltrating DCs also overexpressed BTLA and TIM-3 compared to DCs from paired nontumoral tissue. Finally, in vitro functional experiments showed that ligand-mediated engagement of BTLA and TIM-3 receptors significantly reduced the secretion of effector cytokines by DC subpopulations. Our findings demonstrate that UCa induces local and systemic overexpression of BTLA and TIM-3 by DCs that may result in their functional inhibition, highlighting these receptors as potential targets for UCa treatment.

Patient summary: We investigated the expression and function of a panel of inhibitory receptors in dendritic cells (DCs), an immune cell subpopulation critical in initiation of protective immune responses, among patients with urothelial carcinoma. We found high expression of BTLA and TIM-3 by blood and tumor DCs, which could potentially mediate decreased DC function. The results suggest that BTLA and TIM-3 might be new targets for urothelial carcinoma treatment.

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Immune responses are tightly regulated by activatory and inhibitory receptors (IRs), also called immune checkpoints. Engagement of IRs on interaction with their cognate ligands leads to dimming of T-cell receptor signaling, resulting in a reduction in immune responses to antigens [1]. IR expression has been associated with T-cell exhaustion in autoimmune diseases, chronic infections, and cancers, and with T-cell impotence in tumor eradication [2]. Over the past few

http://dx.doi.org/10.1016/j.eururo.2016.10.009
0302-2838/

years, therapeutic use of humanized antibodies against IRs or their ligands has shown unprecedented clinical results in patients with solid tumors, particularly for muscle-invasive bladder cancer (MIBC) [1,3], demonstrating the great potential of such approaches and leading to a breakthrough therapy designation by the US Food and Drug Administration. Although IRs have been extensively studied in T-cell subpopulations, almost no data are available on IR expression and function by dendritic cell (DC) subsets in humans. DCs are key players in the initiation and regulation of immune responses. DCs are able to uptake, process, and present tumor antigens to other immune cells, so they are crucial in priming or activating antitumor T-cell responses that eventually lead to tumor-cell killing. Thus, DCs are prominent targets in cancer immunotherapy strategies [4]. Human circulating DCs can be broadly categorized into two groups: $CD11c^{neg}CD123^{+}$ plasmacytoid DCs (pDCs) and conventional $CD11c^{+}CD123^{neg}$ DCs (cDCs) [5]. Among cDCs, two subpopulations have been identified according to expression of CD1c (also known as BDCA-1) and CD141 (also known as BDCA-3 or thrombomodulin) [5]. $CD141^{+}$ DCs have prominent capacities to cross-present antigens after their uptake, and thus may play a major role in inducing antitumor immune responses [5,6]. We conducted the first analysis of the expression and function of several well-known IRs on DC subsets from healthy donors (HDs) and patients with urothelial cancer (UCa).

Expression of PD-1 (also known as CD279), CTLA-4 (CD152), BTLA (CD272), CD160 (BY55), and TIM-3 (CD366) was first assessed via flow cytometry of circulating DC subpopulations from HDs and UCa patients. pDC and conventional $CD1c^{+}$ and $CD141^{+}$ DCs were identified using a combination of phenotypic markers (Supplementary Fig. 1), and IR expression was determined. PD-1, CTLA-4, and CD160 were not expressed by any DC subtypes from HDs or UCa patients (data not shown). By contrast, BTLA was observed in all DC subsets, albeit at a very low level in $CD1c^{+}$ DCs, and TIM-3 was only expressed by $CD1c^{+}$ and $CD141^{+}$ DCs from HDs. Comparison to UCa patients showed that BTLA was significantly overexpressed by $CD141^{+}$ DCs and pDCs, whereas only a slight increase in TIM-3 expression was observed in $CD141^{+}$ DCs (Fig. 1A). This result suggests that the bladder tumor microenvironment may increase BTLA and TIM-3 expression by DCs. To gain more insight into BTLA and TIM-3 expression by DCs, we segregated the data for UCa patients into two groups according to disease stage (Supplementary Table 1): non-MIBC (NMIBC) and MIBC. Significantly higher TIM-3 expression was only found in $CD141^{+}$ DCs from MIBC patients compared to HDs, suggesting that UCa-mediated overexpression of TIM-3 is later than for BTLA, which was overexpressed in $CD141^{+}$ DCs and pDCs from patients in both groups (Supplementary Fig. 2).

Seeking further evidence that BTLA and TIM-3 expression may be altered by the bladder tumor microenvironment,

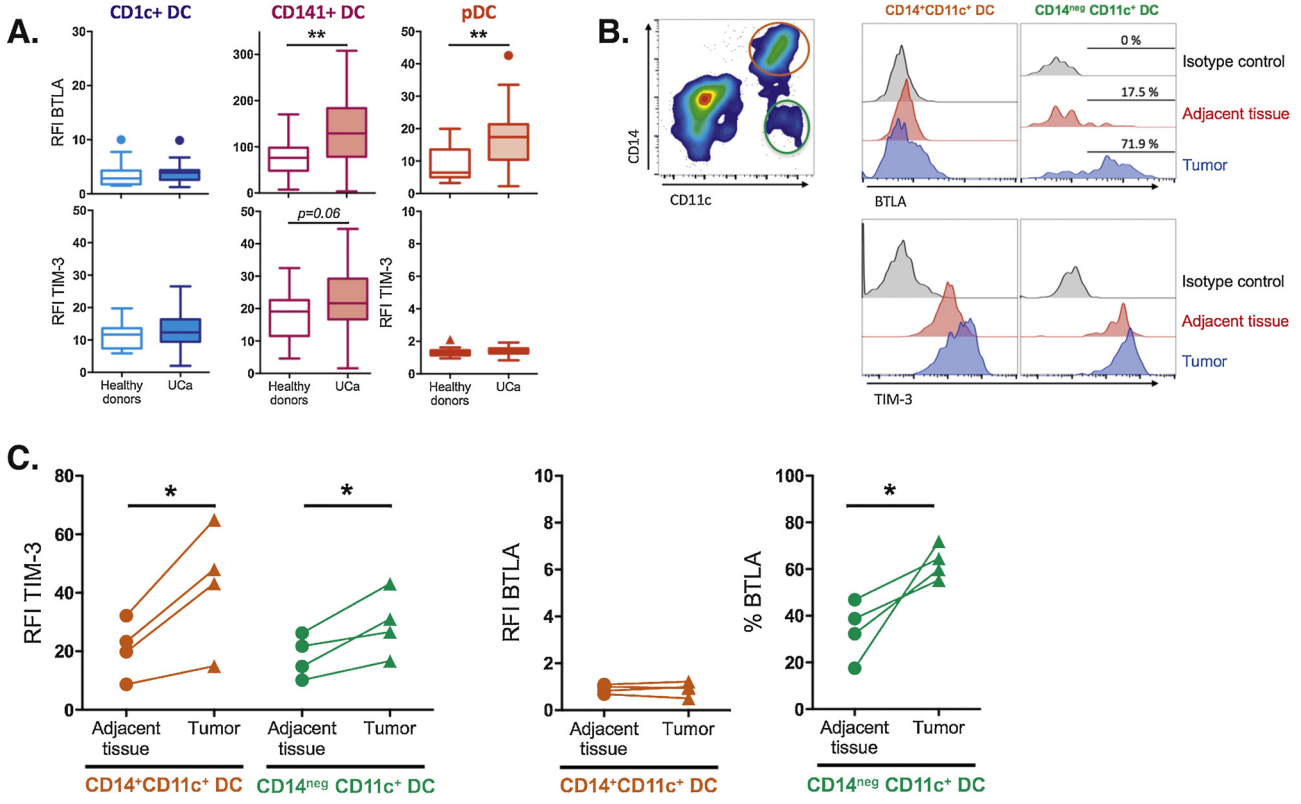

Fig. 1 – Overexpression of BTLA and TIM-3 in circulating and tumor-infiltrating dendritic cell (DC) subsets. (A) Comparison of BTLA and TIM-3 expression in $CD1c^{+}$ DCs, $CD141^{+}$ DCs, and $CD11c^{neg}CD123^{+}$ plasmacytoid DCs (pDCs) from peripheral blood mononuclear cells (PBMCs) from healthy donors ($n = 15$) and patients with urothelial carcinoma (UCa; $n = 40$). (B) Representative example of BTLA and TIM-3 labeling in $CD14^{+}CD11c^{+}$ and $CD14^{neg}CD11c^{+}$ DCs infiltrating the bladder from patients with muscle-invasive bladder cancer. (C) Quantification of BTLA and TIM-3 expressed by bladder-infiltrating DCs. * $p < 0.05$; ** $p < 0.01$. RFI = ratio of mean fluorescence intensity for specific staining versus isotype Ig control, except for BTLA in $CD14^{neg}CD11c^{+}$ DCs (expressed as a percentage of positive cells, since a bimodal population was observed).

Please cite this article in press as: Chevalier MF, et al. Immunoregulation of Dendritic Cell Subsets by Inhibitory Receptors in Urothelial Cancer. Eur Urol (2016), http://dx.doi.org/10.1016/j.eururo.2016.10.009