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Peripheral Circulating CD45RA^{-}FOXP3^{hi} T Regulatory (T_{Reg}) II Cells Provide a Window into the Activity of Intratumoral T_{Reg} Cells

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Abstract

The immune landscape of cancer determines its responsiveness to immunotherapy. Tumors infiltrated with $CD8^+$ T cells (immune-active tumors) are more likely to respond to immunomodulatory agents. However, immune activation often is counterbalanced by strong immunosuppressive mechanisms that are necessary to maintain homeostasis but consequentially can facilitate the survival of cancer cells in the immunocompetent host, a concept defined as compensatory immune suppression. T_{Reg} cells contribute to compensatory immune suppression, and therapies targeting the immunosuppressive T_{Reg} population are being actively explored. Wang *et al.* characterize a subset of peripheral circulating $CD45^{-}FOXP3^{hi}$ T_{Reg} II cells that phenotypically and functionally parallel the activity of their intratumoral counterparts. The findings are paradigm shifting and may provide a potential liquid002Dbased tool to evaluate the immunosuppressive activity of intratumoral T_{Reg} cells; they may also allow temporal assessment of whether the fine balance between immune rejection versus tolerance is achieved with various applied therapies.

Tumors infiltrated with $CD8^+$ T cells (immune-active tumors) are more likely to respond to immunotherapy than tumors where such infiltrates are absent (immune-desert tumors) or are limited to the margins of the cancer nests (immune-excluded). Immune activation, in turn, is evolutionarily dependent upon the coexistence of immune-suppressive mechanisms that inadvertently can facilitate the survival of an immunogenic entity in an immunocompetent host, a concept defined as compensatory immune resistance [1,2]. Wang *et al.* describe a subset of circulating $CD45^{-}FOXP3^{hi}$ T_{Reg} II cells that phenotypically and functionally parallel intratumoral T_{Reg} cells a finding that will have several important clinical implications [3]. (i) T_{Reg} II ($CD45RA^{-}FOXP3^{hi}$) cells are increased in the tumor microenvironment (TME) compared with the broader distribution of T_{Reg} cell subsets in the peripheral circulation. (ii) The T cell receptor (TCR) repertoire of intratumoral T_{Reg} overlaps with that of circulating T_{Reg} II T cells but not of other T_{Reg} subtypes. (iii) CD25 expression is higher in both the circulating and intratumoral T_{Reg} II cells, and may represent a principal mechanism of immune suppression by competing for and/or consuming interleukin (IL)-2. (iv) Localization of T_{Reg} II cells is mediated by the CCL1/CCR8 axis,

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although other chemokine receptors are selectively overexpressed, including CCR4, CCR5, and CXCR6, but not CCR2, CCR10, or CXCR3. (v) T_{Reg} II cells are more sensitive to stimulation with immunosuppressive cytokines such as transforming growth factor (TGF)-β or IL-10, and are less sensitive to immunostimulatory cytokines such as IL-6 and inter-feron (IFN)-γ. Sensitivity to these cytokines in pretreatment conditions is associated with worse relapse-free survival (RFS) in breast cancer patients. The predictive value is enhanced when parameters from the four cytokines are combined into a cytokine signaling index (CSI), whereas no correlation with RFS was noted when the CSI of other T_{Reg} subpopulations was tested. (vi) The percentage of intratumoral T_{Reg} II cells interacting with tumor-associated macrophages (TAMs) is higher among patients with worse RFS and was strongly associated with the CSI of circulating T_{Reg} II cells. Moreover, T_{Reg} II cell frequency correlates with a reduced prevalence of CD^+ T cells, suggesting that interactions between T_{Reg} II cells and TAMs cooperate in creating a strong immune-sup-pressive TME, perhaps by augmenting the production of CCL2. (vii) The ratio of CD8 T cells/T_{Reg} II cells is inversely correlated with the percentage of T_{Reg} II cells adjacent to TAMs. This last observation may represent a driving mechanism of immune suppression because low CD8/T_{Reg} ratios have previously been associated with poor clinical outcomes [4,5].

Several thought-provoking questions arise from this study. First, what is the role of the circulating tumor-derived T_{Reg} II cells? T_{Reg} II cells express CCR8 – does the chemotactic CCL1/CCR8 axis play an ongoing role in peripheral circulating T_{Reg} II cells? The authors [3] suggest that 'peripheral blood T_{Reg} II cells represent a major source of intratumoral T_{Reg} II cells'. Given that the TCR repertoire of circulating T_{Reg} II cells parallels that of intratumoral T_{Reg} cells, the origin of the T_{Reg} II cells could be through primary exposure to the cancer cells and/or to antigen-presenting cells (APCs) within the TME, and tumor-derived T_{Reg} II cells then enter the circulation after being 'educated' in the TME and/or tumor-involved draining lymph nodes. Given the continued expression of CCR8 by the circulating immunosuppressive T_{Reg} II cells, these specific T_{Reg} cells could then be recruited to cancer cells seeded at distant metastatic sites and may play an important role in propagating a systemic immune suppressive microenvironment (Figure 1). If this is the case, are the circulating T_{Reg} II cells a crucial immune cell population that should be targeted to reverse systemic immune suppression? Furthermore, the observation that the peripheral T_{Reg} II cells and intratumoral T_{Reg}s have shared TCR clonal populations brings forth another interesting consideration: can immunogenic tumor antigens expressed by the tumor be identified and tracked over time by characterization of the TCRs expressed on the peripheral circulating T_{Reg} II population? This would have significant implications for cellular and/or vaccine-based therapies without a need for invasive tumor biopsy.

The study also highlights the need to investigate the mechanism of T_{Reg} interactions with TAMs. Is there a spatial polarization of such interactions that could explain the phenomenon of immune exclusion, a frequent immune phenotype whose etiology remains largely elusive [6]. According to the immunologic constant of rejection (ICR) signature [7], CCR5 and CXCR3 and their respective chemokine ligands are coordinately expressed in immunogenic tumors and are also enriched with immune-suppressive compensatory mechanisms [1,2,8]. However, the selective expression of CCR8 and coex-pression of CCR5, but not CXCR3, by T_{Reg} II cells – and the reverse in $CD8^+$ T cells – suggests that these fundamental

chemoattractive vectors in the TME may play different roles by attracting distinct immune cell populations, respectively immune-suppressive T_{Reg} II cells through CCR5 versus immune-effector $CD8^+$ T cells and other immune-effector cells through CXCR3. This may result in different T_{Reg} II cells/$CD8^+$ T cell ratios in distinct patients experiencing various degrees of clinical response.

Wang *et al.* report their findings in the context of breast cancer patients, and it would be of interest to assess the applicability of their findings to other tumor types. It is increasingly appreciated that $CD4^+$ $FoxP3^{hi}$ T cells are prevalent in areas of tumor inflammation, often outnumbering $CD8^+$ T cells [9,10], suggesting that they may play a dominant role in immune suppression. In oral tongue squamous cell carcinoma (OTSCC) we observed a high prevalence of programmed cell death protein-1 (PD-L1)-expressing cancer and/or stromal cells, whereas programmed cell death (PD)-1 expression was observed in $CD4^+$ tumor-infiltrating lymphocytes in the presence of $PD\text{-}L1^+$ TAMs [9]. In OTSCC as well as in human papilloma virus-positive oropharyngeal carcinomas [11], congregates of PD1/PD-L1 expressing cells are observed at the periphery of the cancer nests in immune-excluded tumors [9]. This pattern is presumed to result from inductive expression of PD-L1 by cancer and/or stromal cells caused by IFN-γ secreted by T cells upon encounter with cancer cells at the tumor margins as a dynamic functional barrier to T cell infiltration. It can be hypothesized that the T_{Reg} II cells described by Wang *et al.* [3] may also explain observations in head and neck cancers [9,11]. Specifically, chemokine secretion in parallel with checkpoint upregulation in response to IFN-γ secretion by tumor antigen-specific T cells may result in the accumulation of different subtypes of immune cells in the TME, and that by targeting the T_{Reg} population one can overcome the immune-excluded phenotype. Studies on crosstalk between $CD8^+$ T cells and myeloid cells to assess immune responsiveness are actively being explored [12]; however, the work of Wang *et al.* brings forward the concept of whether T_{Reg} II cells and TAM crosstalk may be at the crux of changing the immune landscape. Finally, several common and distinctive markers have been suggested that connect or distinguish intra-tumoral and circulating T_{Reg} cells (Table 1). These will need to be validated by future studies in other tumor types.

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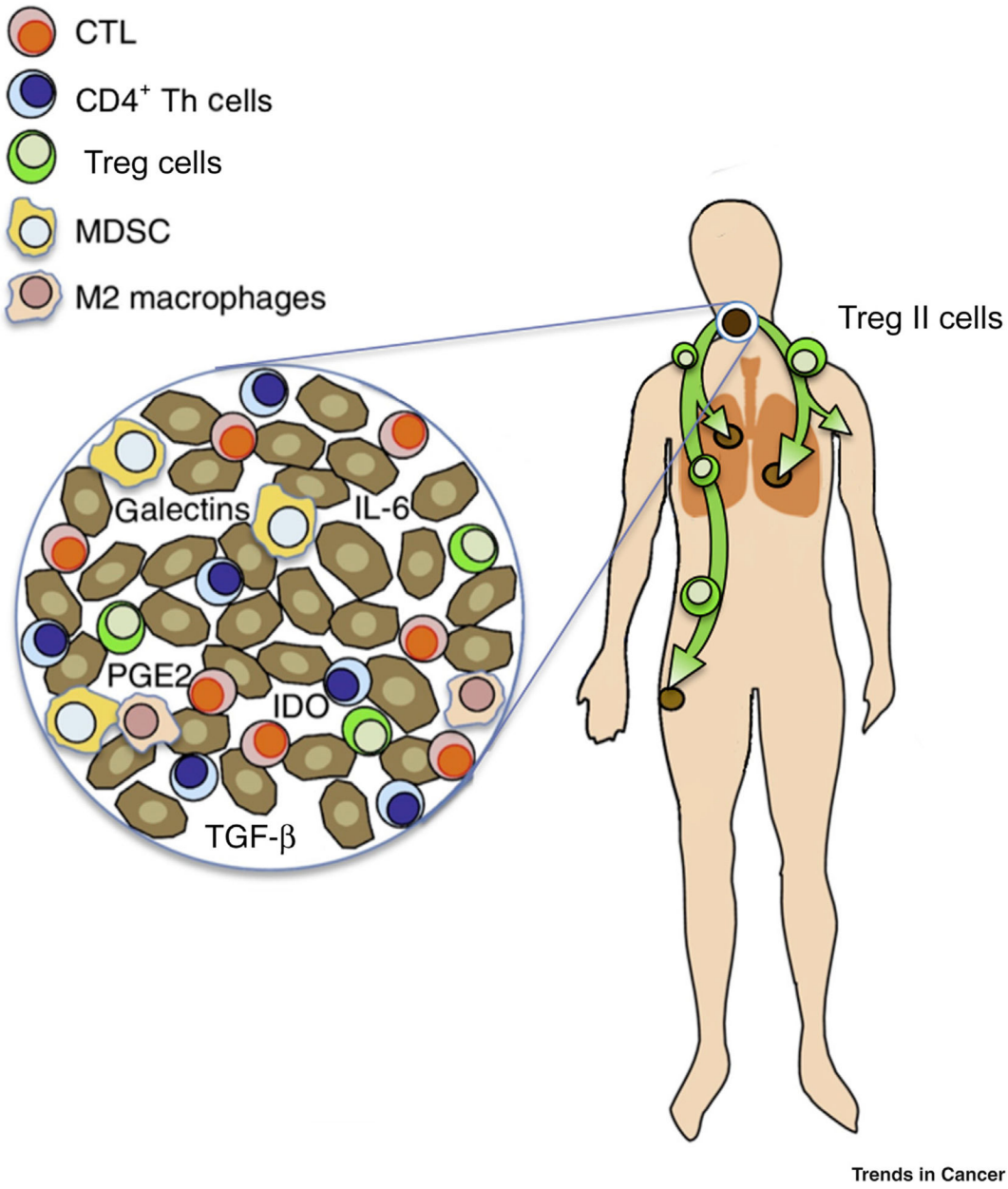

Figure 1. Schematic of Hypothesized Systemic Immunosuppressive Effects of T Regulatory (T_{Reg}) II Cells.

The T cell receptor (TCR) repertoire of circulating T_{Reg} II cells parallels that of intratumoral T_{Reg} cells. Thus, it is hypothesized that T_{Reg} II cells could be primed through primary exposure to the cancer cells and/or antigen-presenting cells (APCs) within the tumor microenvironment (TME), and tumor-derived T_{Reg} II cells then enter the circulation after being 'educated' in the TME and/or tumor-involved draining lymph nodes. Given the continued expression of CCR8 by the circulating immunosuppressive T_{Reg} II cells, these specific T_{Reg} cells could then be recruited to cancer cells seeded at distant metastatic sites and may play an important role in propagating a systemic immune-suppressive microenvironment. Abbreviations: CTL, cytotoxic T lymphocyte; IDO, indoleamine 2,3-

dioxygenase; IL-6, interleukin 6; MDSC, myeloid-derived suppressor cell; PGE2, prostaglandin E2; Th cell, T helper cell.

Table 1.

Comparative Markers between Peripheral T_{Reg} II Cells and Intratumoral T_{Reg} Cells

Shared markers	
Increased expression	Decreased expression
CD25	LAG 3
CD39	CCR2
CTLA4	CCR10
TIGIT	CXCR3
ICOS	
OX40	
GITR	
HLA-DR	
Helios	
CCR8	
TCR clonal overlap	
Distinct marker expression by circulating T_{Reg} II cells	
IL-2RG	
pSTAT	
PD1	
TIM3	
CCR4	
CCR5	
CXCR6	