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Following up tumor-specific regulatory T cells in cancer patients

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Regulatory T cells (Treg) represent a subpopulation of immunosuppressive cells that preferentially expand during tumor progression. The primary role of Tregs is to dampen antitumor effector T-cell responses, but they also modulate inflammatory reactions and promote angiogenesis. Until now, the great majority of studies analyzed the total number of Tregs without focusing on their antigen specificity, due to the lack of available analytic tools. Philipp Beckhove's group has now reported a high frequency of endogenous Tregs directed against the self antigen mammaglobin (mam) in primary breast carcinoma patients.[1] The authors detected the presence of these cells by a functional assay based on the amplification of anti-mam effector T cells after Treg depletion. In addition, they confirmed their results by manufacturing specific tetramers loaded with MHC class II-restricted peptides derived from mammaglobin (mam_{34-48}). A mean frequency of 0.21% anti-mam Tregs was found in the peripheral blood of breast carcinoma patients. The use of HLA class II tetramers is still in an early stage due to paucity of reagents and tools to validate them. Beckhove and colleagues derived specific anti-mam $CD4^+$ T-cell clones to control the specificity of their mam-targeted HLA class II tetramers, significantly reinforcing the strength of the study.

Wang et al. pioneered the detection of tumor-specific Treg directed against the cancer/testis antigen 2 CTAG2 (an homolog of NY-ESO-1 best known as LAGE-1) and against peptides derived from BBX (best known as ARTC1) in tumor-bearing mice.[2] Next, pre-existing Tregs specific for a variety of antigens including differentiation (e.g., gp100, TRP2), cancer-testis (e.g., NY-ESO-1), overexpressed (e.g., CEA, EGFR, MUC1), universal (e.g., telomerase, surviving), and viral (e.g., HPV16-derived) antigens were detected in cancer patients. One striking feature of the Beckhove study is that mam-specific Tregs were detected in the peripheral blood of patients directly ex vivo. Indeed, in most studies, the identification of antitumor Tregs required an in vitro amplification step or the generation of T-cell clones.[3] Usually, specific Tregs are identified within tumor-infiltrating lymphocytes, as they are highly enriched in this compartment as compared with the peripheral blood. Indeed, although an increased frequency of circulating Tregs has been observed in cancer patients, reaching 5–10% of blood $CD4^+$ T cells, Tregs can account for 40–50% of $CD4^+$ T cells infiltrating some human and murine tumors.[3] This said, as specific Tregs have been observed both in peripheral blood and within neoplastic lesions, these cells may interfere with antitumor immune responses at both the induction and effector levels.[4] The Beckhove study clearly demonstrated that the levels of mam-specific Tregs are higher in the blood of breast carcinoma patients than in healthy individuals, as previously observed for Tregs targeting other tumor-associated antigens. It would have been of interest to complete this study by assessing the levels of mam-specific Tregs in the tumor microenvironment of these patients.

Various mechanisms may account for Treg deregulation in cancer patients and their accumulation within neoplastic lesions. The tumor microenvironment favors indeed the conversion of conventional T cells into Tregs, since the presentation of self tumor antigens prevails in the presence of transforming growth factor β (TGFβ), interleukin-10 (IL-10), and vascular endothelial growth factor (VEGF), most likely owing to immature dendritic cells (DCs) and myeloid-derived suppressor cells (MDSCs).[5,6] In addition, specific chemokines produced in the tumor microenvironment such as CCL17 and CCL22 preferentially recruit Tregs.[7,8] There is a debate as to whether intratumoral Tregs reflect the local amplification of natural Tregs or the in situ conversion of conventional T cells (Tconvs).[9,10] Both mechanisms have been reported to occur, and Beckhove et al. showed that the same tumor-associated antigen could be recognized by both Tconvs and Tregs. The study of the TCR repertoire of mam-specific T cells may have helped in distinguishing their precise origin.

It is clear that various subpopulations of Tregs endowed with various clinical significance co-exist in cancer patients.[11] For example, Tregs expressing activation markers such as CCR4 may exert more robust immunosuppressive functions and hence be more closely associated with prognosis than the general Treg population.[8] Although not performed in the study by Beckhove and colleagues, HLA class II tetramers will also allow for an

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extensive phenotyping of tumor-specific Tregs, hence informing strategies to inhibit their function.[12]

Various arguments support the need for rigorously monitoring specific Treg subsets (instead of the whole pool of Tregs) in cancer. First, Beckhove et al. have previously shown that the depletion of Treg efficiently enhanced tumor-associated antigen-specific Tconvs only when Tregs recognizing the same antigen pre-existed, supporting the antigen specificity of optimal Treg-mediated Tconv inhibition.[13] Second, in the course of anticancer vaccination, monitoring specific Treg subsets is highly recommended, as in both mice and humans these vaccines could increase specific Tregs and not only effector cells, an issue that may explain some recent clinical failures.[3] Third, some anticancer vaccines do not modify the pool of Tregs, but decrease the ratio between antigen-specific Tregs and Tconvs, favoring T_H1 immune responses.[14] The importance of Tregs in the clinics has been shown for the first time in a randomized clinical trial testing an anticancer vaccine in renal cancer patients.[15] In this study, a single dose of cyclophosphamide affected predominantly proliferative Tregs, possibly those specific for tumor-associated antigens, and only prolonged the survival of subjects exhibiting vaccine-elicited immune responses.

It remains unclear whether therapeutic avenues to boosting Tconvs (e.g., the blockade of immunological checkpoints, immunogenic chemotherapies) may modulate tumor-specific Tregs. The emergence of new tools to directly access specific Tregs ex vivo will allow for a fine monitoring of these cells in cancer patients before and after therapeutic interventions, and will therefore help the design of future clinical trial.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Schmidt H, Ge Y, Hartmann F, Conrad H, Klug F, Nittel S, et al. HLA class II tetramers reveal tissue-specific regulatory T cells that suppress T-cell responses in breast carcinoma patients. OncoImmunology 2013; In press; http://dx.doi.org/10.4161/onci.24962
2. Wang HY, Wang RF. Regulatory T cells and cancer. Curr Opin Immunol 2007; 19:217-23; PMID:17306521; http://dx.doi.org/10.1016/j.coi.2007.02.004
3. Tanchot C, Terme M, Pere H, Tran T, Benhamouda N, Strioga M, et al. Tumor-Infiltrating Regulatory T Cells: Phenotype, Role, Mechanism of Expansion In Situ and Clinical Significance. Cancer Microenviron 2012; PMID:23104434; http://dx.doi.org/10.1007/s12307-012-0122-y
4. van der Burg SH, Piersma SJ, de Jong A, van der Hulst JM, Kwappenberg KM, van den Hende M, et al. Association of cervical cancer with the presence of CD4+ regulatory T cells specific for human papillomavirus antigens. Proc Natl Acad Sci U S A 2007; 104:12087-92; PMID:17615234; http://dx.doi.org/10.1073/pnas.0704672104
5. Ghiringhelli F, Puig PE, Roux S, Parcellier A, Schmitt E, Solary E, et al. Tumor cells convert immature myeloid dendritic cells into TGF-beta-secreting cells inducing CD4+CD25+ regulatory T cell proliferation. J Exp Med 2005; 202:919-29; PMID:16186184; http://dx.doi.org/10.1084/jem.20050463
6. Terme M, Pernot S, Marcheteau E, Sandoval F, Benhamouda N, Colussi O, et al. VEGFA-VEGFR pathway blockade inhibits tumor-induced regulatory T-cell proliferation in colorectal cancer. Cancer Res 2013; 73:539-49; PMID:23108136; http://dx.doi.org/10.1158/0008-5472.CAN-12-2325
7. Ménétrier-Caux C, Gobert M, Caux C. Differences in tumor regulatory T-cell localization and activation status impact patient outcome. Cancer Res 2009; 69:7895-8; PMID:19808962; http://dx.doi.org/10.1158/0008-5472.CAN-09-1642
8. Pere H, Montier Y, Bayry J, Quintin-Colonna F, Merillon N, Dransart E, et al. A CCR4 antagonist combined with vaccines induces antigen-specific CD8+ T cells and tumor immunity against self antigens. Blood 2011; 118:4853-62; PMID:21908423; http://dx.doi.org/10.1182/blood-2011-01-329656
9. Schreiber TH, Wolf D, Bodero M, Podack E. Tumor antigen specific iTreg accumulate in the tumor microenvironment and suppress therapeutic vaccination. Oncoimmunology 2012; 1:642-8; PMID:22934256; http://dx.doi.org/10.4161/onci.20298
10. Sainz-Perez A, Lim A, Lemercier B, Leclerc C. The T-cell receptor repertoire of tumor-infiltrating regulatory T lymphocytes is skewed toward public sequences. Cancer Res 2012; 72:3557-69; PMID:22573714; http://dx.doi.org/10.1158/0008-5472.CAN-12-0277
11. Mandapathil M, Szczepanski M, Harasymczuk M, Ren J, Cheng D, Jackson EK, et al. CD26 expression and adenosine deaminase activity in regulatory T cells (Treg) and CD4(+) T effector cells in patients with head and neck squamous cell carcinoma. Oncoimmunology 2012; 1:659-69; PMID:22934258; http://dx.doi.org/10.4161/onci.20387
12. Pere H, Tanchot C, Bayry J, Terme M, Taieb J, Badoual C, et al. Comprehensive analysis of current approaches to inhibit regulatory T cells in cancer. Oncoimmunology 2012; 1:326-33; PMID:22737608; http://dx.doi.org/10.4161/onci.18852
13. Bonertz A, Weitz J, Pietsch DH, Rahbari NN, Schlude C, Ge Y, et al. Antigen-specific Tregs control T cell responses against a limited repertoire of tumor antigens in patients with colorectal carcinoma. J Clin Invest 2009; 119:3311-21; PMID:19809157
14. Jandus C, Bioley G, Dojcinovic D, Derré L, Baitsch L, Wieckowski S, et al. Tumor antigen-specific FOXP3+ CD4 T cells identified in human metastatic melanoma: peptide vaccination results in selective expansion of Th1-like counterparts. Cancer Res 2009; 69:8085-93; PMID:19808957; http://dx.doi.org/10.1158/0008-5472.CAN-09-2226
15. Walter S, Weinschenk T, Stenzl A, Zdrojowy R, Pluzanska A, Szczylik C, et al. Multipeptide immune response to cancer vaccine IMA901 after single-dose cyclophosphamide associates with longer patient survival. Nat Med 2012; PMID:22842478; http://dx.doi.org/10.1038/nm.2883