Prognostic significance of regulatory T cells in tumor

Cailin Moira Wilke1,2, Ke Wu3, Ende Zhao2,3, Guobin Wang3 and Weiping Zou1,2

1 Graduate Program in Immunology, University of Michigan, Ann Arbor, MI
2 Department of Surgery, University of Michigan, Ann Arbor, MI
3 Department of Surgery, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

Since entering the immunological stage several decades ago, regulatory T cell biology has been realized as fundamentally important in the prevention of autoimmune conditions, induction of transplant tolerance and the immune response to cancer. The role of regulatory T cells in tumor immunobiology is still being elucidated. Currently, regulatory T cells are implicated in the dampening of antitumor T-cell responses both through direct and indirect means. A number of investigators have demonstrated that regulatory T cell density and location may serve as independent prognostic factors in several types of cancer and are alternately detrimental or beneficial to patient survival. In this article, we will review the characteristics and functional phenotype of classical regulatory T cells, describe their distribution and quantification in tumor-bearing hosts and summarize recent studies investigating the prognostic significance of regulatory T cell number and locality in various cancers.

Regulatory T (TReg) cells are a subpopulation of CD4+ T cells with suppressive functionality. In healthy individuals, perhaps the most important role of regulatory T cells is to maintain immune tolerance to self-antigens, which prevents development of autoimmune disease. TReg cells are also responsible for limiting tissue damage during ongoing and resolving immune responses, maintaining oral and fetomaternal tolerance and restraining asthma and allergy. In settings of organ transplant and cancer, the suppressive function of TReg cells is currently being manipulated to improve patient health and survival. Investigators of transplantation biology are exploring ways to increase the number of alloantigen-reactive regulatory T cells in transplant recipients to minimize grafted tissue damage and prevent organ rejection. In cancer patients, where regulatory T cells contribute to the dampening of the antitumor immune response, combination therapies that include the inhibition of regulatory T cell function have been explored. Although few Stage III trials of TReg inhibition have reached their clinical endpoints, analysis of TReg cells in tumor environments can still yield useful information about patient prognosis and tumor growth, and may eventually lead to new, more successful treatment regimes.

Definition
Regulatory T cells, originally termed suppressive T cells, were first described by Gershon et al.2,3 in the early 1970s as thymus-derived lymphocytes that tolerized bone marrow-derived lymphocytes to antigenic challenge. Research in the laboratory of R.J. North subsequently demonstrated that T cells expressing CD4 and CD25 from tumor-bearing mice abrogated tumor rejection; this suggested the existence of a tumor-suppressor T-cell population4-6. Many years later, after more than a decade of intense skepticism regarding the suppressive cells’ existence, Sakaguchi et al.7 ascertained that the interleukin-2 (IL-2) receptor α-chain (also called CD25) could be used to identify them.7 Later studies in the same laboratory, as well as studies from Rudensky et al., established the transcription factor forkhead box P3 (FoxP3) as both a key intracellular marker of CD4+CD25+ regulatory T cells and necessary factor for development and proper function of these cells,8-10 which was described early on as prevention of autoimmune conditions (e.g., colitis11) and suppression of CD8+ T-cell homeostatic proliferation.12 Beginning with these reports, the field of regulatory T cells has expanded and progressed rapidly. In fact, several distinct regulatory T-cell populations have been proposed, including CD8+ subsets. These include thymically derived CD8+CD25+ T cells that utilize cytotoxic T-lymphocyte–associated antigen-4 (CTLA4) and transforming growth factor β (TGFβ) to suppress cell proliferation and activation,13 as well as a CD8+CD28− T-cell population from the periphery that targets immunoglobulin-like transcripts 3 (ILT3) and 4 (ILT4) on dendritic cells (DCs).14 Our group has identified CD8+ T cells15,16 in human ovarian cancer that secrete the suppressive cytokine interleukin-10 (IL-10). Interestingly, a CD8+ regulatory T cell population specific for heme oxygenase-1 has recently been identified.17 This population, isolated from the peripheral blood of cancer patients, inhibited proliferation, cytotoxicity and cytokine production of other cell immune cells. Groux et al. identified a FoxP3- CD4+ population (termed Treg1 cells), which may also suppress through IL-10 in vitro.18 Weiner characterized a CD4+TGFβ+...
population (T_{reg}) that exerts suppressive action in vivo through TGFβ. Both aforementioned populations are likely derived from the periphery. Classic regulatory T cells (T_{reg}), CD4+CD25+FoxP3+ T cells, differentiate in the thymus and migrate to the periphery. They constitutively express leukocyte common antigen isoform RO (CD45RO), glucocorticoid-induced tumor-necrosis factor receptor–related protein (GITR) and CTLA-4. Fascinatingly, research from Arne Akbar’s group has demonstrated that functional T_{reg} cells may be induced from memory CD4+ T cell populations found in inflamed skin. These antigen-specific CD4+ T cells were isolated, rendered anergic and then tested for suppressive capacity. Interestingly, in parallel with their newly acquired suppressive ability, FoxP3+ mRNA and protein expression in these cells increased profoundly, while CD25, GITR and CTLA-4 expression were all up-regulated. Finally, a recent paper from the laboratory of Shimon Sakaguchi presents the possibility of further categorizing naturally occurring T_{reg} cells into three subgroups: CD45RA+ FoxP3^{hi} resting T_{reg}, termed “iTreg” by the authors, CD45RA− FoxP3^{lo} activated Treg (aTreg) cells and cytokine-secreting CD45RA-FoxP3^{lo} nonsuppressive T cells. Ongoing investigations into phenotype, function and associations with disease states will likely contribute to knowledge of an even wider range of regulatory T cell populations in the future. Regardless, it is important to emphasize that regulatory T cells must be defined not only by phenotypic markers but also by their suppressive activity in vivo.

Distribution in Tumor-Bearing Hosts

In healthy mice and humans, T_{reg} cells are found primarily in the thymus, peripheral blood, lymph nodes and spleen. They constitute 5–10% of the resident CD4+ T cells in each of these organs. In bone marrow, however, T_{reg} cells account for a remarkable 25% of CD4+ T cells. Bone marrow is the preferential site of metastasis for some cancers (such as breast, lung and prostate), suggesting that the suppressive environment here is conducive to tumor growth. In tumors themselves, however, there are a number of ways that T_{reg} cells might accumulate: trafficking to the tumor under the influence of chemokine ligand 22 (CCL22), differentiation or expansion within the tumor stroma and conversion from normal T cells. Many tumors express tumor-associated antigens (TAAs), molecules found not only on tumor cells but also on certain populations of normal cells. The work of several groups has identified multiple mechanisms of suppression by TAA-specific T_{reg} cells. These include induction of IL-10, which can drastically suppress antigen-presenting cell (APC) and T cell function, induction of TGFβ, which may suppress natural killer (NK) cell function, competitive consumption of interleukin-2 (IL-2), which is a survival factor for conventional T cells, perforin- and granzyme-dependent killing of T cells and APCs, CTLA-4 induction of indolamine 2,3-dioxygenase (IDO)-expressing APCs, which suppress T cell activation and promote tolerance, and finally, induction of B7-H4 expression on APCs, which renders them immunosuppressive. Thus, T_{reg} cells target both T cells and APCs to create a generally tolerant tumor microenvironment.

Mouse tumors

Tumor-associated T_{reg} cells have been studied largely with reagents that target T_{reg} cells in tumor-bearing mice. Treatment with CD25-specific antibody (PC61) in vivo suppressed growth of several tumor types. These early studies demonstrated a correlation between reduced T_{reg} numbers and reduced tumor volume. Interestingly, depletion of total CD4+ T cells corroborated these data and lead to improved tumor immunity and rejection of tumors. Several groups confirmed these data with CD25-depletion alone or in concert with other treatments, such as anti-CTLA4 antibody, anti-B7H1 antibodies (Zou et al., unpublished observations), exogenous interferon-α (IFNα) or interleukin-12 (IL-12), adoptive transfer of DCs and irradiated tumor cells. Adoptive transfer of human or mouse T_{reg} cells into mice have also provided a direct functional connection between T_{reg} cell presence and reduced tumor immunity. One study examined B16 melanoma-bearing mice that received tumor-specific CD8+ T cells with either classic T_{reg} cells or with CD25− CD4+ T cells. Tumor immunity was abrogated in mice receiving classic T_{reg} cells, but not CD25− CD4+ T cells. These studies demonstrate that T_{reg} cells inhibit murine TAA-specific immunity.

Human tumors

June et al. observed increased numbers of T_{reg} cells in patients with nonsmall cell lung cancer and ovarian carcinoma when compared to healthy patients. Since this study in 2001, several other groups have made similar observations in the peripheral blood of patients with various types of cancer, including pancreatic and breast cancer, colorectal cancer (CRC), gastric and esophageal cancer, leukemia and lymphoma, melanoma, lung and ovarian cancer and hepatocellular carcinoma.

Quantification of Regulatory T Cells in Tumor

Regulatory T cell numbers may be evaluated in the tumor and tumor microenvironment in multiple ways. It is common for these cells to be identified on the basis of CD25 and/or FoxP3 expression. Quantification may be presented as a percentage of CD4+ T cells or total (CD3+) T cells, or as absolute number per area (such as mm²) in a given tissue, as in the recent study by Haas et al. Additionally, data may take the form of the ratio of regulatory T cells: effector T cells. Typically, tissue samples are investigated either by creating tissue microarrays and using immunohistochemistry or processing the tissue and examining cell populations via flow cytometry. Importantly, it is known that cell types other than T_{reg} cells express what were formerly thought of as unique T_{reg} markers: both CD25 and FoxP3 can be expressed on activated T cells. A recent publication has suggested that
分析 DNA 甲基化的 FoxP3+ 基因可能是一种更有力的方式来识别 Treg 细胞。具体来说，显而易见，狐精蛋白基因（TSDR，Treg 特异性甲基化区域）是去甲基化的。因为这种去甲基化在其他细胞类型中也存在——包括那些已知会表达 FoxP3，像 T 细胞，通过允许 Treg 细胞的 DNA 量化方法，如 PCR。在呈现 Treg 量化数据时，它很重要——考虑在上下文中的数字和位置。淋巴细胞与癌症的联系，如 T 帮助-17（Th17）细胞，肿瘤相关的巨噬细胞（TAMs），和其它 APCs，尤其是 DCs。

**Association with Pathological and Clinical Outcome**

到目前为止，我们已经查看了关于调节 T 细胞类型——量化和功能在肿瘤微环境。大量的数据表明，高 Treg 细胞数在肿瘤微环境中意味着更差的预后。在许多情况下，这是真的。然而，调节 T 细胞在肿瘤内和肿瘤周围的浸润可能取决于肿瘤类型。

**Ovarian cancer**

2003 年由 Zhang 等人发现，在进展期生存率和 5 年总生存率（OS）186 例卵巢癌患者中，根据肿瘤的出现或缺乏内部 CD3+ T 细胞。**82** 内部 T 细胞与预期的或间期的 CD3+ T 细胞相关。内部 T 细胞与延迟复发或死亡率有关。T 细胞的存在与细胞环境的免疫调节作用和增加的肿瘤内部水平的 IL-2 和 IFNγ 高度相关。虽然这个研究没有具体说明肿瘤的预后或临床意义，它支持了肿瘤细胞在肿瘤内存在的概念——理论上的假设，然后，就会抑制内部 T 细胞激活的下降。一个后续的研究来自 Kunle Odunsi 的实验室，表明上皮性卵巢癌患者与更高数量的内膜性 CD8+ T 细胞具有改善的生存期（5 年 vs 26 个月）与较低的生存期。**83** 没有检测到 CD8+ T 细胞的生存关联。发现较高比值的 CD8+/CD4+ TIL 比例有改善的生存（74 个月 vs 25 个月），表明较高比值的 TIL（或亚群）可能减少肿瘤 T 细胞的活性。这与 CD8+ TIL 相关。在肿瘤微环境中， Рас Белов 等人研究了具有更高级肿瘤的患者。他们发现与 T 细胞相关，肿瘤行为可以严重影响病人预后。我们研究了 2004 年的 103 名卵巢癌患者。**84** 我们已经展示了卵巢癌和 TAMs 表达 B7-H4。**85** 我们的数据表明，B7-H4 在肿瘤微环境的 DCs 和肿瘤细胞之间显著相关与 Treg 细胞数目，和 T 细胞在肿瘤中的存在和功能在卵巢癌相关的与生存率降低有关与减少的存活。数据详细说明了其他类型的细胞在 Treg 细胞存在和功能在卵巢癌中相关的与生存率降低有关。这些研究提出了一个更为令人信服的图片，增加了 Treg 细胞在肿瘤和肿瘤细胞的生存中存在和功能。

**Prognostic significance of TReg in tumor**

Kawaida 等人发表在 2005 年的一篇研究中，增加了 CD4+ CD25+ T 细胞在区域淋巴结在胃癌患者，与控制的结节性淋巴结从同一批病人。**85** 功能测试这些细胞确认抑制性活性对应于 Treg 细胞。一个后续的研究由 Kono 等人调查了 CD4+ CD25hi FoxP3 mRNA 表达的 T 细胞在总 PBMC CD4+ T 细胞在 72 名患者与胃癌和 42 名患者与食管癌。**86** 虽然他们没有提供具体的数据以呈现的文本，作者表示在早期和先进的疾病阶段，胃癌（～2% 在 I 期 vs. ～7% 在 IV 期）和食管癌（大约 2.5% 在 I 期 vs. ～8% 在 IV 期）。他们也表示，存在于癌症中，患者的 T 细胞与高级的 CD4+ CD25hi T 细胞显示出较低的生存率（大约 vs. 93% 后 6 年对于胃癌，以及 vs. 55% 在食管癌）与减少的比例。这项研究利用了小样本，但不具体地讨论了分组参数。2008 年由 Mizukami 等人。
demonstrated a relationship between the localization of T_{Reg} cells and clinical outcome in 80 patients with gastric cancer.\textsuperscript{87} Although the populations of Foxp3\textsuperscript{+} cells in patients with Stage IV cancer (107.4 cells per five randomly selected high-magnification fields vs. 47.2) were significantly larger than those with stage I cancer, this study did not find a significant difference between survival of patients with low levels of T_{Reg} cells (fewer than 34.5 cells per five randomly selected fields) and those with high T_{Reg} levels (more than 34.5) in the tumor. Localization patterns of infiltrating Foxp3\textsuperscript{+} cells in the tumor were divided into three groups: a peritumour group (more frequent in Stage I), a diffuse group and a follicular group (defined as patients in whom the population of Foxp3\textsuperscript{+} cells mainly occupied the lymphoid follicles of the submucosal layer compared with any other region of the tumor; more prevalent in Stages II–IV than Stage I). Interestingly, patients with a diffuse pattern of Foxp3\textsuperscript{+} cells had significantly poorer 10-year survival (60%) than patients with a peritumoral pattern (90%). This suggests that T_{Reg} location, rather than number, might be more important when forecasting the survival of patients with gastric cancer. Shortly afterward, Mizukami et al. published another report in which they investigated the frequency of Foxp3\textsuperscript{+} T_{Reg} cells within total CD4\textsuperscript{+} cells, regional lymph nodes and peripheral blood lymphocytes (PBLs) of gastric cancer patients (n = 45). As might be expected, the frequency of T_{Reg} cells in TILs was significantly higher than in normal gastric mucosa (12.4 vs. 4.1%) both in early and late disease. Interestingly, the frequency of CCL17\textsuperscript{+} or CCL22\textsuperscript{+} cells among intratumoral CD14\textsuperscript{+} cells (monocytes and macrophages) was significantly higher than that of normal gastric mucosa, and this frequency correlated significantly with tumor-infiltrating T_{Reg} numbers. The investigators confirmed in an in vitro migration assay that T_{Reg} cells could be induced to migrate by CCL17 or CCL22. This study supports the notion suggested previously by our group\textsuperscript{35} that chemokines secreted by monocytes and macrophages within the tumor environment are important for T_{Reg} trafficking into the tumor. More recently, Haas et al. published an investigation of T_{Reg} Prognostic significance in 52 patients with intestinal-type gastric cardiac cancer.\textsuperscript{77} Although the group found no relationship between the numbers of T_{Reg} cells (or macrophages) infiltrating the tumor and patient survival, they did observe that patients with larger T_{Reg} populations in the tumor stroma (>125.9 Foxp3\textsuperscript{+}TILs/mm\textsuperscript{2}) had a median survival time of 58 months while those with smaller populations (<125.9 Foxp3\textsuperscript{+}TILs/mm\textsuperscript{2} of tissue) had a median survival time of 32 months. Interestingly, they also discovered that patients with higher (above 2.9) stromal CD68\textsuperscript{+} (a glycoprotein expressed on monocytes and macrophages)/Foxp3\textsuperscript{+} cell ratios in primary tumor had shorter median survival time than those with lower ratios. This data again supports the concept of an immunosuppressive and/or tumor-promoting role for APC in the tumor microenvironment. Haas et al. propose that their findings suggest that inflammatory processes within the tumor stroma of gastric cardiac adenocarcinomas may have direct effects on patient outcome. In opposition to the probable protumor role for macrophages, it is feasible that large numbers of stromal T_{Reg} may inhibit local cancer-promoting inflammatory processes. Therefore, it is possible in patients with chronic inflammation-associated cancers such as gastritis-associated gastric adenocarcinoma and ulcerative colitis–associated colon cancer (see discussion below), T_{Reg} may be protective.

**Pancreatic cancer**

In 2006, Hiraoka et al. performed a study of the clinical significance of T_{Reg} in the progression of pancreatic ductal adenocarcinoma.\textsuperscript{88} On investigation of tumor tissue and draining lymph nodes of 198 pancreatic ductal adenocarcinomas, their associated premalignant lesions and 15 non-neoplastic pancreatic lesions, the investigators found an increased T_{Reg} prevalence in the ductal adenocarcinomas compared with that in the stroma of non-neoplastic lesions. This increase significantly correlated with certain clinicopathologic factors, including distant metastasis, advanced tumor stage and higher tumor grade. Interestingly, the investigators demonstrated that infiltration of intraepithelial CD8\textsuperscript{+} cytotoxic T cells into pancreatic ducts was prominent in low-grade premalignant lesions but diminished during the progression of both pancreatic intraepithelial neoplasias and intraductal papillary-mucinous neoplasms. Conversely, numbers of stromal T_{Reg} cells increased during this progression. Patients with a low frequency (less than the average 34.6% of total intratumoral CD4\textsuperscript{+} T cells) of tumor-infiltrating T_{Reg} cells had significantly longer survival than those who had a high frequency (more than the average 34.6%) of intratumoral T_{Reg} cells.

**Anal cancer**

A study by Grabenbauer et al. explored the prognostic significance of T_{Reg} cells and TIL subsets in 38 anal squamous cell carcinoma patients treated with radiochemotherapy.\textsuperscript{90} Although they found no prognostic effects for T_{Reg} or macrophages, the investigators did determine that higher numbers of cytotoxic TIL numbers (>0.6 granzyme B+ TILs per 100 tumor cells) served as indicators of poor prognosis (3-year survival rate of 47 vs. 89% in patients with fewer than 0.6 granzyme B+ TIL per 100 tumor cells). Additionally, 3-year survival rates for patients with low numbers of TILs (defined as ≤3.8 CD3\textsuperscript{+} per 100 tumor cells or ≤1.5 CD4\textsuperscript{+} per 100 tumor cells) were 89 and 95%, respectively, and 54 and 48%, respectively, in cases with high numbers (>3.8 CD3\textsuperscript{+} per 100 tumor cells or >1.5 CD4\textsuperscript{+} per 100 tumor cells). It appears here that lower numbers of TILs indicate better patient outcome. However, the prognostic significance of these cell populations must be considered in light of the fact that the patients examined had already been treated with radiochemotherapy, and it is therefore possible that the
remaining tumor cells may have arisen from radiation-resistant precursors.

Colorectal cancer

A recent study by Salama et al. assessed the survival correlations of CD8+ and CD45RO+ and FoxP3+ T cell frequencies in tumor and normal colonic tissue from 967 patients with Stage II or Stage III CRC.91 The investigators found that CD8+ and CD45RO+ cell densities (cells per mm²) were lower in tumor than in normal tissue, but FoxP3+ cell density was higher. FoxP3+ cells were not associated with any histopathologic features other than tumor stage: interestingly, lower numbers of FoxP3+ cells correlated with more advance tumor stages. Further examination demonstrated that tumor stage, vascular invasion, and FoxP3 tumor stages. Further examination demonstrated that tumor stage, vascular invasion, and FoxP3+ density in normal and tumor tissue all served as independent prognostic factors. High FoxP3+ frequency (more than the median value of 44 FoxP3+ cells/mm²) in healthy tissue was associated with worse prognosis, while higher frequencies of FoxP3+ cells in tumors (more than the median value of 116 FoxP3+ cells/mm²) were associated with better survival. In this study, CD8+ and CD45RO+ T cells did not correlate with patient outcome. Two other studies have contributed to our knowledge of TReg cells in CRC in the past year. Sinicrope et al. investigated the prognostic impact of T Reg and CD3+ T cell numbers within 160 Stage II or III colon cancer patients.92 On comparison with normal colon tissue from the same patients, the investigators determined that densities of both TReg and CD3+ T cell populations were increased in tumor tissue. Although intraepithelial FoxP3+ cell numbers were not prognostic, higher levels of expression were found to correlate with poor tumor differentiation, advanced patient age and, interestingly, female gender. As for CD3+ T cells, patients with smaller intraepithelial populations experienced reduced disease-free survival (DFS). A low intraepithelial CD3+/FoxP3+ ratio (lower than the first quartile value of all patients) also served as a prognostic indicator for reduced DFS. Both of these variables were found to be prognostically stronger for patients with colon carcinoma than either numbers of lymph node metastases or tumor stage. More recently, Frey et al. investigated the prognostic significance of TReg cells in CRC patients after their tumors had been stratified by mismatch repair (MMR) status.93 They examined 1,197 MMR-proficient and 223 MMR-deficient CRCs. Fascinatingly, high FoxP3+ numbers (classified as more than 17 FoxP3+ cells per microarray tissue punch, approximately the same area as one 40× field) in the MMR-proficient patients correlated with early T stage, tumor location (rectal) and better 5-year survival rate. In MMR-deficient CRCs, however, larger FoxP3+ populations were associated with an absence of lymph node involvement and absence of vascularization, along with a better 5-year survival rate. Finally, the investigators determined that an elevated FoxP3+ cell frequency served as an independent prognostic factor in MMR-proficient CRC and could predict enhanced survival in these patients.

Liver cancer

In 2007, Kobayashi et al. examined the infiltration of FoxP3+ TReg and CD8+ T cells in the tumor stroma and nontumorous liver parenchyma of patients with liver cancer.94 Their samples included 323 hepatic nodules (including precursor lesions), early hepatocellular carcinoma (HCC), and advanced HCC, in addition to 39 intrahepatic cholangiocarcinomas and 59 metastatic liver adenocarcinomas. The investigators found that TReg numbers were significantly higher in HCC than in non-tumorous liver, and higher in primary HCC than in metastatic HCC. In both cases, HCC-infiltrating TReg cell density was an independent prognostic factor. TReg frequency was also increased in nontumorous liver (both with and without hepatitis) bearing primary tumors. Higher TReg numbers within tumor tissue correlated with higher tumor grade and tended to correlate (p = 0.064) with fewer infiltrating CD8+ T cells. The patient group with a high prevalence of TReg (greater than 29% of CD4+ T cells) infiltrating HCC showed a significantly lower DFS and overall survival (OS) rate (27.3 and 45.1 months, respectively) than patients with fewer than 29% of CD4+ T cells identified as TReg cells (36.2 and 60.3 months, respectively). Contrastingly, there was no significant difference in the OS or DFS between patients with low numbers of tumor-infiltrating CD8+ T and those with high numbers. Kobayashi et al. observed that during hepatocarcinogenesis, the prevalence of TReg increased, while CD8+ T cell numbers decreased. This work supports the notion that primary hepatic cancers develop in liver that is immunosuppressed by large populations of TReg cells. In the same year, Gao et al. published a manuscript detailing their investigation into the prognostic value of TILs in 302 HCC patients after tumor resection.95 Interestingly, numbers of CD3+, CD4+ and CD8+ TILs were not associated with patient survival. However, fewer intratumoral TReg (<2.24 per 400× field) in combination with more (>17.74 per 400× field) activated CD8+ cytotoxic cells (CTLs, activation as defined by positive Granzyme B staining), served as an independent prognostic factor for both improved DFS and OS. Patients with high numbers of tumor-infiltrating TReg and low numbers of CD8+ CTLs (fewer than 17.74 per 400× field) had 5-year OS and DFS rates of 24.1 and 19.8%, respectively, whereas the group with low TReg and high CD8+ CTLs had rates of 64.0 and 59.4%. Both TReg alone and activated CTLs alone within the tumor served as independent predictors for OS. Patients with low numbers of intratumoral TReg had longer OS (70 months) and DFS (69 months) than did those with high numbers of TReg (higher than 2.24 per 400× field; 51 and 34 months, respectively). Interestingly, the investigators found a correlation between high TReg cell density and both absence of tumor encapsulation and presence of tumor vascular invasion, which suggests an association of TReg cells with tumor invasiveness. If a
CD8+ CTL-heavy balance of CTL and T_{Reg} in the tumor microenvironment is indicative of better patient outcome, then therapy which increases CD8+ number and efficacy while simultaneously depleting T_{Reg} cells would be ideal. In 2006, Cai et al. explored the potential of intratumoral DCs and T cells to serve as prognostic indicators in 123 patients who underwent surgical resection of hepatocellular carcinoma. Although the investigators did not find a significant correlation between the number grade of infiltrating immune cells in HCC nodules or pericancerous tissues and DFS, they observed that an absolute number of DCs in HCC nodules of 25 or more per ten HPF did correlate with DFS. However, 28 or more DCs per ten HPF in pericancerous tissues had no correlation with survival. As might be expected, more DCs alone or with T lymphocytes (CD3+, CD45RO+ or CD8+), or more CD8+ T lymphocytes alone in HCC nodules strongly correlated to longer tumor-free survival time. It is important to consider this study because it explores numbers and locations of immune cells other than T_{Reg} cells that likely interact with T_{Reg} in the tumor microenvironment. In 2009, we published a study of B7-H1 and PD-1 expression in HCC patients in which we determined not only that B7-H1 expression on Kupffer cells (KC) was increased in tumor tissues compared with surrounding nontumor liver tissues, but also that this expression correlated with poor survival. Additionally, numbers of PD-1+ CD8+ T cells were higher in tumor tissues than in non-tumor tissues, and B7-H1+ KCs and PD-1+ T cells colocalized in the HCC stroma. PD-1+ CD8+ T cells had decreased proliferative ability and effector function, but these attributes were rescued on PD-1/B7-H1 blockade. In summary, it is clear from the aforementioned studies that T_{Reg} are not the only prognostic marker of survival in HCC patients. Zhang et al. recently published a study investigating the prognostic potential of Th17 cells in 178 patients with hepatocellular carcinoma. The investigators found that Th17 cell numbers were higher in tumors of HCC patients than in non-tumor tissue, and that these Th17 displayed an effector memory phenotype. It was also determined that intratumoral frequency of IL-17–producing cells, which correlated proportionally with tumor microvessel density, could serve as an independent prognostic factor for OS and DFS. Other studies have documented a proangiogenic role for IL-17. In HCC, intratumoral density of Th17 cells is negatively associated with patient outcome.

Head and neck cancer

In 2006, a study from the laboratory of Eric Tartour investigated the prognostic value of various tumor-infiltrating CD4+ T-cell populations in 84 untreated patients with head and neck squamous cell carcinoma. The investigators found that larger populations of tumor-infiltrating CD4+ CD69+ (activated) T cells (greater than 2.6 cells per field using a 40× objective) correlated with both better local control of the tumor and longer patient survival. Interestingly, higher numbers of intratumoral regulatory Foxp3+ CD4+ T cells (more than 1.5 cells per 40× field) were also positively associated with and served as an independent prognostic factor for better regional control of the tumor. CD4+ CD69+ T cells made up the only population within the tumor that significantly influenced OS: more infiltration correlated with better patient outcome. In head and neck cancer, T_{Reg} cells may enact better local tumor control through suppression of inflammatory intermediates.

Breast cancer

Also in 2006, Bates et al. performed experiments to assess the clinical significance of T_{Reg} cells in breast cancer patients with pure ductal carcinoma in situ (DCIS; n = 62), invasive breast cancer (n = 237) or from samples of normal breast tissue (n = 10). The investigators found increased T_{Reg} numbers in in situ and invasive breast carcinomas when compared with normal breast tissue, and larger T_{Reg} populations in invasive tumors than in DCIS. Increased levels (greater than or equal to 15 positively-stained cells per 1 mm diameter invasive tumor cores) of T_{Reg} cells distinguished both patients with DCIS at increased risk of relapse and patients with invasive tumors who would go on to have shorter DFS and OS. High-grade tumors, patients with lymph node involvement and estrogen receptor (ER)-negative tumors all displayed significantly larger numbers of T_{Reg} cells. Patients with larger T_{Reg} populations in ER+ tumors were categorized as high risk. In this study, high numbers of T_{Reg} cells identified patients at risk of relapse after 5 years. Bates et al. recommend T_{Reg} as a novel marker for identifying late-relapse patients who might be good candidates for aromatase therapy (which suppresses estrogen production) after tamoxifen treatment.

Lymphoma

Research from the lab of Miguel Piris in 2005 explored the relevance of T_{Reg} and CTL (defined by TIA-1 and Granzyme B) populations in the reactive background of Hodgkin’s lymphoma (HL) samples in the prognosis of 257 patients with classic HL (cHL). Previous research reported by Oudejans et al. that increased numbers of CTLs were associated with poor patient outcome was met with skepticism. The 2005 report showed that a smaller population of Foxp3+ cells (lowest quartile of total patient numbers) combined with higher numbers of CTL (highest quartile) in the infiltrate served as an independent prognostic factor that negatively influenced event-free survival (EFS) and DFS in cHL patients. Alvaro et al. tested four cases in which patients relapsed and discovered that these samples tended to have more TIA-1+ cells and a lower proportion of Foxp3+ cells than at the time of diagnosis. The results of this investigation suggest that a combination of more CTLs with small numbers of Foxp3+ cells in the reactive background may predict a poor outcome in cHL patients. Three years later, Karube et al. analyzed the expression of Foxp3 in adult T-cell leukemia and lymphoma. Interestingly, 60 (36%) of the 169 cases examined had Foxp3...
expression in lymphoma cells. On closer examination, the investigators found that FoxP3+ and FoxP3− leukemia/lymphoma cases did not differ in clinical stage, age distribution, lactate dehydrogenase and calcium in serum or in overall survival. However, a larger proportion of FoxP3+ cases (8/34) suffered from severe infection; while in FoxP3− cases, only two of 62 patients did so. Karube et al. also demonstrated that FoxP3 expression in adult T-cell leukemia/lymphoma indicated certain morphological features (including chromosome abnormalities) and concluded that this TReg marker is associated with patient immunosuppression.

Melanoma

In 2007, Miracco et al. analyzed 66 vertical growth phase primary cutaneous melanomas for correlation of TReg cell presence with recurrence potential. The investigators discovered that the percentage of TReg within tumor parenchyma, at its periphery, and among TILs at the tumor–stroma boundary, was significantly higher in patients that experienced recurrence than in those that did not. Interestingly, many of the TRegS identified in these samples were found in close proximity to TAMS, the presence of which has been correlated to poor prognosis in patients with advanced melanoma. Although the Miracco study did not analyze such parameters as distant metastases and patient survival, the preliminary data point to the possibility of using TReg quantification as a prognostic indicator in melanoma.

Conclusions

Although regulatory T cells share functionality and mechanisms of suppression across cancers, it is important to recognize the individual (tumor-specific) nature of the TReg component of any given tumor microenvironment. As we have reviewed above, TReg play an important role in the development and maintenance of tumors and in the abrogation of the immune responses against them. In many cancers, elevated TReg cell presence and number imply a worse prognosis for the patient in question. However, this is not always the case. As the studies of gastric, colorectal and anal cancer suggest, it seems that TReg also have a different role. In these reports, increased TReg populations within the tumor tissue seemed to be beneficial. It is interesting to note that all of these cancers are localized to the gastrointestinal tract, portions of which are sites of the most rapid cell turnover in the human body. It is possible that TReg cells in this environment are more crucial for restraining inflammation (and thus preventing angiogenesis and other developments beneficial to tumor growth and survival) than for shutting off the host's response to the tumor. Chronic inflammation is often linked to cancer development in the gastrointestinal system. Further investigation into this phenomenon is warranted. In addition to tumor location, then, it is important to consider TReg populations in the context of their upstream and downstream mediators, as well as alongside their cohorts in the tumor microenvironment. It is prudent to acknowledge the recently identified population of myeloid-derived suppressor cells (MDSC), the known functions of which thus far include inhibition of T cell activation, proliferation, migration and cytokine production through a variety of mechanisms. As many studies have described, other T cell subsets and APCs (notably macrophages and dendritic cells) are significant in their relationships to intratumoral TReg cells in the recruitment of TReg into the tumor, influence of TReg function within the tumor environment and in the molecules they (as target cells) upregulate in response to TReg signaling. For example, IDO, a molecule induced in APC via CTLA-4 expression on TReg has been shown to diminish local CD8+ T cell infiltration and responses to allogeneic target cells in vitro and in vivo. Interestingly, Sørensen et al. identified IDO-specific cytotoxic effector CD8+ T cells in the peripheral blood and tumor microenvironment of some cancer patients, demonstrating that the immune system can mount responses against at least some of the mechanisms designed to suppress it. This affirms the importance of evaluating intratumoral TReg presence in the context of other T cells, especially CD8+ T cells. Indeed, no single molecule or cell examined thus far within the tumor environment can serve as an absolutely independent indicator of patient survival. Therefore, it will be necessary to examine multiple tumor-associated cell populations in tandem with well-defined pathological, clinical and genetic parameters to more accurately predict patient outcome.

References

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