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Cutting Edge: Th17 and Regulatory T Cell Dynamics and the Regulation by IL-2 in the Tumor Microenvironment

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Th17 cells play an active role in inflammation and autoimmune diseases. However, the nature and regulation of Th17 in the context of tumor immunity remain unknown. In this study, we show that parallel to regulatory T (Treg) cells, IL-17+ CD4+ and CD8+ T cells are kinetically induced in multiple tumor microenvironments in mice and humans. Treg cells play a crucial role in tumor immune pathogenesis and temper immune therapeutic efficacy. IL-2 is crucial for the production and function of Treg cells. We now show that IL-2 reduces IL-17+ T cell differentiation in the tumor microenvironment accompanied with an enhanced Treg cell compartment in vitro and in vivo. Altogether, our work demonstrates a dynamic differentiation of IL-17+ T cells in the tumor microenvironment, reveals a novel role for IL-2 in controlling the balance between IL-17+ and Treg cells, and provides new insight of IL-17+ T cells in tumor immune pathology and therapy. The Journal of Immunology, 2007, 178: 6730–6733.

The range of effector CD4+ T cell lineages has been expanded with a description of an IL-17-producing subpopulation called Th17. Th17 cells and IL-17 play an active role in the inflammation and autoimmune diseases in murine systems (1–8). IL-23 and IL-1 may be important for amplifying and stabilizing the Th17 phenotype in chronic inflammation (2, 4–6, 9–12). Dendritic cells transduced with IL-23 promote antitumor immunity (13). Strikingly, it was also reported that IL-23 may promote tumor incidence and growth (14). Although these data may suggest a potential impact of Th17 cells on tumor, the nature, regulation, and role of Th17 in the context of tumor immunity remain unknown both in mice and humans.

Materials and Methods

Mice and tumors

All mouse procedures were performed in accordance with institutional protocol guidelines at the University of Michigan under an approved protocol. TRAMP C57BL/6 mice (8- to 10-wk-old males) and wild-type C57BL/6 mice (4- to 8-wk-old females) were obtained from The Jackson Laboratory. Prostate tumor developed in TRAMP C57BL/6 mice was used for T cell phenotyping. The B16 mouse melanoma (B16), and the head and neck squamous cell carcinoma (SCC7) cell lines were obtained from American Type Culture Collection. Mouse 3-methylcholanthrene-induced fibrosarcomas (MCA207) were maintained in vivo by serial subcutaneously transplantation in C57BL/6 mice and were used within the eighth transplantation generation. B16, HN, and MCA tumors were established in C57BL/6 mice by intradermal inoculation of 1.5 × 106 tumor cells. For experiments requiring two tumors on the same mouse, tumors were given on the left and right flank. In some cases, C57BL/6 mice bearing B16 were received by i.p. injection of IL-2 every 3 days (5 μg/animal). Tumor, blood, lymph nodes, and spleen were collected for analyzing T cell phenotype and cytokine profile as we described (15, 16).

Human tumors

Fresh human tumor tissues were obtained from previously untreated patients with epithelial ovarian carcinomas, renal cell carcinoma, and pancreatic carcinoma in stage III or IV. Patients gave written, informed consent. The study was approved by local Institutional Review Boards.

In vitro culture system

T cells were isolated from the spleen of C57BL/6 mice with a commercial kit (Stem Cell Technology) and stimulated with 2.5 μg/ml anti-CD3 and 1.2 μg/ml anti-CD28 (BD Biosciences) for 3 days in the presence of cytokines TGF-β1 (10 ng/ml), IL-6 (10 ng/ml), IL-2 (50 ng/ml) (all obtained from R&D Systems), or neutralizing anti-IFN-γ mAbs (3 μg/ml, S4B6; BD Biosciences). Cells were subjected to T cell phenotyping.

T cell phenotype and cytokine profile

T cells were collected from tumor tissues and multiple organs were stimulated for 4 h with leukocyte activation cocktail (BD Biosciences) in the presence of either GolgiStop (BD Biosciences). Cells were first stained extracellularly with anti-CD4, anti-CD8, and anti-CD90 for mouse or anti-CD3 for human T cells (BD Biosciences), then were fixed and permeabilized with Perm/Fix solution (eBioscience), and finally were stained intracellularly with anti-IFN-γ (BD Biosciences), anti-IL-17 (BD Biosciences and eBioscience), and anti-FOXp3 (eBioscience). Samples were acquired on a LSR II (BD Biosciences), and data were analyzed with DIVA software (BD Biosciences).

Statistical calculations

Differences in cell surface and intracellular molecule expression were determined by an χ2 test, with p < 0.05 considered significant.

Results and Discussion

In the first experimental setting, we studied IL-17+ T cells in multiple mouse and human tumors. We initially evaluated the potential existence and organ distribution of IL-17+ T cells in normal vs mice bearing advanced B16 melanoma. We found that the levels of IL-17+ T cells were limited (<1.5%) in different compartments in normal mice. Interestingly, the levels of...
IL-17+ T cells (3–8%) were significantly increased in blood, bone marrow, and spleen in mice bearing advanced B16 melanoma, but not in the tumor draining lymph nodes (Fig. 1a). More strikingly, the highest levels of IL-17+ T cells (average: 12%, range: 2–45%) were found in the tumor tissues (Fig. 1a).

To determine whether it is tumor-type specific, we examined IL-17+ T cells in mice bearing other tumors. We detected a substantial amount of IL-17+ T cells in advanced head and neck tumor, MCA207 fibrosarcomas and prostate cancer (Fig. 1b). We analyzed the phenotype of IL-17+ T cells. We observed that both CD4+ T cells (Th17) and CD8+ T cells expressed IL-17 in four types of tumors studied. The IL-17 expression was comparable between CD4+ and CD8+ T cells. Furthermore, both CD4+ IL-17+ T cells and CD8+ IL-17+ T cells barely expressed IFN-γ (Fig. 1).

Th17 cells have not been studied in human subjects with cancer. We next examined the potential existence and distribution of IL-17+ T cells in normal healthy donors vs multiple cancer patients. Consistent with our observations in mice (Fig. 1, a and b), we showed that there were limited IL-17+ T cells (<1.2%) in peripheral blood in normal donors. However, the levels of IL-17+ T cells were significantly increased in peripheral blood in patients with advanced ovarian carcinoma. Whereas the highest levels of IL-17+ T cells were detected in the tumor tissues in patients with advanced ovarian (including tumor tissue and malignant ascites fluid), pancreatic, and renal cell carcinoma (Fig. 1c).

High levels of regulatory T (Treg) cells are found in the tumor microenvironment and Treg cells play a crucial role in tumor immunity (16–20). We further studied the kinetic distribution of IL-17+ T cells and the relationship with CD4+FOXP3+ Treg cells in tumor and multiple organs in mice bearing B16 melanoma. We observed that the number of CD4+IL-17+ T cells and CD4+FOXP3+ Treg cells (Fig. 2, a and b) was gradually and synchronically increased in the tumor microenvironment during tumor development. However, there were limited IL-17+ CD8+ T cells in the early stages of tumor growth with greater numbers of IL-17+CD8+ T cells appearing in later stages of tumor development (Fig. 2a). Although the trend of increase was similar between Treg cells and IL-17+CD4+ T cells in the tumor, the prevalence of Treg cells was significantly and constantly higher than that of IL-17+ T cells in the tumor (Fig. 2, a and b).

Tumor-associated Ag-specific T cells may be primed in the tumor draining lymph nodes. We further compared Th17 and Treg cells in tumor draining lymph nodes. Tumor draining lymph nodes harbored a significant Treg cell population and Treg cells in tumor draining lymph nodes increased gradually during tumor development (Fig. 2, c and d). Strikingly, the number of IL-17+ T cells remained limited during tumor development in the tumor draining lymph nodes, including advanced tumor stages (Figs. 1a and 2, c and d).

The first part of our findings has demonstrated for the first time several features of these tumor-associated IL-17+ expressing T cells. 1) Th17 and Treg cells are synchronically increased following tumor development. Both populations reach the maximal levels in advanced tumors. The kinetic distribution of Treg cells and IL-17+ T cells suggests their close relationship in the tumor. The cytokine cocktail of TGFβ and IL-6 promotes Th17 differentiation (21–23). In the murine autoimmune system Treg cell-derived TGFβ supports Th17 cell differentiation.

3 Abbreviations used in this paper: Treg, regulatory T; MCA, 3-methylcholanthrene.
High levels of TGFβ and IL-6 are often found in advanced tumors (19). Cytokines in the tumor microenvironment should support Th17 differentiation within the tumor. However, the levels of Treg cells are significantly and constantly higher than IL-17\(^+\) T cells in the tumor environment. It suggests that IL-17\(^+\) T cell differentiation may be inhibited by unknown factor(s) in the tumor microenvironment. 2) In advanced tumors there are IL-17\(^+\) CD4\(^+\) T cells (Th17 cells) as well as substantial amounts of IL-17\(^+\) CD8\(^+\) T cells. The data suggest the potential role of a previously unappreciated IL-17 expressing T cell population, IL-17\(^+\) CD8\(^+\) T cells in tumor immunopathogenesis. It remains to be defined whether IL-17\(^+\) CD8\(^+\) T cells are a unique feature in tumors. 3) IL-17 expressing T cells are largely found in the tumor, particularly in advanced tumors, not in the tumor draining lymph nodes. It suggests that IL-17\(^+\) T cells may be predominantly differentiated in the tumor microenvironment, rather than in the draining lymph nodes. 4) Similar dynamic distribution and compartmentalization of IL-17\(^+\) T cells are observed in multiple mouse and human tumor models. It suggests a potential broad involvement of IL-17\(^+\) T cells in tumor pathogenesis. Thus, our data provide the first evidence of the existence of the IL-17 expressing T cells in mouse and human tumors including both CD4\(^+\) and CD8\(^+\) T cells, and demonstrate the kinetic distribution and relationship between Treg cells and IL-17\(^+\) T cells.

IL-2 is crucial for the production and function of Treg cells in mice (24–26) and cancer patients (27, 28). IL-2 is used to boost IL-2 profoundly reduced the percentage of IL-17\(^+\) T cells in vivo in mice bearing B16 tumor. Spleen T cells were cultured for 3 days with TGFβ and IL-6 in the presence of IL-2 or neutralizing anti-IL-2 mAb. The resulting T cells were analyzed with LSR II for the expression of IL-17 and IFN-γ. a. Results were expressed as the mean ± SEM of IL-17 and IFN-γ cells in T cells (n = 6, *p < 0.01). b and c. Effects of IL-2 on IL-17\(^+\) IFN-γ- and FOXP3\(^+\) CD4\(^+\) T cell differentiation in vitro. Spleen T cells were cultured 3 days with TGF-β in the presence of IL-2 or neutralizing anti-IL-2 mAb. The resulting T cells were analyzed with LSR II for the expression of FOXP3. Results were expressed as the percentage of FOXP3\(^+\) cells in CD4\(^+\) T cells (n = 6, *p < 0.01). d and f. Effects of IL-2 on IL-17\(^+\) IFN-γ- and FOXP3\(^+\) CD4\(^+\) T cell differentiation in vivo. B16 melanoma bearing mice were treated with IL-2 as described in Materials and Method. T cells were obtained from day8 tumor tissues and tumor draining lymph nodes, and analyzed with LSR II for the expression of IL-17, IFN-γ, and FOXP3. Results were expressed as the mean percentage of IL-17\(^+\) IFN-γ- (a) or CD4\(^+\) FOXP3\(^+\) cells ± SEM in CD4\(^+\) T cells (f) (n = 5–8 per group, *p < 0.05). LNs, lymph nodes.

In sharp contrast to the effects of IL-2 on IL-17\(^+\) T cells, exogenous IL-2 resulted in 2-fold increase of the absolute Treg cell numbers (218 ± 31%, compared with control) and of the percentage of Treg cells in CD4\(^+\) T cells mediated by TGFβ (Fig. 3d). Blockade of IL-2 with neutralizing anti-IL-2 mAb reduced Treg cells in the culture (Fig. 3d). The data demonstrate the opposite effects of IL-2 on Th17 and Treg cell differentiation in vitro in the murine system.

We next examined the in vivo effects of IL-2 on IL-17\(^+\) T cell and Treg cell differentiation in vivo in mice bearing B16 tumor. Tumor bearing mice were treated with IL-2. We observed that IL-2 significantly reduced IL-17\(^+\) T cells in the tumor, but not in the draining lymph nodes, whereas FOXP3\(^+\) T cells were significantly enhanced in tumor and tumor draining lymph nodes (Fig. 3, e and f). The dose of IL-2 used in this experiment had no significant effects on B16 melanoma growth (data not shown). The data demonstrate the opposite effects of IL-2 on IL-17\(^+\) T cell and Treg cell differentiation in vivo in tumor bearing host. TGFβ has been proposed to be the link between Th17 and Treg cells in mouse autoimmune diseases (21–23). We now propose that IL-2 is a new link between these two T cell types. Treg and Th17 cells play active roles in autoimmune diseases, allergy, organ transplantation, and tumor. Therefore, our data reveal a novel regulatory mechanism for IL-2: IL-2 may play a central role in balancing Treg cells and IL-17\(^+\) T cells in multiple diseases.

Altogether, our work has demonstrated a dynamic differentiation of IL-17\(^+\) T cells including CD4\(^+\) T cells and CD8\(^+\) T cells in the tumor microenvironment in multiple mouse and human tumors. We have further identified a novel role for IL-2 in regulating the IL-17\(^+\) T cell pool. It has been reported that IL-23 promotes tumor incidence and tumor growth (14). However, dendritic cells transduced with IL-23 elicit potent tumor immunity (13). IL-23 is associated with the development of Th17 cells in autoimmune disease models (9–12). It is predicted that IL-17\(^+\) T cells in the tumor microenvironment may contribute to tumor pathogenesis.

Disclosures
The authors have no financial conflict of interest.

References


