T-Regulatory Cells Shift from a Protective Anti-Inflammatory to a Cancer-Promoting Proinflammatory Phenotype in Polyposis

Elias Gounaris,1 Nichole R. Blatner,1 Kristen Dennis,1 Fay Magnusson,3 Michael F. Gurish,4 Terry B. Strom,5 Philipp Beckhove,6 Fotini Gounari,2 and Khashayarsha Khazaie1

1Division of Gastroenterology and Robert H. Lurie Comprehensive Cancer Center, Northwestern University Feinberg School of Medicine; 2Committee on Immunology, Department of Medicine, The University of Chicago, Chicago, Illinois; 3Department of Immunology, Center for Cancer Immunology, M. D. Anderson Cancer Center, University of Texas, Houston, Texas; 4Division of Rheumatology, Immunology, and Allergy, Departments of Medicine and Pathology, Brigham and Women's Hospital and Harvard Medical School; 5The Transplant Research Center, Division of Immunology, Harvard Medical School, Boston, Massachusetts; and 6Translational Immunology Unit, Deutsches Krebsforschungszentrum, Heidelberg, Germany

Abstract

T-regulatory (Treg) cells play a major role in cancer by suppressing protective antitumor immune responses. A series of observations (from a single laboratory) suggest that Treg cells are protective in cancer by virtue of their ability to control cancer-associated inflammation in an interleukin (IL)-10–dependent manner. Here, we report that the ability of Treg cells to produce IL-10 and control inflammation is lost in the course of progressive disease in a mouse model of hereditary colon cancer. Treg cells that expand in adenomatous polyps no longer produce IL-10 and instead switch to production of IL-17. Aberrant Treg cells from polyt-ridden mice promote rather than suppress focal mastocytosis, a critical tumor-promoting inflammatory response. The cells, however, maintain other Treg characteristics, including their inability to produce IL-2 and ability to suppress proliferation of stimulated CD4+ T cells. By promoting inflammation and suppressing T-helper functions, these cells act as a double-edged knife propagating tumor growth.

Introduction

T-regulatory (Treg) cells are an obstacle for immune surveillance and immune therapy of cancer. Hallmarks of these cells are expression of the transcriptional factor Foxp3 and the interleukin (IL)-2 receptor α subunit (CD25) together with their inability to produce IL-2 (1). Treg cells suppress CD4 T cells in part through competition for the development of adenomatous polyps (10). Yet, there is also precedence for differentiation of Treg cells to produce IL-10 and control inflammation is lost in the course of progressive disease in a mouse model of hereditary colon cancer. Treg cells that expand in adenomatous polyps no longer produce IL-10 and instead switch to production of IL-17. Aberrant Treg cells from polyp-ridden mice promote rather than suppress focal mastocytosis, a critical tumor-promoting inflammatory response. The cells, however, maintain other Treg characteristics, including their inability to produce IL-2 and ability to suppress proliferation of stimulated CD4 T cells. By promoting inflammation and suppressing T-helper functions, these cells act as a double-edged knife propagating tumor growth.

Materials and Methods

Chloroacetate staining. Paraffin sections (5 μm), after deparaffinization with xylene (three, 5 min each) and rehydration with gradually decreasing solutions of ethanol (100%, 95%, and 70%), were stained with naphthol-AS-D chloroacetate and counterstained with hematoxylin Gill's II. Preparation of intestinal monocellulars. Mononuclear cells (MNC) were prepared by chopping with blades the intestines and incubation of 25 mL suspension in RPMI 1640 with 10 units of collagenase type IV (Worthington Biochemical Corp.) for 20 min at 37°C with agitation. MNCs were collected from the interface of a 40% and 60% discontinuous Percoll gradient, washed, and resuspended in PBS plus 0.2% bovine serum albumin for analysis. Mast cell progenitor assay and Treg anti-inflammatory activity assay. Briefly, 10,000 MNCs in 100 μL of medium [RPMI 1640 complete
treated APCΔ468 polyps (*n* = 11; *P* = 0.0002, unpaired *t* test with Welch’s correction; Fig. 1B), and had diminished frequencies of mitotic cells (3.4 ± 0.20% versus 5.3 ± 0.52%, respectively; *n* = 8; *P* = 0.0072, unpaired *t* test with Welch’s correction; Fig. 1B). These observations are the first independent confirmation of earlier reports suggesting that nTreg cells derived from healthy donors are protective to the host and detrimental to polypl growth, causing regression of the lesions by promoting death and hindering division of aberrant epithelial cells.

**nTreg adoptive transfer reduces focal mastocytosis.** We have reported that mast cells are crucial hematopoietic components for polypl development (10). Here, we tested the notion that adoptive transfer of nTreg cells into mice with established polyps results in a drop in the frequencies of polypl-infiltrating mast cells and mast cell progenitors (MCP). To this aim, we transferred nTreg cells from healthy donors to polypl-bearing mice as before. Three weeks after transfer, we checked the frequency of resident intestinal mast cells and progenitors. Paraflin sections of intestines from these mice were stained with chloroacetate esterase to quantify the average number of mature mast cells per field. On average, polyps of

**Results**

**nTreg cells protect against polyplosis.** To examine the effect of nTreg cells on polyplosis, we performed adoptive transfer of CD4ΔCD45RBΔlowCD25Δhigh cells from healthy C57BL/6 mice into APCΔ468 mice. As early as 2 days after i.v. transfer of 1 × 10⁶ Treg cells from C57BL/6 mice to polypl-bearing APCΔ468 mice, the cells were readily detectable in the intestine of recipient mice (data not shown). By 3 weeks after adoptive transfer, the recipient mice had few polyps of smaller sizes (50 ± 13 polyps of 0.98 ± 0.23 mm mean diameter; *n* = 11) compared with untreated age-matched APCΔ468 mice of 4 months of age (76 ± 6.2 polyps of 1.9 ± 0.08 mm mean diameter; *n* = 11). By 6 weeks after transfer, the sizes and numbers of polyps continued to diminish (38 ± 5.1 polyps of 0.21 ± 0.32 mm mean diameter), suggesting active regression of the lesions (*P* < 0.001, one-way ANOVA with Bonferroni’s multiple comparison test; Fig. 1A). The remaining polyps seemed regressive, with 10 ± 0.78% of the total cells displaying apoptotic activity compared with 5.4 ± 0.56% of the total cells in un-

**MNC staining and flow cytommetry analysis.** All staining reactions were preceded by a 10-min incubation with a blockade mixture made of 2.4G2 supernatant (Fe block) and 10% rat and mouse sera (Jackson ImmunoResearch Laboratories). Dead cells were systematically excluded by 4′,6-diamidino-2-phenylindole staining thereafter for 20 min on ice. For intracellular Foxp3 (Biolegend), IL-2, IL-10, and IL-17 stainings, and cell sorting were performed by using FACSAria, FACSCanto, and MoFlo instruments. Single-cell data analyses used the FlowJo software (Tree Star).

**TUNEL and bromodeoxyuridine staining.** Cryosections (10 μm) were stained with ApopTag red fluorescent in situ detection kit (Qiagenome) to detect apoptosis, and mitotic cells were revealed by bromodeoxyuridine staining. Cell numbers were calculated with the NIH software ImageJ (nuclei counter, and color threshold plug-ins).

**ELISA and multiplex ELISA.** ELISA was performed according to the manufacturer’s instructions (eBioscience). Multiplex ELISA was performed according to the manufacturer’s instructions using a customized set of analyte detection from Millipore. The Luminex 100 was used to acquire the results that are analyzed with the software of the machine.

**Light microscopic data images.** Microscopic images were collected with a Leica DCC camera. For multicolored images, the ImageJ plug-in “threshold color” was used to identify cells of the color of interest, which were then counted with the plug-in “nuclei counting.”

**Statistical analysis.** The statistical analyses were performed with the use of the Prism 4 software. ANOVA one-way parametric with Bonferroni post hoc test and 99% confidence intervals or unpaired one-tailed *t* tests with Welch’s correction were used.

![Figure 1. Adoptive transfer of Treg cells in APCΔ468 mice causes polyps to regress and reduces mastocytosis.](image-url)
APCΔ468 mice had a 25-fold more mast cells compared with healthy intestines of wild-type (wt) C57BL/6 mice when counted per 200× field (APCΔ468 = 25 ± 1.6 mast cells, wt = 1.0 ± 0.16 mast cells, n = 24 fields, n = 6 mice). The nTreg adoptive transfer reduced the mean number of polyptide mast cells by at least 4-fold (5.3 ± 0.69 mast cells, n = 24 fields, n = 6 mice, 99% confidence; P = 0.0001, one-way ANOVA with Bonferroni’s multiple comparison test; Fig. 1C and D). A comparable drop in the frequency of MCps was observed (Fig. 1D). The average frequency of MCps in the intestine of APCΔ468 mice was 7-fold higher than the frequency of MCps in the intestine of age-matched wt C57BL/6 mice (APCΔ468 = 883 ± 46 colonies/106 MNCs, n = 12; wt = 119 ± 9.4 colonies/106 MNCs, n = 9; Fig. 1D). nTreg adoptively transferred APCΔ468 mice showed a 3-fold drop in the frequency of intestine resident MCps compared with untreated APCΔ468 mice, approaching double the number of MCps in wt mice (treated APCΔ468 = 227 ± 15 colonies/106 MNCs, n = 10, 99% confidence; P = 0.0001, one-way ANOVA with Bonferroni’s multiple comparison test; Fig. 1D). These observations are consistent with nTreg cell control of tumor-infiltrating mast cells.

**APCΔ468 Polyps are enriched with CD4+CD25+Foxp3+ cells.** Because adoptive transfer of a limited number of nTreg cells into APCΔ468 mice hindered polyptide in the intestine, we expected the recipient mice to be deficient either in the number or function of endogenous Treg cells. To investigate this, we isolated CD4+ lymphocytes either from pools of microdissected polyps or from the entire small bowel and stained for cell surface expression of CD25 and intracellular Foxp3. Surprisingly, CD4+CD25+Foxp3+ cells were significantly more abundant in the polyp-ridden APCΔ468 mice compared with age-matched healthy mice.

CD4+CD25+Foxp3+ T-cell frequencies were elevated in the mesenteric lymph nodes (MLN) of APCΔ468 mice compared with age-matched healthy mice [19 ± 1.0%, n = 7, compared with 13 ± 0.62%, n = 7; P = 0.0002, unpaired t test with Welch’s correction; Fig. 2A (a and b) and B]. The difference was significantly greater when comparing adenomatous polyps with the healthy small intestine [22 ± 0.98%, n = 11, compared with 4.6 ± 0.9%, n = 7; P < 0.0001, unpaired t test with Welch’s correction; Fig. 2A (c–e) and B]. Treg frequency dropped in tissue adjacent to the polyps relative to the polyps [17 ± 1.6%, n = 11; P = 0.0069, unpaired t test with Welch’s correction; Fig. 2A (d and e) and B] but was still significantly higher than in healthy intestine (P < 0.0001). The larger spleens of polyptide APCΔ468 mice contained elevated frequencies and markedly higher numbers of CD4+CD25+Foxp3+ T cells compared with the spleens of wt mice (8.3 × 106 ± 1.8 ± 106 cells compared with 2.4 ± 1.8 ± 106 cells; P = 0.01, unpaired t test with Welch’s correction; Fig. 2B and C). In contrast, we found similar frequencies of Treg cells in peripheral lymph nodes that did not drain the intestine, such as the cervical lymph nodes (Fig. 2B, neck LN).

These observations established that frequencies and absolute numbers of CD4+CD25+Foxp3+ T cells were elevated in the polyp-ridden mice, raising the intriguing question of why, in spite of their increased numbers, cancer-associated mastocytosis was unhindered. We therefore proceeded to check for Treg functions. To distinguish activated CD4 T cells from Treg cells, we crossed APCΔ468 mice with Foxp3-GFP reporter mice (20). T cells were isolated from the spleen and intestine of aged APCΔ468-Foxp3-GFP mice, stimulated with anti-CD3 and anti-CD28 for 3 days, and then stained for CD4, CD25, and intracellular IL-2. Treg cells do not produce IL-2 but constitutively express the IL-2α receptor (CD25) and effectively compete with helper CD4 T cells for IL-2 and hence for proliferation. As expected, CD4+Foxp3+ cells derived from control wt C57BL/6 mice failed to produce IL-2, whereas intracellular IL-2 was readily detected in Foxp3+ cells (Fig. 3A). CD4+Foxp3+ cells derived from the intestine or spleen of APCΔ468 mice also did not produce IL-2. Additionally, we performed a widely used standard in vitro assay that measures the ability of Treg cells to inhibit proliferation of CD4 helper T cells in the absence of exogenous IL-2. Treg cells from both spleen and intestine of polyptide APCΔ468-Foxp3-GFP mice readily suppressed proliferation of anti-CD3-stimulated and anti-CD28–stimulated CD4 T cell in coculture experiments (Fig. 3B and C). Together, these results indicate that Foxp3-GFP cells from polyp-bearing APCΔ468 mice share morphologic characteristics with and behave as Treg cells.

**CD4+CD25+ Foxp3+ cells in polyptide mice are functionally altered.** We had previously reported that adenomatous polyps are infiltrated with proinflammatory cells, including mast cells (10). The observation that mast cell frequency is controlled by nTreg cells is in line with the role of nTreg cells in regulation of
inflammation (21) and control of inflammation-induced cancer (18). There are reports of defective Treg cells in arthritis, a chronic autoimmune inflammatory disease (22, 23). In our current animal model, accumulation of endogenous CD4+CD25+Foxp3+ cells in polyps in progressive disease was consistent with a functional failure. IL-10 is critical for suppression of inflammation (24) and cancer by Treg cells (18). To test possibility of defects in Treg function related to IL-10, we stimulated CD4+CD25+Foxp3+ cells from the intestine of APCΔ468 Foxp3-GFP mice with anti-CD3 and anti-CD28 for 3 days and stained for IL-10. Approximately 4% of Treg cells from spleen and >40% from intestine of healthy control C57BL/6 mice produced IL-10 (Fig. 4A). In contrast, Treg cells from the spleen of APCΔ468 Foxp3-GFP mice were devoid of IL-10 (Fig. 4A). IL-10–expressing Treg cells were reduced by at least an order of magnitude in the healthy intestine tissue marginal to the polyps in comparison with intestine of healthy mice (Fig. 4). Adenomatous polyps contained even fewer IL-10–expressing Treg cells (Fig. 4A).

We concluded that the endogenous Treg cells in polytumor-bearing mice were defective in secretion of IL-10. Based on the crucial role of IL-10 in control of inflammation and the accumulation of Treg cells in adenomatous polyps, we predicted that the poly-infiltrating Treg cells either were unable to control inflammation or were proinflammatory and thus functionally distinct from Treg cells from healthy mice. To further characterize polyp-infiltrating Treg cells, we tested the cells for the synthesis of T H1 (IFN-γ) or T H2 (IL-4) cytokines but failed to detect either (data not shown). However, up to a quarter of CD4+CD25+Foxp3+ cells derived from APCΔ468 mice expressed IL-17 after 3 days of ex vivo stimulation (Fig. 4B). Isotype control antibody for the cytokines did not stain (Fig. 4B). These observations were consistent with polyposis being a T H17–driven disease.

T H17 cytokines are suppressed by adoptive transfer of healthy Treg. Treg cells adoptively transferred from healthy mice homed to the intestine and expressed IL-10 (Fig. 5A), in contrast to the endogenous Treg cells in the same mice (Fig. 5A and B). As reported before (19), the adoptive transfer of Treg cells resulted in suppression of polyposis, and we have already mentioned that it causes significant reductions in the frequencies of gut-infiltrating mast cells and progenitors (see Fig. 1). To further investigate the effect of Treg transfer on inflammation, we carried out multiplex ELISA on serum of diseased or age-matched healthy control mice for key cytokines associated with T H1, T H2, or T H17 responses. T H1 cytokines (IL-12 and IFN-γ) were lowered with the exception of TNF-α that was elevated in the sera of APCΔ468 mice compared with C57BL/6 (Fig. 5C). T H2 cytokines showed a disparate pattern, with IL-13 being elevated and IL-4 reduced (Fig. 5C). IL-17 was markedly elevated (Fig. 5C). We focused on T H17 cytokines and TNF-α, monitoring with standard ELISA mice that had received CD4+CD25+ nTreg cells from healthy donors. Treg transfer resulted in a clear drop in the level of serum IL-6, IL-17, IL-23, and TNF-α as early as 3 weeks after adoptive transfer, as detected by standard ELISA assays (Fig. 5D). Together, these observations showed that adoptively transferred Treg cells home to the intestine, secrete IL-10 and control inflammation, and are distinct from the endogenous Treg cells that did not produce IL-10. This raised the question of the function of endogenous Treg cells about inflammation.

CD4+Foxp3+ cells from tumor-bearing mice are proinflammatory. The CD4 T-cell proliferation assay that is classically used to test Treg activity is primarily based on competition for IL-2 and is not sufficiently relevant to inflammation. To test the effect of Treg cells on inflammation, we devised a new in vitro assay that relied on the ability of Treg cells to suppress the differentiation and expansion of mast cells ex vivo. The assay was based on the MCp assay reported by us earlier (10) but included IL-2 and exogenous T cells.

To avoid complications due to endogenous Treg cells, we crossed the APCΔ468 mice to the Rag2−/− background and used...
these as donors of MNCs for the MCp assay. The MNCs were then mixed with CD4+Foxp3+ Treg cells sorted from the spleens of healthy, polyp-ridden, or IL-10−/− mice. MNCs with or without T cells were then plated in limiting dilution into 96-well dishes with medium containing IL-2, SCF, and IL-3. MCp-derived mast cell colonies were scored after 11 to 15 days (Fig. 6A). Addition of CD4+Foxp3-GFP+ cells from healthy Foxp3-GFP mice to the cultures significantly reduced the frequency of mast cell colonies (APCΔ468×Rag−/−, 1,600 ± 100 MCps/10⁶ MNCs; APCΔ468×Rag−/− in the presence of 5 × 10⁴ nTreg cells, 1,100 ± 120 MCps/10⁶ MNCs; P = 0.0014). However, addition of CD4+Foxp3-GFP+ cells derived from polyp-ridden mice almost doubled the number of progenitor-derived mast cell colonies (2,700 ± 170 MCps/10⁶ MNCs; P = 0.0002). Because these Treg cells could not produce IL-10, we tested the role of IL-10. Treg cells from healthy mice failed to control MCp differentiation and expansion when they were depleted of IL-10 either through adding anti-IL-10 (2,000 ± 170 MCps/10⁶ MNCs; P = 0.058) or by using IL-10−/− mice as source of Treg cells (1,800 ± 200 MCps/10⁶ MNCs; P = 0.2468; Fig. 6A). These observations suggested that Treg cells may be controlling the natural frequency of mucosal MCps. To test this, we depleted MNCs isolated from the intestine of healthy mice (B6, 220 ± 28 MCps/10⁶ MNCs) from CD4+ (740 ± 160 MCps/10⁶ MNCs; P = 0.0427) or CD25+ T cells (950 ± 130 MCps/10⁶ MNCs; P = 0.0002). In both cases, we observed marked increase in the mast cell colonies (Fig. 6B). Addition of anti-IL-10 produced a similar positive effect, arguing that the suppression by Treg cells required IL-10 (760 ± 170 MCps/10⁶ MNCs; P = 0.0078, all analyzed with unpaired t test with Welch’s correction). Based on these observations, we conclude that mast progenitor frequencies in the gut are controlled by Treg cells in a process that requires IL-10. In dysplasia, Treg cells are rendered incapable of producing IL-10 and convert to a proinflammatory phenotype while maintaining their expression of Foxp3 and ability to compete for IL-2. The altered functional status of Treg cells in tumor-bearing mice favors tumor progression.

Discussion

Polyposis is reversed through the adoptive transfer of Treg cells from healthy mice to polyp-ridden mice. We show that this is in
part due to Treg suppression of focal mastocytosis, a critical tumor-promoting inflammatory response (10). Because Treg cells are known to expand in cancer, it is perplexing why the transfer of a limited number of Treg cells to mice harboring preneoplastic lesions should have such dramatic protective results. We show for the first time that the diseased recipients harbor functionally altered Treg cells. These express Foxp3 and suppress CD4 T-cell expansion but do not produce IL-10 and in the polymp microenvironment produce IL-17. CD4+Foxp3+ T cells derived from polymp-ridden mice promote rather than suppress mast cell maturation and expansion and are therefore “protumor” T cells that support cancer inflammation and tumor growth. While this manuscript was in preparation, a report documented the suppression of mast cell degranulation and allergic responses by Treg cells (17). This is consistent with earlier observations suggesting that Treg cells in arthritic patients fail to suppress inflammation and that a major benefit of anti-TNF treatment is the recovery of Treg functions (23).

The present report is the first to show that Treg cells are altered in the preneoplastic stage and that this alteration specifically affects mast cell expansion. Treg cells are important in immune homeostasis in the intestine. Loss of production of IL-10 is a major functional defect that could explain the inability of the Treg cells to suppress focal mastocytosis and systemic inflammation in the polymp-ridden mice. IL-10 is well accepted as a major regulator of mucosal immune responses in mice (24). Interestingly, IL-10 can also be protective in carcinogenesis, causing profound inhibition of tumor establishment, growth, and metastasis (25). Much less is known about the role of IL-10 in control of TH17-driven inflammation. Our observations are consistent with IL-10 mediating the Treg suppression of MCp differentiation/expansion and maintenance of gut homeostasis.

Our report is also the first to propose a pathologic function for the earlier reported but thus far poorly studied “Foxp3/IL-17 double-positive cells” (9) in cancer promotion. Adenomatous polyps are immediate precursors to intestinal and colonic carcinomas, and therefore, we propose that the shift in function of CD4+CD25+ T cells is likely to be an early event in colon cancer. At this point, we cannot rule out the possibility that the CD4+Foxp3+IL-17+ cells are generated elsewhere and targeted to the polyps. However, we failed to identify such cells in the thymus, spleen, or lymphatics, including the MLN, leaving the possibility open that these are bona fide Treg cells that are converted to produce IL-17 in the preneoplastic stromal microenvironment. Whether this is a true conversion of nTreg cells or of naive CD4 T cells remains to be clarified.

Recent studies have shown the plasticity and ready conversion of Foxp3+ cells to a CD4+Foxp3+IL-17+ phenotype (26, 27). The elevated levels of IL-6, IL-1β, and IL-23 in sera of polymp-ridden mice are consistent with the notion of Treg conversion to Th17 lineage; however, the persistent expression of Foxp3 raises new questions. Treg differentiation from naive CD4 T cells needs TGF-β but is inhibited by IL-6 (28). In contrast, differentiation of naive CD4 T cells into Th17 cells requires local production of TGF-β and IL-6, with IL-23 being necessary for prolonged stabilization (28, 29). The concurrent expression of Foxp3 and production of IL-17 is intriguing. Foxp3, a key transcription factor expressed by Treg cells, antagonized the function of ROR-γt, which is essential for the Th17 phenotype (4). Nevertheless, the concomitant expression of IL-17 and

![Figure 5](image-url)

**Figure 5.** Elevated levels of proinflammatory cytokines in sera of APCΔ468 mice are corrected after adoptive transfer of nTreg cells from healthy mice. A, adoptively transferred Treg cells were explanted from the intestine of polymp-ridden mice 3 wk after transfer and analyzed by FACS for expression of IL-10; a representative contour plot is shown. B, summary of three independent experiments; transferred and endogenous Treg cells were distinguished by expression of Lys.1 versus Lys.2 and staining for intracellular Foxp3. C, sera from 4-mo-old APCΔ468 and age-matched wt mice were analyzed with multiplex ELISA for 10 cytokines. The results of three independent experiments conducted in triplicate were used to calculate the positive or negative changes using the wt values as baseline. D, standard ELISA used to measure the level of Th17-associated cytokines 3 wk after adoptive transfer of 1 × 106 nTreg cells from healthy donors to polymp-ridden APCΔ468 mice of 2.5 to 3 mo of age. Sera from untreated age-matched APCΔ468 mice were used as control. White columns, APCΔ468; black columns, C57BL/6; gray columns, Treg-treated APCΔ468.
Foxp3 by CD4 T cells has been reported before (9, 30). Several recent studies have shown that Treg and TH17 cells are simultaneously enriched in tumors. Needle aspirates from localized adenocarcinoma and peripheral blood from patients undergoing prostatectomy show skewing of T cells toward both a Treg and TH17 phenotype (31). Cells double positive for IL-17 and Foxp3 have been observed in mismatch repair proficient colorectal cancer (32). Our observations are consistent with the findings and extend these by showing that the CD4+Foxp3+IL-17+ T cells can have a potent proinflammatory and therefore tumor-promoting role.

We have noticed high levels of IL-17 and IL-23 in the sera of polyp-ridden mice and that polyp-infiltrating CD4+Foxp3+ cells express IL-17. Based on these findings and the ability of these Treg cells to promote mast cell expansion, we propose the term protumor T cells to describe the CD4+Foxp3+IL-17+ T cells. A plethora of recent data has emerged on the prevalence of IL-17-producing T cells in cancer. Tumor-secreted lactic acid is reported to promote T17 differentiation (33). IL-17-producing CD8 T cells promote growth of transplanted tumors in mice by suppressing apoptosis (34). IL-17–producing T cells promote inflammation and activate the p38, c-Jun NH2-terminal kinase, and extracellular signal-regulated kinase pathways in xenograft transplanted human gastric cancer cells (35). IL-17 is up-regulated in prostate cancer (36), and IL-17–positive cells are enriched in the bone marrow of myeloma patients (37). Up-regulation of IL-17 in progressive cancer is consistent with a pathogenic role in tumor promotion. However, we cannot rule out the possibility that expression of IL-17 by tumor-specific cytotoxic cells may be in other circumstances protective.

An intimate link between IL-17 and focal mastocytosis in cancer is emerging. IL-17 is associated with the increased production of SCF, a critical mediator of mast cell expansion and maturation (38). Mast cells in turn promote IL-17–dependent recruitment of secondary inflammatory cells (39). In an earlier study with APCDelta468 mice (10), we provided evidence for mast cells being causatively involved in the progression of preeoplastic adenomatous polyps (10). Animal modeling and studies with human tumor samples suggest that the tumor-promoting role of mast cells is not unique to polyposis. Angiogenesis has been a focus of attention as a potential mechanism of action of mast cells in promoting skin cancer (40) and pancreatic islet cell dysplasia (41) in mice genetically predisposed to these diseases. Similar observations were reported with transplantable tumor cell lines, where secretion of SCF tumor by tumor cells seemed to be responsible for homing of mast cells (42). Mast cells are common in several human cancers, including Merkel cell carcinoma (43), breast (44), lung (45), and Hodgkin’s lymphoma (46). Mast cell infiltration correlates well with tumor angiogenesis and metastases in gastric cancer (47), colorectal cancer (48), pulmonary adenocarcinoma (45), renal cell cancer (49), and prostate cancer (50). Altogether, these observations justify the choice of mast cells as candidate proinflammatory cells etiologically linked with tumor progression.

Based on our findings, we propose that early in cancer Treg cells are functionally diverted into protumor T cells and play an important role in shaping the microenvironment of developing tumors. A major contribution of such tumor-promoting T cells to cancer is the escalation of cancer-associated inflammation. At the same time, the cells intercept tumor-specific T cells, providing a dual advantage to the developing tumor. These observations provide the rationale for targeting Treg cells and their interaction with mast cells for therapeutic intervention in dysplasia and cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

Received 1/27/09; revised 4/1/09; accepted 4/15/09; published OnlineFirst 6/30/09.

Grant support: RO1-CA104547. K. Khazaie is the recipient of American Cancer Society Research Scholar grant 113422 RSG and Zell Family Award of Robert H. Lurie Cancer Center.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.


35. Zhou Y, Toh ML, Zrioual S, Miossec P. IL-17A versus IL-17F induced intracellular signal transduction pathways and modulation by IL-17RA and IL-17RC RNA interference in AGS gastric adenocarcinoma cells. Cytokeine 2007;38:157–64.


T-Regulatory Cells Shift from a Protective Anti-Inflammatory to a Cancer-Promoting Proinflammatory Phenotype in Polyposis

Elias Gounaris, Nichole R. Blatner, Kristen Dennis, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/69/13/5490

Supplementary Material
Access the most recent supplemental material at:
http://cancerres.aacrjournals.org/content/suppl/2009/07/21/69.13.5490.DC1

Cited articles
This article cites 50 articles, 25 of which you can access for free at:
http://cancerres.aacrjournals.org/content/69/13/5490.full.html#ref-list-1

Citing articles
This article has been cited by 25 HighWire-hosted articles. Access the articles at:
/content/69/13/5490.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.