Brief report

Endogenous IL-17 contributes to reduced tumor growth and metastasis

Ilona Kryczek,1 Shuang Wei,1 Wojciech Szeliga,1 Linhua Vatan,1 and Weiping Zou1

1Department of Surgery, University of Michigan, Ann Arbor

It has been reported that ectopically expressed interleukin-17 (IL-17) in tumor cells suppresses tumor progression through enhanced antitumor immunity in immune competent mice or promote tumor progression through an increase in inflammatory angiogenesis in immune-deficient mice. The role of endogenous IL-17 in tumor immunity remains undefined. Here we showed that tumor growth and lung metastasis were enhanced in IL-17–deficient mice, associated with decreased interferon-γ+ natural killer cells and tumor-specific interferon-γ+ T cells in the tumor draining lymph nodes and tumors. Together with the published data showing that in vitro transforming growth factor-β and IL-6–polarized Th17 cells induce tumor regression, our work supports the notion that endogenous IL-17 or Th17 cells may play a protective role in tumor immunity.

Introduction

Interleukin-17 (IL-17) and Th17 cells play an active role in inflammation and autoimmune diseases in murine systems.1-9 Th17 cells are found in both mouse and human tumors.10-12 However, the role of endogenous IL-17 in tumor immunopathogenesis remains undefined. In this report, we evaluated tumor growth and metastasis in IL-17–deficient mice. We provide novel insight into the role of IL-17 in tumor immunity.

Methods

Mice

C57BL/6 mice were purchased from The Jackson Laboratory. IL-17–deficient C57BL/6 mice were previously described.13 MC38 cells were injected subcutaneously or intravenously (105) into IL-17–deficient and normal C57BL/6 mice. Subcutaneous tumor size was measured twice weekly using calipers fitted with a Vernier scale. Tumor volume was calculated based on 3 perpendicular measurements.14 At day 30 after intravenous tumor inoculation, mice were killed and lung metastatic foci were quantified as we described.15 The study was approved by the Committee on Use and Care of Animals at the University of Michigan.

Cell purification and staining

Single-cell suspensions were made from tumor tissues, spleens, non–tumor-draining lymph nodes, and tumor-draining lymph nodes. The phenotype and cytokine profile of each individual immune cell subset were determined by flow cytometric analysis (fluorescence-activated cell sorter).10,11 Natural killer (NK) and T cells were enriched using paramagnetic beads (StemCell Technologies).

ELISPOT assay

MultiScreen filtration plates (96-wells/plate; Millipore) were coated with 4 μg/mL anti–mouse interferon-γ (IFN-γ) monoclonal antibody (clone R4-6A2; BD Biosciences). Spleen NK cells and lymph node T cells were added (2 × 105 cells/well) and stimulated for 24 hours with or without irradiated (60 Gy) mouse MC38 or YAC-1 cells (5 × 104 cells) or with IL-2 (50 ng/mL), or phorbol-12-myristate-13-acetate (5 ng/mL) and ionomycin (500 ng/mL). Bonded IFN-γ was detected by biotinylated rat anti–mouse IFN-γ monoclonal antibody (clone XMG1.2; BD Biosciences) followed by antibiotin alkaline phosphatase. Spots were visualized with 5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium alkaline phosphatase substrate and counted using the ImmunoSpot analyzer (Cellular Technology). Data are reported as an average number of spots per 105 responders plus or minus SEM of triplicate samples.

Statistical calculations

The Mann-Whitney U test was used to determine pairwise differences, and the χ2 test was used to determine differences between groups. A P value less than .05 was considered significant.

Results and discussion

We studied the effect of IL-17 on tumor growth in IL-17–deficient mice. MC38, a murine colon cancer cell line, was subcutaneously inoculated into the wild-type and IL-17–deficient mice. The IL-17–deficient mice exhibited an accelerated tumor growth compared with the control mice (P < .01; Figure 1A). We further compared the metastatic potential of MC38 cells by intravenously inoculating cells into wild-type and IL-17–deficient mice. We observed more metastatic foci of tumors in the lungs of IL-17–deficient mice (59 ± 8) than in control mice (12 ± 5; P = .01; Figure 1B). These data suggest that endogenous IL-17 may play a protective role in tumor immunity.

We next examined the phenotype and cytokine profile of tumor-infiltrating immune cells in tumor-bearing mice. We observed similar levels of CD4+FOXP3+ T cells, CD8+ T cells, and FOXP3–CD4+ and IL-10–CD4+ T cells in the tumor microenvironments in both IL-17–deficient and wild-type mice (Figure 2A-B;
supplemental Table 1, available on the Blood website; see the Supplemental Materials link at the top of the online article. However, the levels of tumor-infiltrating IFN-γ+ T cells were significantly higher in wild-type mice than IL-17−/− mice (Figure 2C). We also examined tumor-draining lymph nodes. Although the levels of CD8+ T cells and FOXP3+ T cells were comparable (supplemental Table 1), there were fewer NK cells (P < .01; Figure 2D), IFN-γ+ NK cells, IFN-γ−CD4+ T cells, and IFN-γ−CD4− T cells in the tumor-draining lymph nodes in the IL-17−/− mice than the control mice (P < .05; Figure 2E). We further evaluated MC38-specific effector T-cell responses and NK-cell activity against YAC-1 in these mice. ELISPOT assay demonstrated that tumor-specific IFN-γ− T cells and IFN-γ− NK cells were decreased in tumor-draining lymph node T cells in the IL-17−/− mice (Figure 2F top panel; P < .001 for all). There were fewer than 4 nonspecific spots in NK-cell assay and fewer than 20 nonspecific spots in T-cell assay in the absence of specific stimuli. Altogether, the data indicate that both innate immunity and tumor specific T-cell immunity are weakened in the tumor-bearing IL-17−/− mice.

To determine whether the reduced NK- and T-cell responses in the tumor-bearing IL-17−/− mice are specifically associated with tumor inoculation and development, we examined and compared the number and activities of NK and T cells in non–tumor-bearing IL-17−/− mice and wild-type mice. The absolute number and the percentage of CD4+ and CD8+ T cells, NK cells, and CD4+FOXP3+ regulatory T cells (Tregs) were comparable in spleens and lymph nodes in non–tumor-bearing IL-17−/− and wild-type mice (supplemental Table 1). The expression of
IFN-γ was moderately reduced in IL-2-activated NK cells in IL-17–deficient mice (P > 0.05) and was moderately reduced in IL-2-activated NK cells in IL-17–deficient mice (P < 0.05), compared with wild-type mice (Figure 2F bottom panel). Altogether, the data suggest that the reduced function of NK and T cells is manifested upon in vivo tumor challenging in IL-17–deficient mice.

The role of IL-17 or Th17 cells in tumor pathogenesis is not well defined. Recombinant IL-17 has no significant effect on MC38 proliferation, cell cycle, and apoptosis (supplemental Figure 1). Forced overexpression of IL-17 ectopically in tumor cells can either suppress tumor progression through enhanced antitumor immunity in immune-competent mice,16,17 or promote tumor progression through an increase in inflammatory angiogenesis in immune-deficient mice.18,19 These studies focus on the effects of exogenous IL-17 in established mouse tumor cell lines. Our data demonstrate that tumor growth in subcutaneous and lung metastasis are enhanced in IL-17–deficient mice and accompanied with reduced IFN-γ+ NK cells and tumor-specific IFN-γ+ T cells in the tumor-draining lymph nodes and tumors. It suggests that endogenous IL-17 positively impacts on tumor immunity. Along this line, IL-17 has been reported to be a potent activator of innate immunity.15,20 Notably, IL-17–deficient mice do not show a global immunodeficiency without appropriate challenging. T-cell proliferation and cytokine production are basically normal in response to in vitro mitogenic stimulation, and acute graft-versus-host responses remain intact in IL-17–deficient mice.13 However, in IL-17–deficient mice, T-cell responses are reduced in chronic immune responses, such as contact hypersensitivity, delayed-type hypersensitivity, and airway hypersensitivity response priming.13 On in vitro stimulation, the numbers of IFN-γ+ T cells are slightly and IFN-γ+ NK cells moderately reduced in non–tumor-bearing IL-17–deficient mice. It indicates a potential defect of NK- and T-cell function in IL-17–deficient mice. This defect is further amplified by MC38 tumor challenging. Although our data emphasize the importance of endogenous IL-17 in antitumor immunity in MC38 tumor-bearing mice, it remains to be determined whether endogenous IL-17 is involved in regulating tumor immunity in other tumor models, particularly multiple human epithelial carcinomas. Furthermore, it will be important to dissect how endogenous IL-17 affects the numbers of functional NK and effector T cells in tumor-draining lymph nodes and tumor environment.

Given the close relationship between endogenous IL-17 and Th17 cells, although our study does not focus on the role of Th17 cells in tumor immunity, it is probable that Th17 cells may affect tumor pathology in humans. For example, adoptively transferred transforming growth factor-β and IL-6–polarized murine transgenic T cells induce tumor regression.21 Thus, our work supports the notion that endogenous IL-17 and/or Th17 cells may promote protective tumor immunity.

Acknowledgments

The authors thank Dr Ikawara of the University of Tokyo for providing the IL-17–deficient mice.

This work was supported in part by the National Cancer Institute (grants CA123088, CA099985) (W.Z.).

Authorship

Contribution: I.K., S.W., W.S., and L.V. performed research; and I.K., S.W., and W.Z. analyzed data and wrote the paper.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

Correspondence: Weiiping Zou, University of Michigan School of Medicine, 1150 W Medical Center, MSRB II, C560, Ann Arbor, MI; e-mail: wzou@med.umich.edu

References


2. Weaver CT, Harrington LE, Mangan PR, Gavrieli M, Murphy KM. Th17, an effector CD4 T cell lineage with regulatory T cell ties. Immunity. 2006;24:677-688.


Endogenous IL-17 contributes to reduced tumor growth and metastasis

Ilona Kryczek, Shuang Wei, Wojciech Szeliga, Linhua Vatan and Weiping Zou