IL-17 Promotes Tumor Development through the Induction of Tumor Promoting Microenvironments at Tumor Sites and Myeloid-Derived Suppressor Cells

Donggou He, Hui Li, Nabiha Yusuf, Craig A. Elmets, Jun Li, John D. Mountz and Hui Xu

J Immunol 2010; 184:2281-2288; Prepublished online 29 January 2010;
doi: 10.4049/jimmunol.0902574
http://www.jimmunol.org/content/184/5/2281
IL-17 Promotes Tumor Development through the Induction of Tumor Promoting Microenvironments at Tumor Sites and Myeloid-Derived Suppressor Cells

Donggou He,* Hui Li,* Nabiba Yusuf,*†§ Craig A. Elmets,*†§ Jun Li,§ John D. Mountz,†§ and Hui Xu*†*

The role of immune responses in tumor development is a central issue for tumor biology and immunology. IL-17 is an important cytokine for inflammatory and autoimmune diseases. Although IL-17–producing cells are detected in cancer patients and tumor-bearing mice, the role of IL-17 in tumor development is controversial, and mechanisms remain to be fully elucidated. In the current study, we found that the development of tumors was inhibited in IL-17R–deficient mice. A defect in IFN-γR increased tumor growth, whereas tumor growth was inhibited in mice that were deficient in both IL-17R and IFN-γR compared with wild-type animals. Further experiments showed that neutralization of IL-17 by Abs inhibited tumor growth in wild-type mice, whereas systemic administration of IL-17 promoted tumor growth. The IL-17R deficiency increased CD8 T cell infiltration, whereas it reduced the infiltration of myeloid-derived suppressor cells (MDSCs) in tumors. In contrast, administration of IL-17 inhibited CD8 T cell infiltration and increased MDSCs in tumors. Further analysis indicated that IL-17 was required for the development and tumor-promoting activity of MDSCs in tumor-bearing mice. These data demonstrate that IL-17–mediated responses promote tumor development through the induction of tumor-promoting microenvironments at tumor sites. IL-17–mediated regulation of MDSCs is a primary mechanism for its tumor-promoting effects. The study provides novel insights into the role of IL-17 in tumor development and has major implications for targeting IL-17 in treatment of tumors. The Journal of Immunology, 2010, 184: 2281–2288.

Immune responses have paradoxical roles in tumor development (1, 2). On the one hand, immune responses play a key role in immune surveillance for prevention of tumor development. Numerous studies indicate that antitumor immune responses are able to prevent and eliminate tumors. On the other hand, however, immune responses, especially in a form of chronic inflammation, promote tumor development in many cases (3, 4). A prominent feature of tumor-promoting immune responses is the increased number of myeloid-derived suppressor cells (MDSCs) in the blood, spleen, and bone marrow (BM) and abundant infiltration of MDSCs at the tumor site (5–8). Heavy infiltration of MDSCs has been considered as a major cause for immunosuppression at tumor sites (5, 8, 9). MDSCs are considered as an immature form of myeloid cells that are mostly identified as CD11b and Gr-1 double-positive cells in mice (7, 10). MDSCs are able to suppress antitumor immune responses and promote tumor growth (5, 8). Recent studies have shown that MDSCs are composed of two subpopulations, which suppress T cell responses by different mechanisms (11, 12). Inflammatory cytokines and tumor-derived mediators have been reported to regulate MDSCs (5–8). However, mechanisms for the development and function of MDSCs remain to be fully elucidated.

IL-17 is an inflammatory cytokine secreted by CD4 Th17 and CD8 Tc17 cells (13–17). Six IL-17 family members (IL-17A–F) have been described, and the prototype member of the family is IL-17A, often termed IL-17 in literature. The receptor for IL-17A and IL-17F, IL-17R, is expressed ubiquitously (14). IL-17 plays an important role in the regulation of leukocyte migration in inflammatory reactions, and a defect in IL-17R decreases the expression of cytokines and chemokines and reduces the infiltration of inflammatory cells, especially neutrophils (15, 18–22). The role of IL-17 in inflammatory and autoimmune diseases has been studied extensively (13, 15, 16, 23).

Although IL-17–producing cells are detected in cancer patients and tumor-bearing mice (24–27), the role of IL-17 in tumor development is controversial (21, 28–34). Recent reports indicate that tumor growth is increased in IL-17−/− mice and that the mechanism is associated with IFN-γ–producing NK and T cells (31, 34). It implicates that IL-17–mediated responses are protective against tumor development. However, another recent report shows that tumor growth is suppressed in IL-17−/− and IL-17/IFN-γ double-knockout mice (30). A mechanism is that IL-17 induces the production of IL-6 by tumor cells, which in turn promotes tumor growth in a Stat3–dependent pathway. Interestingly, the report shows that the production of IFN-γ by tumor-infiltrating T cells from IL-17−/− mice is increased (30). Although IFN-γ plays a role in the regulation of antitumor immune responses (35–37), CTL activity of tumor-specific T cells is an important mechanism for T cell-mediated tumor rejection. It is not examined whether IL-17 regulates the CTL activity of tumor-specific CD8 T cells. Moreover, it is largely unknown whether

*Department of Dermatology, †Department of Medicine, and ‡Skin Disease Research Center, University of Alabama, Birmingham, AL 35294; and §Birmingham Veterans Affairs Hospitals Medical Center, Birmingham, AL 35233

Received for publication August 7, 2009. Accepted for publication December 20, 2009.

This work was supported by National Institute of Arthritis and Musculoskeletal and Skin Diseases Grant AR46256 and Skin Diseases and National Institute of Allergy and Infectious Diseases Grant AI071041 (to H.X.). This investigation was conducted in a facility constructed with support from Research Facilities Improvement Program Grant C06 RR15490 from the National Center for Research Resources, National Institutes of Health.

Address correspondence and reprint requests to Dr. Hui Xu, VHS66B, 1670 University Boulevard, University of Alabama, Birmingham, AL 35294. E-mail address: xuhui@uab.edu

Abbreviations used in this paper: Ad, adenovirus; Arg-1, arginase-1; BM, bone marrow; BM-DC, bone marrow-derived dendritic cell; MDSC, myeloid-derived suppressor cell; MMP9, matrix metalloproteinase 9; PCNA, proliferating cell nuclear Ag.

Copyright © 2010 by The American Association of Immunologists, Inc. 0022-1767/10/$16.00
IL-17–mediated effects on innate immune cells, such as MDSCs, play a role in tumor immune responses. It is often observed that T cells from tumor patients retain the ability to respond to tumor Ags. However, immune responses in peripherals are not correlated with tumor rejection (38–40). Mechanisms for immunosuppression include failure of innate T cell infiltration into tumors and presence of regulatory T cells and immunosuppressive myeloid cells at tumor sites (8, 41–43). The infiltration of immune T cells in tumors is associated with good prognosis (44), whereas infiltration of MDSCs is associated with poor prognosis (5, 8). Although it is well documented that IL-17 is an important cytokine for the regulation of leukocyte infiltration in inflammatory tissues (15, 18–21), it remains to be examined whether IL-17 plays a role in the regulation of immune responses at tumor sites.

In the current study, we examined the effect of endogenous IL-17 on the growth of tumors in mice that were deficient in IL-17 receptor A (IL-17R/−/−). Additionally, anti–IL-17 Abs and exogenous IL-17 cytokine were applied to further confirm the effect of IL-17 on tumor development. We examined IL-17–mediated effects on the infiltration of tumor-specific T cells and myeloid cells in tumors and determined the role of IL-17 in the development of MDSCs in tumor-bearing mice. Our findings indicate that IL-17 promotes tumor development through the induction of tumor-promoting responses at tumor sites and enhancement of MDSCs.

Materials and Methods

**Mice**

IL-17R/−/− mice on C57BL/6 background were provided by Amgen (Thousand Oaks, CA). IFN-γR/−/− (C57BL/6 background) and wild-type C57BL/6 mice were purchased from The Jackson Laboratory (Bar Harbor, ME). All cell lines were cross-bred to IFN-γR/−/− mice to generate double-knockout mice in our laboratory. The gene phenotype was routinely confirmed by PCR using specific primers. All animal procedures were performed according to National Institutes of Health guidelines, and the protocols were approved by the Institute Animal Care and Use Committee of the University of Alabama.

Transplanted tumor models: Protocols for inoculation and measurement of tumors in transplanted tumor models were reported previously (45). Mouse lymphoma cell line EL4, E.G7-OVA (EL4 transfected with OVA gene), prostate tumor cell line Tramp-C2, and melanoma cell line B16-F10 were purchased from the American Type Culture Collection. Tumor cells were injected s.c. in mice, and tumor growth was monitored every 3 d. Tumor sizes were calculated with the formula: tumor size = L × W 2/2 (L, long diameter; S, short diameter; H, height).

To examine the effect of IL-17 on tumor growth, mice were treated i.v. with adenovirus (Ad)-encoding GFP or mouse IL-17A (Ad–IL-17) (109 PFU/mouse) (provided by Dr. J. K. Kolls, Division of Pediatric Pulmonary Medicine, Allergy and Immunology, University of Pittsburgh, Pittsburgh, PA) as described previously (46). Two days later, the mice were inoculated with E.G7-OVA tumor cells.

To examine the effect of neutralizing IL-17 on tumor growth, mice were inoculated s.c. with E.G7-OVA tumor cells and treated i.p. with normal rat IgG or a rat anti-mouse IL-17 mAb (100 μg/mouse) (TC11-18H10 and anti–IL-17A; Southern Biotechnology Associates, Birmingham, AL) on days 0 (the day of tumor inoculation), 1, 6, 10, and 14.

To examine effects of MDSCs on tumor growth, MDSCs were purified from spleens of tumor-bearing mice by using anti-CD11b Ab-coupled magnet beads according to the manufacturer’s instructions (MACS system; Miltenyi Biotec, Auburn, CA). The purity of the cells was >90% as assessed by flow cytometry. The cells were cocultured with E.G7-OVA tumor cells (4 × 105/mouse) in wild-type mice. In experiments using B16 tumors, MDSCs (2 × 107/mouse) were cocultured s.c. with B16 melanoma cells (1 × 107/mouse) in wild-type mice. Tumor growth was monitored.

**Immunohistochemical staining of tissues**

Immunohistochemical staining of tissue sections was described in our previous studies (17). Frozen tissue sections (5 μm) were made for staining of CD8-positive cells. Paraffin-embedded sections (6 μm) were made for staining of anti-proliferating cell nuclear Ag (PCNA)-positive cells. The Abs were purchased from Santa Cruz Biotechnology (Santa Cruz, CA) and BD Biosciences (San Diego, CA). Pictures were taken microscopically (×10 objective) with a digital camera (Olympus, Melville, NY). Positive cells were counted in 10 fields of each group. Average numbers of positive cells per field were calculated and analyzed statistically.

**Flow cytometry analysis**

To examine tumor infiltrating cells, tumor tissues were cut into small pieces and digested in RPMI 1640 medium containing collagenase D (2 mg/ml) (Sigma-Aldrich, St. Louis, MO), Dnase I (50 μg/ml) (Sigma-Aldrich), and 10% FCS. To detect MDSCs, cell suspensions of tumors and spleens on blood leukocytes were stained with Alexa648-labeled CD11b and allophycocyanin-labeled Gr-1 Abs (BD Biosciences). The percentage of cells that expressed a high level of both CD11b and Gr-1 was analyzed in a flow cytometer (FACSCalibur; BD Biosciences) as described previously (17). To detect tumor Ang OVA–specific CD8 T cells in tumors, tumor cell suspensions were stained with Alexa680-labeled anti-CD8 Ab and PE-labeled OVA/MHC class I tetramers, which bind to OVA specific CD8 T cells (Terasaki et al.; Beckman Coulter, Fullerton, CA). CD8 T cells were gated, and the percentage of the tetramer-positive cells was analyzed by flow cytometry.

**Assays for T cell functions**

CD8 T cells from spleens of tumor-bearing mice were purified by using the MACS system according to the manufacturer’s instructions (Miltenyi Biotec, Auburn, CA). In our hands, this procedure results in >90% selection of CD8 T cells. Bone marrow-derived dendritic cells (BM-DCs) were generated from BM of C57BL/6 as described in our previous report (17) and pulsed with OVA (100 μg/ml) for overnight.

To detect cytokine production by tumor-specific T cells, spleen cells from tumor-bearing mice were stimulated with OVA-pulsed BM-DCs for 4 d (2 × 107 T cells plus 2 × 105 DCs/ml). Our preliminary data showed that the stimulation of T cells with OVA-pulsed BM-DCs for 4 d in vitro resulted in high levels of cytokine-producing cells and cytokines in cultures compared with cultures for 1, 2, or 3 d (data not shown). Concentrations of cytokines in supernatants were measured by cytokine specific ELISA as described in our previous studies (22). To detect cytokine-producing T cells, cells were harvested 4 d after cultures and stimulated with PMA (50 ng/ml) and ionomycin (500 ng/ml) in the presence of GolgiStop (BD Pharmingen, San Diego, CA) for 4–6 h. Intracellular cytokines were stained, and cytokine-producing cells were analyzed in a flow cytometer as described in our previous report (17).

To examine the CTL activity of tumor-specific CD8 T cells, CD8 T cells were purified from spleens of E.G7-OVA tumor-bearing mice and stimulated in vitro with OVA-pulsed BM-DCs for 5 d (2 × 107 T cells plus 2 × 105 DCs/ml). E.G7-OVA tumor cells were labeled with 5 μM CFSE and used as control cells. E.G7-OVA and EL4 cells were mixed at 1:1 ratio and used as target cells. The CD8 T cells, which were harvested after the in vitro stimulation, were incubated with mixed target cells (E.G7-OVA/EL4) (1 × 105/well) at ratios of 100:1, 50:1, 25:1, and 1:1 (control) for 4 h. Each sample was quadruplicated. The specific lysis of target cells was calculated with the formula as reported (47). The percent-specific cytotoxicity = 100 × (1 – [E.G7-OVA/EL4] experimental/[E.G7-OVA/EL4] control).

To examine the effect of MDSCs on T cell activation, CD4 T cells were purified from naive wild-type mice by the MACS system and labeled with a fluorescent dye CFSE (Molecular Probes, Eugene, OR) at 5 μM for 20 min. The CFSE-labeled CD4 T cells were then placed in cultures with purified MDSCs from wild-type or IL-17R/−/− tumor-bearing mice (2 × 105 T cells plus 1 × 106 MDSCs/ml). CD4 T cells from tumor-free wild-type mice served as controls. The cultures were stimulated with plate-bound anti-CD3 (20 μg/ml; BD Pharmingen) and anti-CD28 (20 μg/ml; BD Pharmingen) Abs for 4 d. T cells that were not stimulated with the Abs served as negative controls. The division of CD4 T cells was analyzed by flow cytometry.

**TUNEL assay**

Apoptotic cells were detected by TUNEL assay as described in our previous report (45). Briefly, tumor samples were fixed in 10% formalin, and sections (5 μm) were made. TUNEL assay was performed using a commercial apoptosis detection kit according to the manufacturer’s instructions (Promega, Madison, WI). Sections were counterstained with DAPI and photographed microscopically with a ×10 objective. The number of apoptotic cells was counted, and results from 10 fields of each group were calculated for statistical analysis.

**Real-time quantitative RT-PCR**

The expression of mRNA was quantified by real-time RT-PCR as described in our previous report (22). Briefly, MDSCs were purified from spleen of tumor-bearing mice by using the MACS system and stimulated with LPS.
(5 μg/ml) for overnight. Total RNA was isolated from the cells by using TRIzol Reagent according to the manufacturer’s instructions (Invitrogen Life Technologies, Carlsbad, CA). Real-time RT-PCR was performed with iQ SYBR Green Supermix Kit in a MyiQ real-time quantitative PCR system according to the manufacturer's instructions (Bio-Rad, Hercules, CA). The expression level of cytokines was normalized to the housekeeping gene GAPDH in each sample. The sequences for primers were as follows: arginase-1 (Arg-1), forward, 5'-AGGAGAGGACCTTCCAAGC-T3'; and reverse, 5'-CTTGCTTCCCCGATGTGACG-3'; S100A8, forward, 5'-GCGAGTCCTCTTGGAAGG-3'; S100A9, forward, 5'-CTTGCTTCTTCCAAAGATTGGGG-3'; S100A9, forward, 5'-AGCCAACGACCATACAGA-3'; and GAPDH, forward, 5'-AATGTGAAGGTGCTGTGAAC-3', and reverse, 5'-GAAGATGGTGATGGGCTTCC-3'.

Statistical analysis

All data are presented as means ± SEM. The two-tailed Student t test was applied for statistical analysis with p < 0.05 being considered statistically significant.

Results

Tumor growth is inhibited in IL-17R−/− mice

The effect of IL-17 on tumor growth is controversial (21, 29–31). It is not yet reported whether a defect in IL-17R affects tumor development. To examine the role of IFN-γ in IL-17–mediated regulation of tumor development, we have generated IL-17R/IFN-γR double-knockout mice. Mice were inoculated s.c. with a high-immunogenic cell line E.G7-OVA (4 × 10⁶ cells/mouse). Results showed that tumor growth was increased in IFN-γR−/− mice, whereas it decreased in IL-17R−/− mice compared with wild-type mice (Fig. 1D). In the double-knockout mice that were deficient in both IL-17R and IFN-γR, tumor growth was significantly reduced compared with wild-type mice (Fig. 1D). This suggests that the IL-17R deficiency inhibited the growth of high-immunogenic tumors. Moreover, although the deficiency in IFN-γR signals enhanced tumor growth, the IL-17R deficiency reversed the susceptibility of IFN-γR−/− mice to tumor development. To examine immunological mechanisms for IL-17–mediated effects on tumor growth, E.G7-OVA tumor cells were applied for statistical analysis with p < 0.05 being considered statistically significant.

To further determine whether IL-17 promoted tumor growth, wild-type mice were injected i.v. with Ad–IL-17 or Ad-GFP (10⁹ PFU/mouse) and then inoculated with E.G7-OVA tumor cells. Results showed that the treatment with Ad–IL-17 significantly increased tumor growth compared with control mice that were treated with Ad-GFP or left untreated (Fig. 2A). In contrast, treatment of wild-type mice with a neutralizing anti-IL-17 Ab significantly inhibited the growth of E.G7-OVA tumors compared with controls that were treated with rat IgG (Fig. 2B).

Analysis of IL-17–mediated effects on tumors

To examine effects of the IL-17R deficiency on tumors, tumor tissues from tumor-bearing mice were collected and subjected to analysis. Results showed that the number of proliferating cells in tumors, which were stained with PCNA Ab, was significantly reduced compared with wild-type animals (Fig. 3A). In contrast, the number of apoptotic cells, which were detected by TUNEL assay, was significantly increased in IL-17R−/− tumors (Fig. 3B). These results suggest that the deficiency in IL-17R inhibits tumor cell proliferation and enhances apoptosis.

The immunity at tumor sites is important for the fate of tumors and the infiltration of T cells is closely associated with prognosis (5, 8, 9, 44). We found that the infiltration of CD8 T cells in tumors, which are major effector cells for tumor rejection, was significantly increased in IL-17R−/− mice compared with wild-type animals (Fig. 3C). The infiltration of CD8 T cells in tumors of IFN-γR−/− was hardly affected, whereas it was significantly increased in the double-knockout mice that were deficient in both IL-17R and IFN-γR. The inhibitory effect of IL-17 on CD8 T cell infiltration in tumors was supported by further experiments showing that the treatment of mice with Ad–IL-17 reduced the infiltration of CD8 T cells in tumors (Fig. 3D). The effect of IL-17 on T cell infiltration appeared to be specific for CD8 T cells, because no significant difference was found in CD4

FIGURE 2. Administration of IL-17 promotes, whereas neutralization of IL-17 inhibits tumor growth in wild-type mice. A, Wild-type mice were treated i.v. with Ad–IL-17 or Ad-GFP (10⁹ PFU/mouse) or left untreated (none). Two days later, the mice were inoculated with E.G7-OVA tumor cells, and tumor growth was monitored (n = 5; *p < 0.01). B, Wild-type mice were inoculated with E.G7-OVA tumor cells and injected i.p. with normal rat-IgG or a rat anti-mouse IL-17 mAb (100 μg/mouse) on days 0, 1, 6, 10, and 14 (n = 4; *p = 0.01). Control mice were left untreated (none). The data show means ± SEM of tumor size and are representative of two to three independent experiments.

FIGURE 1. A defect in IL-17R inhibits tumor development. Mice were inoculated s.c. with tumor cells and tumor sizes were monitored. A, Lymphoma cell line E4, 2 × 10⁶ cells/mouse (n = 5). B, Melanoma cell line B16-F10, 1 × 10⁶ cells/mouse (n = 5). C, Prostate tumor cell line Tramp-C2, 2 × 10⁶ cells/mouse (n = 5). D, E.G7-OVA tumor cells, 4 × 10⁶ cells/mouse (n = 5). Data are presented as the mean tumor size ± SEM and are representative of two to four independent experiments. *p < 0.05; **p < 0.01.

The Journal of Immunology 2008 180: 2283-2289
T cell infiltration in tumors of wild-type and IL-17R−/− mice (data not shown).

IL-17–mediated effects on tumor-specific T cells

On the basis of the increased infiltration of CD8 T cells in tumors, we further characterized tumor-infiltrating CD8 T cells. Results showed that the majority of tumor-infiltrating CD8 T cells bound to OVA/MHC class I tetramers (>70%) (Fig. 4A). There was not a remarkable difference in the percentage of tumor-specific CD8 T cells between wild-type and IL-17R−/− mice, although the infiltration of CD8 T cells was significantly increased in IL-17R−/− mice compared with wild-type animals (Fig. 4B). Further analysis of tumor-infiltrating CD8 T cells did not show a significant change in the expression of CTL-related molecules perforin, Fas ligand, and granzyme B between wild-type and IL-17R−/− tumor-bearing mice (data not shown).

To examine whether the IL-17R deficiency had an effect on T cell function, spleen cells of E.G7-OVA tumor-bearing wild-type and IL-17R−/− mice were collected and stimulated with OVA-pulsed BM-DCs for 4 d. Results showed that CD4 and CD8 T cells that produced IFN-γ were reduced, whereas IL-17–producing CD4 T cells were increased (Fig. 5A). Furthermore, ELISAs showed that IL-17 was increased, whereas IFN-γ was reduced in culture supernatants of IL-17R−/− T cells compared with those of wild-type T cells (Fig. 5B). However, there was not a significant difference in T cell numbers between wild-type and IL-17R−/− mice (data not shown), a result consistent with previous reports (19, 22).

IL-17 is required for the development of MDSCs

An increased number of MDSCs in spleen, blood, and tumors is a hallmark of major immunological abnormalities in cancer patients and tumor-bearing animals (5–7). MDSCs are considered as an immature form of myeloid cells, which are mostly identified as CD11b and Gr-1 double-positive cells in mice (7, 10). We found that the percentage of CD11b/Gr-1 double-positive MDSCs in the spleen of IL-17R−/− tumor-bearing mice was lower than that of wild-type tumor-bearing mice (Fig. 6A). The deficiency in IFN-γR had little effect on MDSCs, whereas MDSCs in the double-knockout mouse that were deficient in both IL-17R and IFN-γR were

---

**FIGURE 3.** IL-17–mediated responses at tumor sites. E.G7-OVA tumor cells were inoculated s.c., and tumor tissues were harvested for analysis. A, Paraffin sections were prepared and stained with anti-PCNA Abs. Samples were counterstained with hematoxylin (blue), and PCNA-positive cells (brown) were counted microscopically. B, Paraffin sections were applied for the TUNEL assay and counterstained with DAPI (blue). Apoptotic cells (green) were counted microscopically. C, Frozen tissue sections were stained with Alexa488-labeled anti-CD8 Ab (green) and counterstained with DAPI (blue). The number of CD8 T cells was counted microscopically. D, Wild-type mice were treated with Ad-IL-17, Ad-GFP, or left untreated and then inoculated with E. G7-OVA tumor cells as described in Fig. 2A. Frozen tumor sections were stained with anti-CD8 Ab, and the number of CD8 T cells was counted microscopically as described above. A–D, Original magnification×10. The data show means±SEM of positive cells per field (n = 10; *p < 0.05; **p < 0.01) and are representative of two to three independent experiments.

---

**FIGURE 4.** IL-17–mediated effects on infiltrating CD8 T cells in tumors. A, Tumor cell suspensions were stained with anti-CD8 Ab and MHC class I/OVA tetramers. The left panel shows CD8 T cells in tumor suspensions. The right panel shows the percentage of tetramer-positive cells in the gated CD8+ cells. B, Statistical analysis shows a significant increase of tumor-infiltrating CD8 T cells in IL-17R−/− mice (n = 5; **p < 0.01). The majority of tumor-infiltrating CD8 T cells are specific for OVA/MHC class I tetramers. There is not a significance between wild-type and IL-17R−/− mice (n = 4). The data show means±SEM and are representative of two independent experiments.
The Journal of Immunology

2285

The role of IL-17 in the regulation of tumor immune responses has not yet to be fully understood. In the current study, we have used IL-17R−/− and IL-17/IFN-γR double-knockout mice to specifically examine mechanisms for IL-17–mediated effects on the phenotype and function of MDSCs in tumor-free naive mice (data not shown).

**IL-17 regulates the phenotype and function of MDSCs**

The tumor-promoting function of MDSC is associated with increased activities of Arg-1, MMP9, and S100A8/A9 (7, 10, 50). To examine mechanisms for IL-17–mediated effects on the phenotype of MDSCs in tumor-bearing mice, MDSCs were purified from spleens of tumor-bearing mice and stimulated with LPS in vitro for overnight. Our initial experiments showed a very low expression level of the molecules by freshly isolated MDSCs in vitro. Further analysis showed that MDSCs in the spleen, blood, and tumors of IL-17R−/−, and the double-knockout mice was significantly reduced compared with wild-type controls (Fig. 6B).

In further experiments, results showed that spleens from tumor-bearing mice that were treated with Ad-IL-17 (as described in Fig. 2A) contained a significantly higher level of MDSCs than those from the mice that were treated with Ad-GFP or untreated control (Fig. 6C). In contrast, the number of MDSCs in the spleen of tumor-bearing mice, which were treated with anti-IL-17 Ab (as described in Fig. 2B), was significantly reduced compared with controls that were treated with normal rat IgG (Fig. 6D). The deficiency in IFN-γR or IL-17R did not have a significant effect on the number of MDSCs in tumor-free naive mice (data not shown).

In experiments examining the tumor promoting function of MDSCs, results showed that coinjection of MDSCs purified from wild-type E.G7-OVA tumor-bearing mice significantly increased tumor growth, whereas coinjection of MDSC from IL-17R−/− tumor-bearing mice did not affect tumor growth compared with control mice that were not injected with MDSCs (Fig. 8A). In contrast, MDSCs from wild-type tumor-bearing mice that were treated with Ad-IL-17 showed a reduced tumor-promoting effect of MDSCs from IL-17R−/− and IL-17/IFN-γR double-knockout mice to a significant greater extent than those from control wild-type tumor-bearing mice that were treated with Ad-GFP (Fig. 8B).

Furthermore, similar results were also observed in mouse B16 melanoma model, which showed a reduced tumor-promoting effect of MDSCs from IL-17R−/− B16 tumor-bearing mice compared with those from wild-type counterparts (Fig. 8C). However, the tumor-promoting activity of MDSCs was dependent on the presence of tumors because coinjection of MDSCs from naive tumor free IL-17R−/− or wild-type mice did not have a significant effect on tumor growth compared with control animals that were not injected with MDSCs (Fig. 8D).
examine the role of IL-17–mediated immune responses in tumor development. Results indicate that IL-17 promotes tumor growth, whereas the blockade of IL-17R inhibits tumor growth and even reverses the susceptibility of IFN-γR−/− mice to tumor development. IL-17 inhibits the infiltration of CD8 T cells but increases the infiltration of MDSC in tumors, a characteristic of a tumor-promoting microenvironment at tumor sites. Furthermore, IL-17 is required for MDSC–mediated tumor-promoting activity but had little effect on the CTL activity of tumor-specific CD8 T cells, although a defect in IL-17R reduces IFN-γ production by T cells. These data demonstrate that IL-17–mediated responses, especially at tumor sites, promote tumor development and provide insights into novel mechanisms by which Th1 and Th17 responses regulate tumor immunity.

A line of evidence indicates that IL-17 has tumor-promoting effects, especially in the context of inflammations (28, 51, 52). A recent report shows that the growth of tumors is inhibited in IL-17−/− and IFN-γ/IL-17 double-knockout mice (30). However, conflicting recent reports show that tumor growth is increased in IL-17−/− mice (31, 34). In the report by Wang et al. (30), IL-17 induces IL-6 production by tumor cells and stromal cells that express IL-17R. IL-6 promotes tumor growth in a Stat-3–dependent pathway. In the report by Kryczek et al. (31), IFN-γ–producing NK and T cells are reduced, which is considered to be a mechanism for the increased tumor growth in IL-17−/− mice. Martin-Orozco et al. (34) showed that application of Th17 cells increased IFN-γ–producing CD8 T cells in lungs with metastatic B16 tumors. Interestingly, Wang et al. (30) show that the infiltration of CD8 T cells in tumors and IFN-γ production by tumor-infiltrating T cells are increased in IL-17−/− mice. The report by Wang et al. (30) and our studies used the mouse melanoma cell line B16 and found similar results in IL-17−/− and IL-17R−/− mice, whereas Kryczek et al. (31) used MC38 sarcoma and Martin-Orozco et al. (34) used B16 metastasis model. It is possible that IL-17 may have different roles in different tumors and tumor models. Additionally, different mouse models (IL-17−/− versus IL-17R−/−) may also contribute to the discrepancy. Notably, the studies reporting protective roles of IL-17 in tumor immunity...
imply that the stimulation of IFN-γ production by IL-17 is important for IL-17–mediated antitumor immunity, and the reduction of IFN-γ production and IFN-γ–producing cells is a primary mechanism for the deficient antitumor immunity in IL-17−/− mice (30, 34). Our results suggest that IFN-γ appears to play a minor role in IL-17–mediated regulation of tumor development because tumor growth is inhibited in the IL-17R/IFN-γR double-knockout mice (Fig. 1D). This result is consistent with Wang’s report (30) showing that tumor growth is inhibited in IL-17/IFN-γ double-knockout mice. Our studies implicate that IL-17–mediated tumor promotion is associated with the inhibition of CD8 T cell infiltration in tumors and enhancement of MDSC development and function. Importantly, the CTL activity of tumor-specific CD8 T cells is not significantly affected by the IL-17R deficiency, although IFN-γ production is inhibited (Fig. 5). The inhibition of MDSCs is a critical mechanism for the suppression of tumor growth in IL-17R−/− and IL-17R/IFN-γR double-knockout mice. Additionally, certain studies are required to further determine why IL-17 has different effects on modulating tumor development in different tumor models. An important issue is the effect of IL-17 responses on the function of tumor-infiltrating CD4 and CD8 T cells, which is under our ongoing studies.

MDSCs are considered as an immature form of myeloid cells, which are present in the blood, spleen, and BM of normal mice, and are increased in infectious diseases and tumors (5–8, 10). MDSCs play important roles in the suppression of immune responses and promotion of tumor growth (5, 6, 8). Although IL-17 production is increased in inflammatory reactions and considered as an inflammatory cytokine-promoting tumor development (28, 51, 52), it was not known whether IL-17 had an effect on MDSCs in tumor-bearing hosts. Our studies have implicated that IL-17 is required for the development of MDSCs in tumor-bearing mice. A defect in IL-17R reduces the number of MDSCs in the blood, spleen, and tumors. This is further supported by our data showing that the administration of exogenous IL-17 increases the number of MDSCs in wild-type tumor-bearing mice, whereas neutralization of IL-17 in wild-type tumor-bearing mice reduced the number of MDSCs. It will be very interesting to examine whether IL-23 mediated promotion of tumor development, which is associated with an increased level of IL-17 and myeloid cell infiltration in tumors (28), is linked to IL-17–mediated regulation of MDSCs.

MDSCs inhibit immune responses and promote tumor growth by complexes of mechanisms (6, 53). We show that MDSCs from wild-type tumor-bearing mice exhibit high levels of Arg-I, A100/ A8/A9, and MMP9 molecules, which are known to be mediators for MDSC-mediated immunosuppression and tumor promotion (5, 7, 8, 50). However, MDSCs from IL-17R−/− tumor-bearing mice express a low level of the molecules, which is almost comparable to that of MDSCs from tumor-free naive mice. Accordingly, coapplication of MDSCs from IL-17R−/− tumor-bearing mice does not have a significant effect on tumor growth. In contrast, the treatment with exogenous IL-17 increases the expression level of the molecules and enhances the tumor-promoting activity of MDSCs. Furthermore, MDSCs from IL-17R−/− tumor-bearing mice do not have a significant immunosuppressive effect on T cell activation compared with the wild-type counterparts. These data demonstrate that IL-17 signals are required not only for the development but also for the tumor-promoting and immunosuppressive activity of MDSCs.

Heavy infiltration of MDSCs has been considered as a major cause for immunosuppression at tumor sites (5, 8, 9). The infiltration of immune T cells in tumors is associated with good prognosis (44), whereas infiltration of MDSCs is associated with poor prognosis (5, 8). Our data show that although IL-17 does not have inhibitory effects on tumor-specific T cells or even stimulates IFN-γ production by T cells, its opposite effects on the infiltration of CD8 T cells and MDSCs in tumors imply an important mechanism for IL-17–mediated tumor-promoting microenvironments at tumor sites. This is supported by our data showing that proliferation is inhibited, whereas apoptosis is increased in the tumors of IL-17R−/− mice (Fig. 3). It is to note that the tumor growth is increased, whereas the number of MDSCs is not significantly affected in IFN-γR−/− mice. Because IFN-γ signals are known to have antitumor activities (35–37), the deficiency in IFN-γ–mediated antitumor immunity may lead to the increased tumor growth. However, the reduction of MDSCs provides an interpretation for the suppression of E.G7-OVA tumor growth in IL-17R/IFN-γR−/− compared IFN-γR−/− mice. A recent report shows that MDSCs are able to inhibit the migration of T cells by downregulating the expression of L-selectin by T cells (54). Further experiments are required to determine whether IL-17–mediated opposite effects on CD8 and MDSC infiltration is dependent on the interaction of MDSCs with CD8 T cells or the induction of specific chemokines for the cells.

In summary, our studies have demonstrated that IL-17 promotes tumor growth. Moreover, a defect in IL-17–mediated responses can reverse the susceptibility to tumor development in mice that are deficient in IFN-γ–mediated antitumor responses. IL-17 induces tumor-promoting microenvironments at tumor sites, and its effects on MDSC represent an important mechanism for the tumor-promoting effect. Our studies provide insights into novel mechanisms by which IL-17 mediates tumor-promoting immune responses at tumor sites. Targeting IL-17 may be developed to new immunotherapeutic strategies for treatment of tumors.

Acknowledgments
We thank Dr. Dr. Jay K. Kolls for providing adenovirus encoding mouse IL-17 and Dr. Hui-Chen Hsu for helpful discussions about experiments.

Disclosures
The authors have no financial conflicts of interest.

References