Interleukin-17 promotes angiogenesis and tumor growth

Muneo Numasaki, Jun-ichi Fukushi, Mayumi Ono, Satwant K. Narula, Paul J. Zavodny, Toshio Kudo, Paul D. Robbins, Hideaki Tahara, and Michael T. Lotze

Interleukin-17 (IL-17) is a CD4 T-cell–derived proinflammatory cytokine. We investigated the effects of locally produced IL-17 by tumors as a means to evaluate its biologic function. Although recombinant IL-17 protein or retroviral transduction of IL-17 gene into tumors did not affect in vitro proliferation, IL-17 transfectants grew more rapidly in vivo when compared with controls. Immunostaining for Factor VIII revealed that tumors transduced with IL-17 had significantly higher vascular density when compared with controls. IL-17 indeed elicited neovascularization in rat cornea. In addition, angiogenic activity present in the conditioned media of CD4 T cells was markedly suppressed by neutralizing monoclonal antibody to IL-17. IL-17 had no direct effect on the growth of vascular endothelial cells, whereas IL-17 significantly stimulated migration. IL-17 also markedly promoted the cord formation of vascular endothelial cells. In addition, IL-17 up-regulated elaboration of a variety of proangiogenic factors by fibroblasts as well as tumor cells. These findings reveal a novel role for IL-17 as a CD4 T-cell–derived mediator of angiogenesis that stimulates vascular endothelial cell migration and cord formation and regulates production of a variety of proangiogenic factors. Furthermore, they suggest that inhibition of biologic action of IL-17 may have therapeutic benefits when applied to angiogenesis-related disorders.

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IL-17 elicited neovascularization in rat cornea assay. Our findings indicate that CD4 T-cell–derived cytokine IL-17 is a novel member of the angiogenic factor family and has a prominent role in angiogenesis-related situations.

Materials and methods

Mice and reagents

Female C57BL/6 mice were purchased from Taconic Farms (Germantown, NY). Mouse IL-17 cDNA was kindly supplied by Schering-Plough (Kenilworth, NJ). Mouse IL-17 and antimouse IL-17 monoclonal antibodies (mAbs) were provided by Mr John Abrams (DNAX Research Institute, Palo Alto, CA) and also purchased from Genzyme (Cambridge, MA). Human IL-17, human basic fibroblast growth factor (bFGF), and antimouse IL-17 mAb were purchased from R&D Systems (Minneapolis, MN). Suramin and human vascular endothelial growth factor (VEGF) were purchased from KURABO (Osaka, Japan).

Cells and cell cultures

MCA205 is a weakly immunogenic murine fibrosarcoma. MC38 is a poorly immunogenic murine colon adenocarcinoma. These cells were maintained in RPMI 1640 with 10% fetal calf serum (FCS), 2 mM t-glutamine, 1 mM sodium pyruvate, 5 × 10−5 M 2-mercaptoethanol, 0.1 mM nonessential amino acids (NEAA), 100 IU/mL penicillin, and 100 μg/mL streptomycin (all from Life Technologies, Grand Island, NY), which are designated as complete medium (CM). Human umbilical vein endothelial cells (HUVECs) and human microvascular endothelial cells (HMVECs) were purchased from Clonetics (San Diego, CA) and maintained in Endothelial Cell Basal Medium (EBM) with 12 μg/mL bovine brain extract, 1 μg/mL hydrocortisone, 10 ng/mL epidermal growth factor, 50 μg/mL gentamycin, 50 ng/mL amphotericin-B, and 2% or 5% FCS (all from Clonetics). Primary culture of fibroblasts was established from the lung of 7-week-old C57BL/6 mice. Peripheral blood mononuclear cells were isolated using Lymphoprep (Nycomed Pharma As, Oslo, Norway) from blood from healthy volunteers. CD4 T cells were purified using CD4 T-Cell Enrichment Column (R&D Systems) according to the manufacturer’s instructions. CD4 T cells were cultured for 84 hours in RPMI 1640 with 5% FCS, 2 mM t-glutamine, 1 mM sodium pyruvate, 0.1 mM NEAA, 100 IU/mL penicillin, and 100 μg/mL streptomycin in the presence of a mixture of phorbol myristate acetate (PMA) (1 nM) and ionomycin (3 mM sodium pyruvate, 0.1 mM NEAA, 100 IU/mL penicillin, and 100 μg/mL streptomycin) (both from Sigma, St Louis, MO). Conditioned media of CD4 T cells, which contained approximately 7.1 ng/mL IL-17, were concentrated 40 times using Centricron (Millipore, Bedford, MA).

Construction of recombinant retroviral vector carrying mouse IL-17

A retroviral vector DFG-mIL-17-IRES-Neo was constructed as follows. A retroviral vector termed MFG.13 IRES-Neo cassette, consisting of an EMCV IRES (Encephalomyocarditis Virus IRES) and neomycin phosphotransferase gene (neo) (Stratagene, La Jolla, CA), was subcloned into the BamH1 site of MFG-MIL-7. Transfecting DFG-MIL-7-IRE5-Neo proviral construct into ecotropic packaging cell line BOSC2314 (provided by Drs Pear and Baltimore, Rockefeller University, New York, NY) generated retroviral supernatant. The titer of the retroviral supernatant used in the subsequent experiments was 1.0 to 1.2 × 105 CFU/mL. Target cells were infected with 2 mL DFG-MIL-17-IRE5-Neo retroviral supernatants in the presence of polybrene (8 μg/mL) and selected in CM with 800 μg/mL G418. As a control, a retroviral vector carrying only neomycin phosphotransferase gene (G418, provided by GTI, Germantown, MD) was used.

Flow cytometric analysis

Surface expression of MHC class I (H-2Kb), CD56, and CD44 antigens on tumor cells was examined by a fluorescence-activated cell sorter scan (Becton Dickinson, San Jose, CA) by using the monoclonal antibodies conjugated with fluorescein isothiocyanate (FITC) or phycoerythrin (PE) purchased from PharMingen (San Diego, CA).

In vitro cell growth assay

To evaluate the effect of IL-17 on the proliferation of vascular endothelial cells, HUVECs or HMVECs were suspended in EBM with 50 μg/mL gentamycin, 50 ng/mL amphotericin-B, and 2% FCS and were plated at 1.5 × 105 cells in 10-cm culture dish coated with collagen (Becton Dickinson, Bedford, MA). After 24 hours (on day 0), medium was then replaced with EBM containing 50 μg/mL gentamycin, 50 ng/mL amphotericin-B, and 2% FCS with or without cytokines. The cell number was counted on day 5.

3-(4,5-dimethylthiazol-2-yl)-2,5-dimethyltetrazolium bromide (MTT) assay

Cells (1 × 103) were seeded into 96-well flat-bottomed plates and cultured in RPMI 1640 containing 3.5% FCS with or without 0.5 to 500 ng/mL IL-17. On day 5, cells were washed with medium, and 100 μL MTT (Sigma) solution (2.5 mg/mL in RPMI 1640 with 10% FCS) was added to each well. Plates were incubated for 90 minutes. Next, MTT solution was removed and 50 μL dimethylsulfoxide (DMSO) (Sigma) was added to each well to solubilize formazan crystals formed in viable cells. The absorbance was read at a wavelength of 590 nm on enzyme-linked immunosorbent assay (ELISA) plate reader.

In vivo evaluation of tumor growth

Mice were inoculated subcutaneously in the right flank with $1 \times 10^5$ MCA205WT, MCA205Neo, or MCA205IL-17 or with $2 \times 10^5$ MC38WT, MC38Neo, or MC38IL-17. In some experiments, mice received 500 rad of whole-body irradiation. After 24 hours, tumor cells were inoculated. Tumor volume (cubic millimeter) was calculated by using the formula $a^2b/2$ (a = largest diameter; b = smallest diameter).

Histologic examination and quantitation of vessel density

Tumor tissues were harvested, fixed in 10% buffered formalin, processed, and sectioned with a microtome. Sections were stained with hematoxylin and eosin or stained for Factor VIII. Sections were deparaffinized in xylene, dehydrated in graded concentrations of ethanol, and then rinsed in distilled water. Sections were digested with protease XXIV (Sigma), and then washed with distilled water. Endogenous peroxidase activity was blocked by 3% aqueous hydrogen peroxide, and nonspecific proteins were blocked by using protein blocker (Shannond Lipshaw, Pittsburgh, PA). Finally, sections were weakly counterstained with hematoxylin. For Factor VIII staining, sections were deparaffinized, rehydrated, and stained with hematoxylin and eosin or stained for Factor VIII.

For Factor VIII staining, sections were washed, avidin biotin complex solution (Vector, Burlingame, CA) was applied. After washing, the sections were incubated with biotinylated goat antirabbit F(ab')2 antibody (Jackson ImmunoResearch, West Grove, PA). After washing, avidin biotin complex solution (Vector, Burlingame, CA) was applied and then developed with 3-amin-9-ethylcarbazole (ScyTek, Logan, UT). Finally, the sections were weakly counterstained with hematoxylin.

Tumor sections were examined in a blinded fashion for the presence of Factor VIII immunolocalization. Factor VIII staining was evaluated by quantifying the number of stained blood vessels in 5 randomly chosen high-power fields (HPFs) ($\times 200$) per tumor. A total of 25 HPFs were examined and counted from 5 tumors of each of the groups. Any distinct area of positive staining for Factor VIII was counted as a single, regardless of size. The results were shown as mean ± SD per square millimeter considering the area of HPF.

Rat cornea assay

The cornea assay was performed as described previously. Briefly, hydron pellets (Interferon Sciences, New Brunswick, NJ) containing phosphate-buffered saline (PBS) alone, 250 or 500 ng mouse IL-17, or 100 ng human...
bFGF were implanted into the cornea of anesthetized rats. In some experiments, hydron pellets containing 250 ng mouse IL-17 and 1 μg control antibody or antimouse IL-17 mAb were implanted into the cornea. After 7 days, the animals were killed, and cornea vessels were photographed. Eight rats were used to assess the effect on neovascularization for each group. The experiment was repeated twice.

**Matrigel plug assay**

Mice were injected subcutaneously at the abdominal midline with 0.5 mL Growth Factor-Reduced Matrigel Matrix (Becton Dickinson) in the presence of 8 U/mL heparin (Sigma) supplemented with conditioned media of CD4 T cells and 20 μg/mL control Ab or neutralizing antihuman IL-17 mAb. After 6 days, Matrigel plugs were removed, fixed with 4% buffered paraformaldehyde, and embedded in paraffin. Sections were stained with hematoxylin and eosin. Vessel area and the total Matrigel area were planimetrically assessed from 3 stained sections per Matrigel plug with the use of National Institutes of Health imaging. The experiment was repeated twice with 5 mice per group in each experiment.

**Table 1. IL-6 production is markedly increased in IL-17–transfected tumor cell lines**

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>IL-6 (pg/mL)</th>
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<tr>
<td>MCA2O5WT</td>
<td>&lt; 7.8</td>
</tr>
<tr>
<td>MCA2O5Neo</td>
<td>&lt; 7.8</td>
</tr>
<tr>
<td>MCA2O5IL-17</td>
<td>238</td>
</tr>
<tr>
<td>MCA2O5IL-17 + mAb</td>
<td>11</td>
</tr>
<tr>
<td>MC38WT</td>
<td>&lt; 7.8</td>
</tr>
<tr>
<td>MC38Neo</td>
<td>&lt; 7.8</td>
</tr>
<tr>
<td>MC38IL-17</td>
<td>146</td>
</tr>
<tr>
<td>MC38IL-17 + mAb</td>
<td>&lt; 7.8</td>
</tr>
</tbody>
</table>

Cells (1 × 10^7/mL) were cultured in CM with or without 20 μg/mL antimouse IL-17 monoclonal antibody. Cell-free supernatants were collected after 48 hours and assayed for the production of IL-6 by ELISA. Tumor cells transfected with IL-17 gene produced more IL-6 in response to IL-17 produced by themselves.

**Figure 1. IL-17 promotes in vivo tumor growth in a nonirradiation-dependent fashion.** (A) Recombinant IL-17 protein or transduction of the IL-17 gene has no direct effect on in vitro growth of tumor cells determined by MTT assay. Each value represents mean ± SD (n = 5). (B-C) Mice were inoculated with MCA2O5WT, MCA2O5Neo, or MCA2O5IL-17 or with MC38WT, MC38Neo, or MC38IL-17. Each value represents mean tumor volume (cubic millimeter) ± SD for 6 or 7 mice per group. The result is a representative of 3 independent experiments. (D) Mice were irradiated with 500 rad whole-body irradiation before inoculation with MCA2O5Neo or MCA2O5IL-17. Each value represents mean tumor volume (cubic millimeter) ± SD for 5 mice per group. The result is a representative of 2 independent experiments.

**Figure 2. Histologic examination of subcutaneous tumor tissues.** Tumor tissues were harvested, fixed in 10% buffered formalin, processed, embedded in paraffin, and sectioned with a microtome. Sections were stained with hematoxylin and eosin or with Factor VIII. (A) Hematoxylin-eosin–stained section from MCA2O5WT (original magnification × 150). (B) Hematoxylin-eosin–stained section from MCA2O5IL-17 (original magnification × 150). (C) Hematoxylin–eosin–stained section from MC38WT (original magnification × 150). (D) Hematoxylin–eosin–stained section from MC38IL-17 (original magnification × 150). (E) Hematoxylin–eosin–stained section from MCA2O5WT (original magnification × 80). (F) Factor VIII–stained section from MCA2O5IL-17 (original magnification × 80). (G) Factor VIII–stained section from MC38WT (original magnification × 80). (H) Factor VIII–stained section from MC38IL-17 (original magnification × 80). Note: IL-17 transfectants were markedly infiltrated with neutrophils and were edematous. (E) Factor VIII–stained section from MC38WT (original magnification × 80). (F) Factor VIII–stained section from MC38IL-17 (original magnification × 80). Note: Immunostaining for the presence of Factor VIII showed that tumors transfected with IL-17 were more markedly vascularized when compared with parental tumors. (I) Factor VIII–stained sections were evaluated by quantifying the number of stained blood vessels in 5 randomly chosen HPFs (× 200) per tumor. Bars represent mean number of vessels ± SD per square millimeter considering the area of HPF (n = 25). The result is a representative of 2 independent experiments. *P < .001; **P < .0001; ***P < .01.
EBM with 2% FCS for 8 hours and plated at 12,000 cells/polycarbonate bronectin (Sigma). Cytokines in EBM with 1% FCS were applied in the lower compartments of the chamber. Cells suspended in EBM with 1% Boyden chamber assay, as described previously.17 Cells were cultured in control Ab versus T cells.

Migration of HMVECs or HUVECs was evaluated by using a modified Boyden chamber assay. Migration was quantified by counting cells in 5 randomly selected microscopic fields (× 200) in each well. For inhibitory assay, neutralizing antihuman IL-17 mAb (20 μg/mL) was added in the lower and upper compartments of the chamber.

Endothelial cord formation

In vitro cord formation was performed according to the manufacturer’s instruction (KURABO).18 Briefly, HUVECs and human dermal fibroblasts were admixed and seeded into 24-well plates in medium with or without cytokines. Cells were incubated for up to 11 days, and the medium was replaced with fresh every 3 days. HUVECs were stained for Factor VIII. The analyses were made by computerized image analysis of the number of pixels occupied by the tubules in a total of 15 random areas from these separate wells.

Cytokine and nitric oxide (NO) assays

Fibroblasts or tumor cells (1 × 10⁶/mL) were cultured in RPMI 1640 containing 3.5% FCS with or without IL-17 for 48 hours. Cell-free supernatants were collected and stored at −70°C. Concentrations of angiogenic factors were measured by ELISA kits (R&D Systems). To assess the production of NO, the concentration of nitrite was measured with the use of the Griess reaction, as described previously.19

Reverse transcription–polymerase chain reaction (RT-PCR)

The primer sequences of the oligonucleotides used for PCR were as follows: β-actin, sense: 5'-TTCTCAATGAGCTGCTGTG-3', antisense: 5'-CAGTGTGTTGCACTAGGCTC-3', IL-17, sense: 5'-ATGGACCCAGGAGAGCTCAT-3', antisense: 5'-TCTAGCTGTCCTGGCAGAACAT-3'; acidic fibroblast growth factor (aFGF), sense: 5'-TGGGACCCAGGAGAGCTCAT-3', antisense: 5'-CTTACAGGTCCTGGCAGAACAT-3'; bFGF, sense: 5'-ACAGCTCAAACAAAAGTCTCCA-3', antisense: 5'-TCAGCTTCTATGCACTGAC-3'; transforming growth factor (TGF)-β, sense: 5'-CTAACTGTTGAGGCCGACAA-3', antisense: 5'-CCGTTCATGCTGATGGATGGT-3'. Total RNA was extracted by using RNAzolTM (TEL-TEST, Friendswood, TX) according to manufacturer’s instruction. Total RNA (4 μg) was applied for the synthesis of cDNA with SuperScriptTM RNaseH-Reverse Transcriptase (Life Technologies). PCR was performed in a DNA Thermal Cycler (Perkin-Elmer Cetus, Norwalk, CT) using Taq polymerase (Boehringer Mannheim, Indianapolis, IN).

Statistical analysis

Statistical analysis was performed using an unpaired 2-tailed Student t test with a confidence interval 95% and F tests. Difference was considered significant with a P value less than .05.

Results

Establishment and characterization of IL-17–producing tumor cell lines

We generated a retroviral vector DFG-mIL-17-IRES-Neo as described in “Materials and methods” and used it to transduce the fibrosarcoma MCA205 as well as the colon adenocarcinoma MC38. No expression of either IL-17 mRNA or protein could be detected in MCA205 and MC38 before transfection (data not shown). After G418 selection, expression of IL-17 mRNA by stable transfectants was determined by RT-PCR (data not shown). MCA205IL-17 or MC38IL-17 secretes 153 or 43 ng/10⁶ cells per 48 hours, respectively, determined by ELISA. The biologic activity of expressed protein in the supernatants of MCA205IL-17 or MC38IL-17 was confirmed by its ability to induce IL-6 production (Table 1). Flow cytometric analysis demonstrated that the surface ICAM-1 expression of IL-17 transfectants was slightly enhanced when compared with parental cells (data not shown), which was consistent with a previous report.20 The expression of H-2Kb and CD44 was not altered (data not shown).
IL-17 significantly promotes in vivo tumor growth

IL-17 has no direct effect on proliferation of either MCA205 or MC38 cells determined by MTT assay (Figure 1A). Significant changes were not observed in in vitro growth of IL-17 transfectants when compared with that of parental cells or neomycin phosphotransferase gene transfectants (Figure 1A). When MCA205WT, MCA205Neo, or MCA205IL-17 cells were implanted subcutaneously into syngeneic mice, they all formed solid tumors. However, MCA205IL-17 developed tumors with an increased growth rate compared with MCA205WT or MCA205Neo (MCA205IL-17 versus MCA205WT, P < .008; MCA205IL-17 versus MCA205Neo, P < .0004, on day 25) (Figure 1B). A similar increase in in vivo tumor growth was observed when MC38IL-17 was implanted subcutaneously into the syngeneic animals compared with MC38WT or MC38Neo (MC38IL-17 versus MC38WT, P < .0002; MC38IL-17 versus MC38Neo, P < .0002, on day 30) (Figure 1C).

We next examined whether immunologic mechanisms were involved in the increased in vivo growth of IL-17 transfectants. To address this issue, groups of mice were irradiated and challenged with MCA205Neo or MCA205IL-17 cells. Even in irradiated mice, MCA205IL-17 grew more rapidly when compared with control (MCA205IL-17 versus MCA205Neo, P < .002 [normal mice]; MCA205IL-17 versus MCA205Neo, P < .01 [irradiated mice] on day 28) (Figure 1D). Therefore, irradiation-sensitive cells, presumably immune cells including macrophages, do not primarily mediate the increased in vivo tumor growth induced by locally produced IL-17.

IL-17 markedly enhanced tumor microvascularity

To investigate the mechanisms by which the in vivo growth of tumors transduced with IL-17 was markedly enhanced, we first performed histologic examination of tumor tissues.

Hematoxylin-eosin–stained sections demonstrated that MCA205IL-17 tumors were more infiltrated with neutrophils and were edematous compared with controls (Figure 2A-B). Similar results were found in MC38IL-17 tumors (Figure 2C-D).

Immunostaining for Factor VIII showed that tumor tissues of IL-17 transfectants were more markedly vascularized than parental and Neo-transfected tumors (Figure 2E-H and data not shown). To compare the vascular density, we determined the mean number of blood vessels of Factor VIII–stained sections obtained from 5 independent IL-17 transfectants, Neo transfectants, or parental tumors (Figure 2I). The mean number of microvessels in sections of MCA205IL-17 tumors was significantly higher than that of controls on days 12 and 23 (P < .001). Similar results were obtained in MC38 tumors on days 23 and 30 (P < .01).

IL-17 induces neovascularization in rat cornea

To confirm that IL-17 is angiogenic in vivo, we first examined the formation of new capillary blood vessels in rat cornea assay. Implants containing 100 ng human bFGF as positive control induced angiogenic response (8 positives of 8 corneas tested) (Figure 3B). Although control implants containing PBS gave no angiogenic response (Figure 3A) (no positives of 8 corneas tested), implants containing 250 or 500 ng mouse IL-17 induced significant angiogenic response (8 positives of 8 corneas tested for each dose) (Figure 3C-D). No signs of accompanying inflammatory reaction...
Table 2. IL-17 markedly augments production of proangiogenic factors of tumors

<table>
<thead>
<tr>
<th>Angiogenic factors</th>
<th>MCA205</th>
<th>MCA205 + IL-17</th>
<th>MCA205 + IL-17 + mAb</th>
<th>MC38</th>
<th>MC38 + IL-17</th>
<th>MC38 + IL-17 + mAb</th>
</tr>
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<tbody>
<tr>
<td>VEGF</td>
<td>5.87 ± 0.25</td>
<td>9.88 ± 0.67</td>
<td>5.75 ± 0.37</td>
<td>2.26 ± 0.13</td>
<td>2.7 ± 0.24</td>
<td>2.25 ± 0.18</td>
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<td>KC</td>
<td>1.6 ± 0.13</td>
<td>29.26 ± 1.76</td>
<td>1.57 ± 0.17</td>
<td>0.36 ± 0.07</td>
<td>6.1 ± 0.5</td>
<td>0.38 ± 0.1</td>
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<tr>
<td>MIP-2</td>
<td>10.0 ± 0.4</td>
<td>65.0 ± 4.2</td>
<td>11.0 ± 0.7</td>
<td>11.0 ± 0.8</td>
<td>78.0 ± 3.3</td>
<td>12.1 ± 0.7</td>
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<tr>
<td>PGE1</td>
<td>27.5 ± 2.04</td>
<td>67.0 ± 4.49</td>
<td>27.7 ± 1.77</td>
<td>2.2 ± 0.12</td>
<td>4.5 ± 0.3</td>
<td>2.3 ± 0.15</td>
</tr>
<tr>
<td>PGE2</td>
<td>9.0 ± 1.22</td>
<td>23.1 ± 3.67</td>
<td>10.1 ± 1.7</td>
<td>3.0 ± 0.41</td>
<td>5.8 ± 0.49</td>
<td>2.78 ± 0.57</td>
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<tr>
<td>NO</td>
<td>0.05 ± 0.01</td>
<td>0.09 ± 0.01</td>
<td>0.06 ± 0.01</td>
<td>0.95 ± 0.04</td>
<td>2.98 ± 0.58</td>
<td>0.97 ± 0.05</td>
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Cells (1 × 10^6/mL) were cultured for 48 hours with or without 50 ng/mL IL-17 or 50 ng/mL IL-17 plus 20 μg/mL neutralizing antihuman IL-17 mAb. Cell-free supernatants were collected and assayed for the concentrations of VEGF, KC, MIP-2, PGE1, and PGE2, and NO (pg/mL for VEGF, KC, PGE1, and PGE2; pg/mL for MIP-2; μM for NO). Data were expressed as mean ± SD (n = 3 per data point).

Table 3. IL-17 markedly augments production of proangiogenic factors of fibroblast

<table>
<thead>
<tr>
<th>Angiogenic factors</th>
<th>Fibroblast</th>
<th>Fibroblast + IL-17</th>
<th>Fibroblast + IL-17 + mAb</th>
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</thead>
<tbody>
<tr>
<td>VEGF</td>
<td>2.82 ± 0.18</td>
<td>4.69 ± 0.33</td>
<td>2.77 ± 0.23</td>
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<tr>
<td>KC</td>
<td>1.3 ± 0.05</td>
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<td>1.41 ± 0.12</td>
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<td>MIP-2</td>
<td>36.0 ± 1.3</td>
<td>52.0 ± 2.2</td>
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<tr>
<td>PGE1</td>
<td>0.8 ± 0.35</td>
<td>4.8 ± 0.65</td>
<td>0.87 ± 0.33</td>
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<tr>
<td>PGE2</td>
<td>39.5 ± 2.45</td>
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<tr>
<td>NO</td>
<td>8.1 ± 0.95</td>
<td>13.3 ± 1.0</td>
<td>8.21 ± 1.1</td>
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Cells (1 × 10^6/mL) were cultured for 48 hours with or without 50 ng/mL IL-17 or 50 ng/mL IL-17 plus 20 μg/mL neutralizing antihuman IL-17 mAb. Cell-free supernatants were collected and assayed for the concentrations of VEGF, KC, MIP-2, PGE1, PGE2, and NO (ng/mL for VEGF, KC, PGE1, and PGE2; pg/mL for MIP-2; μM for NO). Data were expressed as mean ± SD (n = 3 per data point).

*P < .05.
Several investigations about the effects of IL-17 on in vivo tumor growth using other tumor model systems have been reported. Hirahara et al\textsuperscript{15} and Benchetrit et al\textsuperscript{16} demonstrated that IL-17 inhibits tumor growth in a T-cell–dependent manner. In contrast, Tartour et al\textsuperscript{17} reported that IL-17 promotes human cervical tumor growth via IL-6–dependent mechanism. Therefore, it appears that IL-17 may be a pleiotropic cytokine with possible tumor-promoting or tumor-suppressing effects. However, although human cervical tumors are generally poorly immunogenic, murine Meth-A fibrosarcoma, P815, and J558L leukemic cells are immunogenic. In addition, MCA205 and MC38 used in our experiments are weakly and poorly immunogenic tumors, respectively. Thus, the effects of IL-17 on in vivo tumor growth may depend largely on the immunogenicity and cell type of tumors.

Macrophages have been thought to play a key role in physiologic and pathologic angiogenesis.\textsuperscript{39} Secretory products such as TNF-\textgreek{a}, IL-8, and VEGF released by activated macrophages have the capacity to influence each phase of the angiogenic process, including modifying the local extracellular matrix, induction of endothelial cells to migrate or proliferate, and inhibition of vascular growth with formation of differentiated capillaries.\textsuperscript{37} In contrast, even though T cells are usually found at the same sites as macrophages in angiogenic processes, including tumors, few reports have analyzed the role of T cells in mediating angiogenesis. We show here that IL-17 secreted by CD4 T cells promotes neovascularization. These findings provide insight into the role of infiltrating CD4 T cells to regulate angiogenesis via elaboration of IL-17.

Although the immune system has been suggested to be an important negative regulator of tumor growth, several experimental models suggest that immune responses could on occasion promote tumor growth.\textsuperscript{38} In particular, there have been reports demonstrating the deleterious effects of CD4 T cells on tumor immunity in several treatment models.\textsuperscript{39-41} We show here that the CD4 T-cell–derived cytokine IL-17 has significant tumor-promoting effects via potentiation of tumor angiogenesis. CD4 T cells infiltrating into tumor tissues may stimulate tumor cells and stromal fibroblasts, in conjunction with tumor-associated macrophages, to elaborate a variety of proangiogenic factors, including VEGF. VEGF also influences the immune system via inhibition of the development of dendritic cells\textsuperscript{42} and subsequently promotes tumor angiogenesis and growth. This could explain in part the reported paradoxical role of CD4 T cells in tumor immunity.

In conclusion, our findings illustrate a novel biologic function of CD4 T-cell–derived cytokine IL-17. IL-17 promotes angiogenesis via not only stimulation of vascular endothelial cell migration but also induced elaboration of a variety of proangiogenic factors that lead to the imbalance between angiogenesis activators and inhibitors present within the vascular microenvironment. Investigation of the physiologic regulation of IL-17 production by CD4 T cells may thus be useful for the treatment in clinical settings characterized by persistent neovascularization. Its production by herpesvirus saimiri also suggests that vascular events, including angiogenesis, are important for virus pathogenesis and persistence.

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