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Cutting Edge: IL-17F, a Novel Cytokine Selectively Expressed in Activated T Cells and Monocytes, Regulates Angiogenesis and Endothelial Cell Cytokine Production¹

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A novel secreted cytokine, termed IL-17F, was cloned using nested RACE PCR. This cytokine bears homology to IL-17. IL-17F was expressed only in activated CD4⁺ T cells and activated monocytes. Recombinant human IL-17F did not stimulate the proliferation of hematopoietic progenitors or the migration of mature leukocytes. However, it markedly inhibited the angiogenesis of human endothelial cells and induced endothelial cells to produce IL-2, TGF-β, and monocyte chemoattractant protein-1. The Journal of Immunology, 2001, 167: 4137–4140.

Cytokines share protein motifs that mediate the structure-function activities of receptor interaction. Examples of this include the four-helix bundle structure of IL-2/IL-10/IL-21/IL-22 (1–3) and the two conserved disulfide bonds of the chemokine family (4, 5). These cytokine motifs share common receptor structures (1–5). A recently described structure shared by several cytokines is the two invariant disulfide bonds shared by IL-17F/IL-17B/IL-17C/IL-17E (6–10).

This structure is unique among cytokines and therefore represents a novel motif for receptor interaction. Consistent with this, the IL-17 receptor is not homologous to any known cytokine receptor (6, 8, 10). Nevertheless, there is recent evidence that this family plays an important role in both the normal immune response and in human immunologic disease. Members of the IL-17 family stimulate T cell proliferation and adhesion molecule expression (9, 10). They induce the expression of a wide variety of other cytokines from various cells such as endothelial or epithelial cells, including IL-6, IL-8, G-CSF, GM-CSF, RANTES, and monocyte chemoattractant protein-1 (9, 10), probably via NF-κB (8, 9, 11). Interestingly, the genome of herpesvirus saimiri encodes an IL-17 homolog (12).

The IL-17 family may play a role in a number of diseases mediated by abnormal immune responses, such as rheumatoid arthritis (13–15), chronic obstructive pulmonary disease (16), psoriasis (17), lupus (18), and multiple sclerosis (19). Interestingly, the IL-17 family may also be important in organ transplant rejection (20, 21) and antitumor immunity (22). The common theme of this family’s characteristics appears to be the regulation of normal vs aberrant T cell response. Because this cytokine motif appears to have a significant part in the immune response, identification of other cytokines bearing this motif is biologically and clinically relevant.

Using RACE PCR we cloned a novel secreted cytokine termed IL-17F that has homology to the IL-17 motif. Recombinant human IL-17F regulates angiogenesis and the expression of other cytokines in endothelial cells.

Materials and Methods

Cloning

A genomic exon with appropriate splice junctions from human chromosome 6 (AL355513) was identified using translated basic local alignment search tool that had the correct cysteine spacing for a member of the IL-17 family, although other amino acid homology was distant. There was no corresponding expressed sequence tag to this exon. Sense and antisense primers were designed for nested RACE PCR (23) using Marathon Ready pooled total leukocyte cDNA (Clontech Laboratories, Palo Alto, CA) according to the manufacturer’s instructions: S1 5’-ggcgtacctgcgtcacc, S2 5’-tcgcatcaccattg, AS1 5’-ggagggacaaggactat, AS2 5’-cactgggctgta caacttc. PCR, using Advantage 2 polymerase (Clontech Laboratories), was performed for 35 cycles, at 94°C for 30 s and then at 68°C for 2 min, with the anchor primers being added after 10 cycles had taken place. PCR products were subcloned into the TOPO blunt vector (Invitrogen, Carlsbad, CA) and sequenced. Once clones were identified that contained the 5’ and 3’ cDNA ends of IL-17F, the complete open reading frame was assembled using overlap extension PCR (24) and the following primers: coding sense 5’-ccagccatggtcaagtacttg and coding antisense 5’-ctgacatggtgatgacag.

Recombinant protein production

The coding sequence of the mature IL-17F (minus the signal peptide) was subcloned into the pCR T7 TOPO TA vector (Invitrogen) and recombinant protein was produced in BL21 cells following isopropyl β-D-thiogalactoside induction. Polyhistidine-tagged IL-17F protein was purified to homo-

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Expression analysis

To analyze the expression pattern of IL-17F, PCR was performed on multiple tissue cDNA panels (Clontech Laboratories) using the coding sense and antisense primers for 35 cycles at 95°C for 30 s, 58°C for 1 min, and 68°C for 1 min. One-tenth volume of the PCR product was size fractionated with agarose gel electrophoresis and then Southern blotted onto a nylon membrane using 0.4 N NaOH. The Southern blot was probed with 32P-end-labeled AS1 and AS2 primers to increase sensitivity and specificity of the expression analysis.

Cytokine induction assay

To test whether rIL-17F stimulated the production of other cytokines, normal HUVECs (Clonetics, San Diego, CA) were treated with IL-17F or Tris protein buffer for 24 h, followed by total RNA isolation using TRIzol (Life Sciences, Gaithersburg, MD). After normalization of the RNA between IL-17F-treated and the Tris protein buffer-treated control, 32P-radioabeled cDNA was synthesized using reverse transcriptase and hybridized to a Human Inflammatory Response Cytokine GEArray membrane (SuperArray, Bethesda, MD). After stringent washing, the subsequent autoradiogram was analyzed using densitometry for induction of cytokine expression.

Hematopoiesis

Ficoll-purified, normal human bone marrow cells obtained under Institution Review Board approval were assessed for granulocyte-macrophage-CFU, erythroid burst-forming unit, and multipotential granulocyte/erythroid/monocyte/megakaryocyte-CFU progenitor cells following treatment with IL-17F at 14 ng/ml or control buffer as we described previously (26).

Angiogenesis

To measure the effect on angiogenesis, recombinant human IL-17F was used in an endothelial capillary tube formation assay as described previously (27). Briefly, 30 HUVECs per sterile microcarrier bead were added together in a culture dish and rocked for 48 h in culture conditions. The HUVEC-coated beads were transferred to a 12-well culture plate containing a fibrin/PBS solution at 20 beads per well. Thrombin was added to each well to stimulate clot formation. Capillary tube formation was stimulated by vascular endothelial growth factor (20 ng/ml), and tubules per microcarrier bead were counted after 72 h of culture.

Results and Discussion

IL-17F gene and protein

The IL-17F cDNA (GenBank accession no. AF384857) was cloned using two rounds of nested RACE PCR from pooled total leukocyte-anchored cDNA. The compiled IL-17F cDNA was 1080 nt in length (Fig. 1). It is located at chromosome 6p12 (AL355513), a region to which no known inherited immunodeficiencies map. There was a consensus AATAA polyadenylation signal 17 nt from the 3′ end. There was a high probability signal peptide from amino acids 1–20, with cleavage between 20 and 21, according to the Center for Biological Sequence Analysis signal peptide prediction server (www.cbs.dtu.dk). There was a total open reading frame of 153 aa. There was an almost perfect fit (6 of 7 aa) to the Kozak consensus for translation initiation (28), with the only deviation being at the −6 position. There were two other in-frame ATG start codon sequences within 20 aa upstream from this translational start site, but they did not match the Kozak consensus for translation initiation and did not contain as good a signal peptide prediction. IL-17F protein is 40% homologous to human IL-17, 39% homologous to open reading frame 13 of HSV2, and 26% homologous to IL-17C. In comparison to IL-17, IL-17F has 26% homologous to IL-17C, IL-17, and IL-17E. Nested RACE PCR was used to clone 5′ and 3′ fragments of the IL-17F cDNA, and then the gene was PCR assembled. The leader sequence is underlined, the polyadenylation signal is bold and underlined, and the conserved cysteines are in bold and italics.

Disulfide bond, and the distinctive function distinguish IL-17F as unique.

Expression

The tissues of origin of IL-17F were analyzed. The expression of IL-17F is low and localized to specific cell subsets and therefore could not be identified using Northern blots of multiple tissues. However, RT-PCR identified IL-17F as being expressed in activated CD4+ T cells and activated monocytes (Fig. 2). IL-17F is not expressed in resting CD4+ T cells, resting or activated CD8+ T cells, resting monocytes, resting or activated B cells, total blood leukocytes, spleen, small intestine, prostate, prostate carcinoma, testis, thymus, or colon. This is somewhat consistent with IL-17, which is expressed in the same tissues as IL-17F but is not expressed in testis or spleen. Proliferating CD8+ T cells and activated monocytes (Fig. 2).

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whose expression can be detected only by RT-PCR. The expression of IL-17 is also restricted to activated T cells (9, 10).

**Angiogenesis**

The role of the IL-17 family in angiogenesis has not been defined, yet many of the cytokines induced by this family are critical regulators of angiogenesis (9, 10). We tested whether 100, 375, or 750 ng/ml IL-17F had any effect on angiogenesis using an endothelial cell capillary tubule formation assay (Fig. 3). At 100 ng/ml, IL-17F inhibited capillary tubule formation by an average of 48%. A dose response was also seen, as 375 ng/ml and 750 ng/ml caused 86 and 95% inhibition, respectively. This may be due to the induction of TGF-β1 described below, which can inhibit angiogenesis (29, 30). These data imply that IL-17F could play a future role in cancer immunotherapy by inhibiting the generation of tumor vascular supply (31).

**Cytokine induction**

Next, the effect of IL-17F on endothelial cytokine gene expression was studied. Treating HUVECs with 140 ng/ml IL-17F for 24 h resulted in changes in cytokine gene expression when compared with buffer-treated control HUVECs (Fig. 4). IL-17F induced the expression of TGF-β1 by 4.7-fold, TGF-β2 by 1.6-fold, monocyte chemoattractant protein-1 by 2.1-fold, lymphotoxin-β by 1.3-fold, and IL-2 by 1.5-fold, when normalized to an actin control. The IL-17 family has previously been shown to induce chemokine expression (9, 10, 32).

**Hematopoiesis**

The effect of IL-17F on hematopoietic progenitor proliferation was tested using colony formation assays. IL-17F indirectly stimulates progenitor proliferation by inducing the expression of G-CSF and GM-CSF by marrow accessory cells (32). IL-17F did not have any effect on granulocyte-macrophage-CFU, granulocyte/erythroid/megakaryocyte-CFU, or erythroid burst-forming unit progenitor proliferation.

**Lymphocyte migration**

The role of the IL-17 family in chemotaxis of lymphocytes has not been reported. Therefore, the effect of 140 ng/ml IL-17F on migration was analyzed using transwell chemotaxis assays (33). IL-17F did not have any effect on the chemotaxis of ARH-77 human B cells, Jurkat human T cells, or THP-1 human monocytic cells (data not shown).

In summary, IL-17F is a novel cytokine that regulates angiogenesis and cytokine production from endothelial cells. Indeed, it is possible that all of the activities of IL-17F are due to its ability to induce cytokine production (34). This raises the intriguing possibility that IL-17F provides general regulation of the cellular immune response by governing the expression of critical cytokines that have much more active stimulatory effects. Such a regulatory function would provide the cellular immune response with an ability to make small changes rapidly in activation status.

**References**

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