Interleukin-17 augments tumor necrosis factor-α-induced granulocyte and granulocyte/macrophage colony-stimulating factor release from human colonic myofibroblasts

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Background. Interleukin (IL)-17 is a newly identified T-cell-specific cytokine. In this study, we investigated the effects of IL-17 on colony-stimulating factor (CSF) release in human colonic subepithelial myofibroblasts (SEMFs). Methods. CSF release and mRNA expression were determined by enzyme-linked immunosorbent assay (ELISA) and Northern blotting, respectively. Nuclear factor (NF)-κB- and activating protein (AP-1)-DNA binding activities were evaluated by electrophoretic gel mobility shift assays (EMSA). Results. Unstimulated cells secreted a small amount of granulocyte G- and granulocyte/macrophage (GM)-CSF, and a considerable amount of M-CSF. IL-17 weakly enhanced G-CSF release, but did not affect GM- and M-CSF release. IL-17 selectively enhanced tumor necrosis factor (TNF)-α-induced G- and GM-CSF release. The combination of IL-17 plus TNF-α induced a marked increase in NF-κB- and AP-1-DNA binding activities. The adenovirus-mediated transfer of a stable form of IκBα and/or a dominant negative mutant of c-Jun markedly inhibited the IL-17 plus TNF-α-induced G- and GM-CSF mRNA expression. Furthermore, a stability study showed that IL-17 plus TNF-α markedly enhanced the stability of G- and GM-CSF mRNA. Conclusions. IL-17 augments TNF-α-induced G- and GM-CSF release via transcriptional and posttranscriptional mechanisms.

Key words: M-CSF, T cells, NF-κB, AP-1

Introduction

Colony-stimulating factors (CSFs) play an important role in controlling the proliferation of hematopoietic progenitor cells and enhancing the functional activities of mature myeloid effector cells.¹ Both granulocyte colony-stimulating factor (G-CSF) and macrophage CSF (M-CSF) are highly lineage-specific in their action, but granulocyte/macrophage CSF (GM-CSF) maintains discrete progenitor cells that consist of macrophages or granulocytes. CSFs are produced by a variety of cells, including monocytes, macrophages, endothelial cells, fibroblasts, and stromal cells, in response to various inflammatory stimuli such as lipopolysaccharide (LPS), tumor necrosis factor (TNF)-α, and interleukin (IL)-1β.¹

Interleukin (IL)-17 is a relatively newly identified T-cell-specific cytokine.²,³ Human IL-17 is a ~20-kDa glycoprotein of 155 amino acids, the sequence of which exhibits close homology to both cytotoxic T-lymphocyte-associated antigen-8 (CTLA-8) and the open reading frame 13 of T-lymphotropic Herpesvirus saimiri (HVS-13). IL-17 secretion is strictly limited to activated CD4⁺ and CD8⁺ T lymphocytes, predominantly memory CD45RO⁺ cells.⁴⁻⁶ Both the Th1 and Th2 subsets of CD4⁺ T cells actively release IL-17. IL-17 induces several genes associated with inflammation, including chemokines, IL-6, leukemia inhibitory factor (LIF), and intercellular adhesion molecule (ICAM)-1.⁷⁻¹⁰ Furthermore, there are an increasing number of reports demonstrating that IL-17 plays a role in the regulation of hematopoiesis. IL-17 directly stimulates G-CSF release from fibroblasts and other cell types.¹¹,¹² IL-17 induces the proliferation and differentiation of CD34⁺ cells isolated from cord blood via the induction of active cytokines (IL-1, G-CSF, IL-6, and interferon [IFN]-γ) from feeder fibroblasts.⁸ The stimulatory effects of IL-17 on granulopoiesis have been reported to depend on a coupled induction of G-CSF and membrane-bound stem-cell factor.¹⁴,¹⁵ Schwarzenberger et al.¹⁶,¹⁷ demonstrated that adenovirus-mediated delivery of IL-17 cDNA to the liver resulted in a marked alteration of granulopoiesis and a rapid increase in se-
rumin G-CSF levels. Thus, IL-17 plays a role in granulopoiesis via the induction of G-CSF. However, it remains unclear how IL-17 modulates the release of the key hematopoietic stimulators, GM-CSF and M-CSF. Furthermore, the interaction between IL-17 and other cytokines in the induction of CSF release has not been investigated.

In patients with intestinal inflammation such as bacterial enterocolitis, or those active-phase with inflammatory bowel disease (IBD), a marked elevation of circulating leukocyte counts is frequently observed. However, the molecular events controlling leukocyte counts remain unclear. To address the mechanisms involved in such a phenomenon, our experiments were designed to investigate CSF secretion from human colonic subepithelial myofibroblasts (SEMFs). Human colonic SEMFs are specialized mesenchymal cells that exhibit the ultrastructural features of both fibroblasts and smooth muscle cells.

We have previously reported that IL-17 modulates various functions of colonic SEMFs. In this study, we focused on the effects of IL-17 on CSF induction in these cells, and found a unique role for IL-17 in the molecular mechanisms controlling CSF release.

Materials and methods

Reagents

Recombinant human IL-1β, IL-17, and TNF-α were obtained from R&D Systems (Minneapolis, MN, USA). All other reagents used in this study were purchased from Sigma Chemical (St Louis, MO, USA).

Culture of human colonic subepithelial myofibroblasts

Primary cultures of human colonic SEMFs were generated and cultured according to methods described previously. Samples of human adult colonic mucosa were obtained from surgical specimens (>5 cm from the tumor margin) from patients undergoing a partial colectomy for carcinoma, with their informed consent. This study was approved by the Ethics Committee of the Shiga University of Medical Science. The studies were performed on passages 3–6 of myofibroblasts isolated from six resection specimens.

Quantification of human CSFs

The amounts of antigenic G-CSF and GM-CSF in the samples were determined by sandwich enzyme-linked immunosorbent assay (ELISA) kits purchased from Bio-Source (Camarillo, CA, USA). The ELISA kits for M-CSF were purchased from R&D Systems. After the collection of supernatants, the number of viable cells was determined by the trypan blue dye exclusion test, and amounts of CSFs were expressed as ng/10^6 living cells.

Northern blot analysis

Northern blotting and hybridization were performed according to a previously described method. Human G-, GM-, and M-CSF cDNA probes were prepared by the reverse-transcription polymerase chain reaction (RT-PCR) method, using the following primers: G-CSF 5′-primer, AACACTGTGCCAACCTCAA (nucleotides 220–239, isolated by Nagata et al.); and 3′-primer, TGGGGAGCACTGATGTAAT (1880–861); GM-CSF 5′-primer, GAATGAAACAGTGAAGTC (191–210, isolated by Lee et al.) and 3′-primer, TTTGCAATTGGCTGATCAG (633–614); and M-CSF 5′-primer, GAAGCTTCAGACTGCAACAG (631–609, isolated by Takahashi et al.) and 3′-primer, GCCATGTGCTGTGTCATCT (1299–1280). The IL-17 receptor (IL-17R) cDNA probe was described in our previous report.

Nuclear extracts and electrophoretic gel mobility shift assays

Nuclear extracts were prepared from cells exposed to IL-1β (5.0 ng/ml), IL-17 (100 ng/ml), and TNF-α (100 ng/ml) for 1.5 h according to the method of Dignam et al.

Consensus oligonucleotides for nuclear factor (NF)-κB (5′-AGT TGA GGG GAC TTT CCC AGC C) and activating protein (AP-1; 5′-CGCTTGATGATGCAG CCGGAA) were purchased from Promega (Madison, WI, USA). The consensus sequences for binding are underlined. Electrophoretic gel mobility shift assays (EMSAs) were performed according to the method previously described. The antisera for the supershift assay were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

Adenovirus-mediated gene transfer

We used a recombinant adenovirus expressing a stable mutant form of IκBα (Ad-IκBΔN), a recombinant adenovirus expressing the dominant negative mutant of c-Jun (Ad-DN-c-Jun), and a recombinant adenovirus containing bacterial β-galactosidase cDNA (Ad-LacZ). The stable mutant form of IκBα (IκBΔN) lacks 54 NH2-terminal amino acids of wild-type IκBα, and is neither phosphorylated nor proteolyzed in response to signal induction, but fully inhibits NF-κB activation.

The dominant negative mutant c-Jun (TAM67) lacks the transactivational domain of amino acids 3 to 122 of wild-type c-Jun, but retains the DNA-binding domain. In preliminary experiments, Ad-LacZ infection of co-