IL-17E, a Novel Proinflammatory Ligand for the IL-17 Receptor Homolog IL-17Rh1

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We report identification of interleukin (IL)-17E, a novel member of the IL-17 family of cytokines. IL-17E is a ligand for the recently identified protein termed EVI27/IL-17BR, which we term IL-17 receptor homolog 1 (IL-17Rh1) in light of the multiple reported ligand-receptor relationships. Murine EVI27 was identified through its location at a common site of retroviral integration in BXH2 murine myeloid leukemias. IL-17Rh1 shows highest level expression in kidney with moderate expression in multiple other organs, whereas IL-17E mRNA was detected at very low levels in several peripheral tissues. IL-17E induces activation of NF-αB and stimulates production of the proinflammatory chemokine IL-8.

IL-171 was identified as a cellular ortholog of a protein encoded by the T lymphotropic herpesvirus Saimiri (1–3). Subsequent characterization has shown that this protein is a potent cytokine that acts to induce proinflammatory responses in a wide variety of peripheral tissues (reviewed in Ref. 4). Recently, two new proteins were identified termed IL-17B and IL-17C that are clearly related to IL-17, establishing that there exists a family of IL-17-like molecules (5). Initial characterization of these proteins suggests that they also are able to induce production of certain proinflammatory cytokines, suggesting that a proinflammatory response may be a common feature of this family of cytokines. Interestingly, they do not appear to be ligands for the IL-17 receptor, suggesting that there exists other molecules that serve as cognate receptors for these factors.

Interest in this family of molecules has increased as it has become apparent that IL-17 may contribute to a number of important medical conditions. In particular, several studies have pointed to a relationship between IL-17 and rheumatoid arthritis (6–10). IL-17 may also play a role in transplant rejection (11–13), psoriasis (14), multiple sclerosis (15), and pulmonary fibrosis (16). Given the potential of IL-17-related molecules to occupy important roles in the control of immune function, there is interest in the identification of other members of this family and the receptors that direct the actions of these molecules through particular target cell populations. In this report we identify a novel member of this family and its receptor.

EXPERIMENTAL PROCEDURES

Isolation of IL-17E and Construction of Expression Vectors—IL-17E (DNA147531) and IL-17Rh1 (DNA115291) cDNA clones were isolated from a human cDNA library and sequenced in their entirety. Fe fusion proteins (immunoadhesins) were prepared by fusion of the entire open reading frames of IL-17B, -C, and E in frame with the Fc region of human IgGl in the eukaryotic expression vector pRK5tkNCEO and the baculovirus vector pHF, a derivative of pVL1393 purchased from Pharmingen. Fusion proteins were transiently expressed in human 293 cells or SF9 insect cells and purified over a protein A column. The extracellular domain of IL-17Rh1 was also as expressed as a C-terminal 8×His tag fusion in baculovirus and purified by nickel affinity column. IL-17E was also expressed as a 8×His tag fusion in Escherichia coli and was purified and refolded. The identities of the purified proteins were verified by N-terminal sequence analysis.

Western Blot, Northern Blot, and Taqman Analysis—Western blot analysis of binding of IL-17E to IL-17Rh1 was performed essentially as described previously (17, 18). For Northern blot analysis, multiple tissue Northern blots (CLONTECH) were probed with a 32P-labeled probe of random-primed IL-17Rh1 cDNA according to manufacturer’s recommendations and exposed to Kodak X-omat (Eastman Kodak Co.) for 72 h. For quantitative PCR analysis (Taqman), total mRNA from human tissues (50 ng) was analyzed as recommended (PerkinElmer Life Sciences) with primers based on the coding sequence of IL-17Rh1.

FACS Analysis—Human 293 cells were transiently cotransfected with expression vectors for green fluorescent protein (GFP) and IL-17Rh1 or IL-17R as indicated. After 24 h, cells were incubated with Fe-tagged ligand as indicated, and binding was revealed with PE-labeled anti-human Fc antibody. FACS curves show PE staining within the cotransfected GFP-positive cell population.

RESULTS

Analysis of human genomic DNA sequence information available in GenBankTM (accession number CNS01DTR) led us to the identification of a putative new member of the IL-17 family. A full-length cDNA was obtained that corresponded to this gene (Fig. 1A). The encoded protein is 177 amino acids and...
is 16–20% identical to IL-17, IL-17B, and IL-17C. We termed this new member of this expanding family IL-17E. The four members share greatest similarity in the C-terminal portion of the molecule with 20–30% amino acid identity and strict conservation of four cysteines. Additional cysteines that may be functionally conserved are present with differences in position. In contrast, there is little conservation apparent in the N-terminal 80 residues. IL-17E mRNA was not detected by Northern blot analysis. However, IL-17E was detected at very low levels in several tissues including brain, kidney, lung, prostate, testis, spinal cord, adrenal gland, and trachea by reverse transcription-PCR using primers designed to distinguish spliced mRNA from genomic DNA (Fig. 1B).

Previous work demonstrated that IL-17B and IL-17C do not bind IL-17R, suggesting that there likely exist additional receptors that serve as cognate pairs for members of this family of cytokines. To identify candidate receptors, expressed sequence tags were examined for sequences related to IL-17R. On the basis of one such group of expressed sequence tags, a cDNA was isolated that encoded a 502-amino acid single transmembrane protein that shares 26% amino acid identity to IL-17R. This molecule appears to be the same gene recently reported as a receptor for IL-17B (20) and independently as EVI27, a cDNA up-regulated by retroviral integration in BXH2 murine myeloid leukemias (21). In light of our data described below that this is also a receptor for IL-17E, we propose that this new receptor be termed IL-17Rh1, a flexible nomenclature to reflect the possibility of additional ligand-receptor relationships within this expanding receptor ligand family. Our cDNAs encoded a protein with a substantially longer C-terminal intracellular domain than that reported by Shi et al. (20) (Close examination of their cDNA reveals the presence of a frameshift relative to our clones.) Our sequence agrees with the sequence reported by Tian et al. (21) with one amino difference (a leucine versus phenylalanine polymorphism at position 468) and shows close homology across full-length of the encoded protein with the murine ortholog, suggesting that this is likely the bona fide sequence. IL-17Rh1 mRNA expression was examined by Northern blot analysis (Fig. 2A) and quantitative PCR (Fig. 2B). Highest levels of expression were observed in kidney with significant expression also observed in liver and other peripheral organs.

To determine whether this new molecule serves as a receptor for members of the IL-17 family, binding studies were conducted. Human 293 kidney cells transfected with an expression vector for IL-17Rh1 bind IL-17E-fc fusion protein (immunoadhesin) but do not show significant binding of IL-17 (Fig. 3A). IL-17E immunoadhesin binding to IL-17Rh1 expressing cells could be completely inhibited by competition with His epitope-tagged IL-17E (not shown). In comparison, cells transfected with expression vector for IL-17R bind IL-17 immunoadhesin but not IL-17E. To examine whether there was direct interac-

**Fig. 1.** Sequence and mRNA expression of IL-17E. A, shown is alignment of the IL-17 family members. The predicted signal sequences are underlined. Conserved cysteines are indicated by bullets, and potential N-linked glycosylation sites are boxed. B, reverse transcription-PCR analysis of IL-17E expression. RNA from the indicated tissues was subjected to reverse transcription-PCR with primers that designed to amplify the entire coding sequence of IL-17E. PCR product was resolved by agarose gel electrophoresis, transferred to nylon membrane, and probed with a 32P-labeled IL-17E cDNA probe.

**Fig. 2.** mRNA expression pattern of IL-17Rh1. A, Northern blot analysis of IL-17Rh1 in selected tissues. B, quantitative PCR analysis of IL-17Rh1 mRNA expression in selected tissues.
tion with members of the IL-17 family, ligand binding studies were conducted with epitope-tagged extracellular domain of IL-17Rh1. We find that this new molecule exhibits robust binding of IL-17E-fc and weak binding to IL-17B-fc but does not bind IL-17-fc or IL-17C-fc (Fig. 3B).

Reasoning that IL-17E may induce NF-κB activity as has been observed with IL-17, we examined whether IL-17E induced activation of a NF-κB-responsive luciferase reporter gene in two human renal cell carcinoma cell lines, 293 and TK-10. (Fig. 4A). Both of these cell lines were found to express endogenous IL-17Rh1 mRNA (not shown). Transfection of the expression vector for IL-17E markedly induced increased luciferase activity. The luciferase activity was induced in a dose-dependent manner and was of similar magnitude to that ob-

**Fig. 3. IL-17E binding to IL-17Rh1.** A, comparison of IL-17 and IL-17E binding to IL-17R and IL-17Rh1. 293 cells were transiently cotransfected with expression vectors for GFP and IL-17R or IL-17Rh1 as indicated. Cells were incubated with IL-17-fc or IL-17E-fc protein as indicated, and binding was revealed with PE-conjugated anti-human Fc antibody. FACS curves show PE staining within the cotransfected GFP-positive cell population.  B, His epitope-tagged IL-17Rh1 extracellular domain was incubated with ligand-Fc fusion protein for members of the IL-17 family (lane 1, IL-17Rh1-His direct load; lane 2, IL-17; lane 3, IL-17B; lane 4, IL-17C; lane 5, IL-17E). Ligand immunoadhesins were immunoprecipitated with protein A beads, and bound IL-17Rh1 was analyzed by Western blot analysis with antibody to the His epitope tag.

**Fig. 4. Induction of NF-κB by IL-17E.** A, human 293 and TK-10 cells were transiently transfected with the NF-κB-responsive luciferase reporter pGL3.ELAM.tk and expression vector for IL-17E as indicated. Luciferase activity was determined as indicated under "Experimental Procedures." B, titration of NF-κB induction by IL-17E. Human 293 cells were transfected with the NF-κB-responsive luciferase reporter pGL3.ELAM.tk and the expression vector for IL-17E as indicated.
served by overexpression of the TNF receptor superfamily member GITR previously shown to be a potent inducer of NF-κB activity (Fig. 4B) (19). NF-κB is thought to mediate a proinflammatory signal suggesting that IL-17E may have proinflammatory action. To examine this possibility we examined production of IL-8, a proinflammatory chemokine previously found to be induced by IL-17. IL-17E induced activation of IL-8 production in TK-10 cells (Fig. 5).

FIG. 5. Effect of IL-17E on IL-8 production. Human TK-10 kidney-derived cell lines were incubated with the indicated concentrations of His-tagged IL-17E, and IL-8 levels were determined by enzyme-linked immunosorbent assay. Shown is the level of IL-8 measured minus the level of IL-8 production observed in the absence of cytokine addition. The experiments were repeated several times with similar results.

DISCUSSION

IL-17Rh1 is the second receptor identified that binds to members of the IL-17 family. The IL-17 receptor family is quite unrelated to other known proteins. The proteins do not possess any recognizable protein structural domains. However, comparison of the two receptors does reveal conservation of many cysteines within the extracellular domain, suggesting that they share similar structure. There are conserved elements within the intracellular domain as well, suggesting that these receptors likely engage similar intracellular machinery. This is supported by the observation that like IL-17 IL-17E signals activation of NF-κB. The regions of conservation within the intracellular domain do not bear obvious similarity to two other receptor families known to activate NF-κB, the IL-1/Toll and TNF receptor families. Details of IL-17 signaling pathways have not yet been well established although numerous intracellular events have been reported including activation of STATs (22), Raf-1 kinase (23), and several mitogen-activated protein kinases (9). The recent demonstration that IL-17 signaling is deficient in TRAF6-deficient cells strongly suggests that members of the TRAF family, known to be involved in both IL-1/Toll and TNF receptor signaling, are also involved in IL-17 receptor signaling (24).

IL-17E induces production of IL-8, a proinflammatory molecule that has also been observed to be induced by IL-17, suggesting that the biological activities of these two cytokines may be similar. The IL-17 receptor has a very broad expression pattern in contrast to the somewhat more restricted mRNA expression pattern of IL-17Rh1. If these molecules mediate generally analogous proinflammatory responses, a key consideration in understanding the function of the different members of the expanding IL-17 cytokine family will be the expression patterns and regulation of the cognate receptors. The observation that IL-17Rh1 may bind two distinct ligands has parallels in other receptor systems that activate NF-κB. Both the IL-1 family and the TNF receptor superfamily have numerous examples of promiscuous interactions between ligands and receptors (reviewed in Refs. 25–28).

The murine ortholog of this new receptor was identified at Evi27, a common site for retroviral integration in BHX2 murine myeloid leukemias. The human IL-17Rh1 maps to 3p21, a region that has been noted to undergo deletion in a variety of cancers, notably renal cell carcinoma (29–32). Interestingly, this region is also a frequently deleted region in chronic myelogenous leukemia (33). A number of other cytokine and growth factor receptors have been implicated as oncogenes, reflecting the important roles these proteins play in control of cell growth and regulation of immune function. Aicardi-Goutières syndrome, an early onset progressive encephalopathy that may present with raised white cell counts or raised levels of interferon-α in the cerebrospinal fluid, also maps to this region (34). It will be of interest to carefully examine the role this cytokine signaling system may play in human disease, particularly cancer and chronic inflammatory conditions.

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