Immunity, Vol. 3, 811–821, December, 1995, Copyright © 1995 by Cell Press

Herpesvirus Saimiri Encodes a New Cytokine, IL-17, Which Binds to a Novel Cytokine Receptor

Zhengbin Yao,* William C. Fanslow,* Michael F. Seldin,† Anne-Marie Rousseau,* Sally L. Painter,* Michael R. Comeau,* Jeffrey I. Cohen,‡ and Melanie K. Spriggs*
*Immunex Corporation
51 University Street
Seattle, Washington 98101
†Departments of Medicine and Microbiology
Duke University Medical Center
Durham, North Carolina 27710
‡Laboratory of Clinical Investigation
National Institutes of Health
Bethesda, Maryland 20892

Summary

Herpesvirus Saimiri gene 13 (HVS13) exhibits 57% identity with the predicted sequence of a T cell–derived molecule termed CTLA8. Recombinant HVS13 and CTLA8 stimulate transcriptional factor NF-κB activity and interleukin-6 (IL-6) secretion in fibroblasts, and costimulate T cell proliferation. An HVS13.Fc fusion protein was used to isolate a cDNA encoding a novel receptor that also binds CTLA8. This receptor is unrelated to previously identified cytokine receptor families. A recombinant soluble receptor inhibited T cell proliferation and IL-2 production induced by PHA, concanavalin A (conA), and anti-TCR MAb. These results define CTLA8 and HVS13 as novel cytokines that bind to a novel cytokine receptor. We propose to call these molecules IL-17, vIL-17, and IL-17R, respectively.

Introduction

Cytokines are soluble proteins with pleiotropic biological activities that mediate immune responses and inflammatory reactions. Cytokines typically exert their biological functions by binding to specific cell surface receptors, which, in turn, activate signal transduction pathways within the cell (Kishimoto et al., 1994). They control activation, proliferation, differentiation, and maturation of a wide variety of cell types (reviewed by Arai et al., 1990; Paul and Seder, 1994). Viral infection often elicits the secretion of cytokines, and their diverse biological functions clearly contribute to host defense mechanisms. In addition to mediating antiviral activities, cytokines can promote the replication and spread of viruses such as human immunodeficiency virus (HIV) (Poli et al., 1990) and human T cell leukemia virus type 1 (HTLV-1) (Greene et al., 1989).

Many animal viruses encode proteins that interfere with normal cytokine function. Viruses use these proteins as countermeasures to host restrictions on viral replication and spread (reviewed by Gooding, 1992; Pickup, 1994; Spriggs, 1994). For example, several poxviruses have been shown to encode soluble receptors for interleukin-1 (IL-1) (Alcamí and Smith, 1992; Spriggs et al., 1992), tumor necrosis factor (TNF) (Smith et al., 1991), interferon (IFN)-α and -β (Colamonici et al., 1995; Symons et al., 1995), and IFNγ (Upton et al., 1992). In vivo studies using mutant viruses in which single genes have been deleted showed that these viral cytokine receptors can function as antagonists of cytokine activity, binding to their respective ligands with high affinity and blocking the interaction of the cytokine with its cellular receptor. In contrast, some herpesviruses encode cytokine receptors that are membrane bound and appear to transduce cellular signals in response to engagement with their ligands (Ahuja and Murphy, 1993; Gao and Murphy, 1994; Schall et al., 1995).

Viruses also modulate the cytokine network by encoding functional homologs of cytokine themselves. For example, Epstein–Barr virus (EBV), a B-lymphotropic herpesvirus, contains an open reading frame (ORF) termed BCRF1, which encodes a homolog of IL-10. This viral IL-10 shares many of the biological properties of human IL-10, including inhibition of monocyte activation and IFNγ synthesis (Hsu et al., 1990).

Herpesvirus saimiri (HVS), like EBV, is a member of the subfamily Gammaherpesvirinae (Roizman, 1992). The γ-herpesviruses are noted for their lymphotropism and host range restriction. HVS is a naturally occurring benign pathogen of squirrel monkeys but is highly oncogenic in many New World primates and induces a fulminant lymphoproliferative syndrome (Fleckenstein and Desrosiers, 1982). HVS is also capable of transforming both simian and human T cells to continuous growth in vitro (Biesinger et al., 1992). The entire nucleotide sequence of HVS has been determined and predicted to encode 76 putative ORFs, which include a complement-regulating homolog and a protein with homology to an IL-8 receptor (Albrecht et al., 1992; Nicholas et al., 1992).

Rouvier et al. (1993) previously identified a rodent cDNA sequence, termed CTLA8, from a T cell hybridoma that was derived from the fusion of a mouse cytotoxic T cell clone and a rat T cell lymphoma. The predicted amino acid sequence of CTLA8 was found to be 57% identical to Herpesvirus saimiri gene 13 (HVS13) (Albrecht et al., 1992). The predicted amino acid sequences suggested that both molecules would have signal sequences and potential N-linked glycosylation sites. Interestingly, the 3′ untranslated region (UTR) of the CTLA8 mRNA contained nucleotide sequences associated with mRNA instability, which are often found in the 3′ UTRs of many cytokines, growth factors, and proto-oncogenes (Shaw and Kamen, 1986). No function for CTLA8 or HVS13 was identified, nor was the exact origin of the CTLA8 molecule described. However, the fact that a gene sequence from a T-lymphotropic virus had homology to a cDNA found in a T cell hybridoma suggested that HVS13 might be derived from the host and play an important role in the virus–host interaction.

In this paper, we report the expression and biological

characterization of both HVS13 and IL-17 (murine CTLA8 [mCTLA8]), and the cloning of a novel surface receptor from mouse T cells that binds to both of these proteins.

Results

Expression of HVS13 and IL-17 (mCTLA8)

To determine whether HVS13 is expressed during HVS infection, HVS-infected owl monkey kidney (OMK) cells were metabolically radiolabeled and immunoprecipitated with an HVS13-specific antiserum. Supernatant and lysates from HVS-infected cells contained four unique proteins with apparent molecular masses of 17, 20, 23, and 26 kDa, which reacted with an HVS13-specific antiserum (Figure 1A). These proteins were not present in mock-infected cells (Figure 1A).

A set of similar-sized proteins was present in supernatant and lysates from CV1/EBNA cells transfected with HVS13 DNA, but not in cells transfected with empty vector (Figure 1B). In the presence of tunicamycin, which inhibits the addition of N-linked oligosaccharides to proteins, the 20, 23, and 26 kDa species disappeared, while the protein of approximately 17 kDa increased (Figure 1B, lanes 2 and 3, 5 and 6), suggesting that the 20, 23, and 26 kDa proteins contained N-linked glycans. The 17 kDa protein most likely represents the unmodified form of the protein, which is consistent with the predicted molecular mass, while the other three proteins likely reflect sequential usage of the three potential N-glycosyl addition sites in HVS13.

To identify the IL-17 (mCTLA8) gene product, CV1/EBNA cells transiently transfected with IL-17 (mCTLA8) cDNA were metabolically labeled in the presence or absence of tunicamycin and the supernatants were analyzed by SDS–polyacrylamide gel electrophoresis (SDS–PAGE). The ^{35}S-labeled supernatant from IL-17 (mCTLA8) expressing cells contained two unique proteins with molecular masses of approximately 17 and 21 kDa (Figure 1C, lane 3). In the presence of tunicamycin, the 21 kDa protein disappeared, leaving only a 17 kDa protein, suggesting that the one potential N-linked glycosylation site in IL-17 (mCTLA8) is utilized (Figure 1C, lane 4).

Molecular Cloning of the IL-17 Receptor

In an effort to identify a receptor for HVS13 and IL-17 (mCTLA8), a chimeric protein, comprising a portion of the Fc region of human immunoglobulin G1 (IgG1) followed by amino acids 19–151 of HVS13 (HVS13.Fc), was constructed. HVS13.Fc protein was expressed, affinity purified, and used to identify potential cell sources for its receptor by flow cytometry. HVS13.Fc was found to bind specifically to the murine thymoma cell line EL4, while a control Fc fusion protein did not (Figures 2A and 2B). An EL4 cDNA library in a mammalian expression vector was screened using a slide-binding autoradiographic method as described (Goodwin et al., 1993). Two cDNA clones (P15 and R11) that specifically bound HVS13.Fc were isolated.

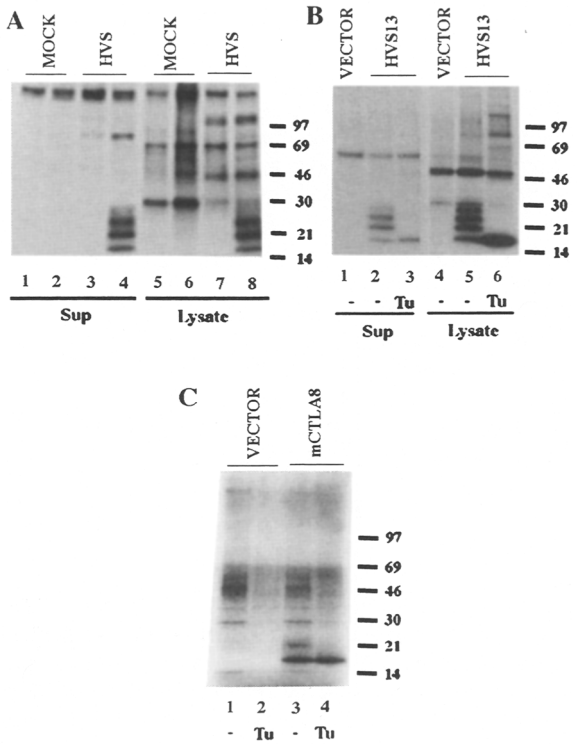

Figure 1. Expression and Processing of HVS13 and IL-17 (mCTLA8)

(A) Immunoprecipitation of HVS13 from HVS-infected cells. HVS-infected or mock-infected OMK cells were labeled with 300 μCi/ml [^{35}S]methionine and cysteine for 16 hr at 37°C. Supernatants (lanes 1–4) or cell lysates (lanes 5–8) were immunoprecipitated with either a preimmune serum (lanes 1, 3, 5, and 7) or an anti-HVS13 polyclonal serum (lanes 2, 4, 6, and 8) and analyzed by 8%–16% SDS–PAGE under reduced conditions.

(B) Immunoprecipitation of HVS13 from transfected cells. CV1/EBNA cells transfected with either HVS13 (lanes 2, 3, 5, and 6) or empty vector (lanes 1 and 4) in the absence (lanes 1, 2, 4, and 5) or presence (lanes 3 and 6) of 20 μg/ml tunicamycin were labeled with 100 μCi/ml [^{35}S]methionine and cysteine for 3 hr at 37°C. Labeled cell supernatants and lysates were harvested, immunoprecipitated and analyzed as described in (A).

(C) Expression of IL-17 (mCTLA8) in transfected cells. CV1/EBNA cells transfected with IL-17 (mCTLA8) DNA (lanes 3 and 4) or empty vector (lanes 1 and 2) in the presence (lanes 2 and 4) or absence (lanes 1 and 3) of 20 μg/ml of tunicamycin were labeled as in (B) and the supernatants analyzed by 8%–16% SDS–PAGE.

To determine whether these cDNAs encoded proteins that also bound mCTLA8, two primers based on the published sequence of IL-17 (CTLA8) (Rouvier et al., 1993) were used in a reverse transcriptase–polymerase chain reaction (RT–PCR) on EL4 RNA to generate a DNA fragment that would encode the mature portion of IL-17 (mCTLA8) (amino acids 20–150). The amplified sequence showed only 92% nucleotide identity to the CTLA8 sequence described by Rouvier et al. (1993). We subsequently isolated and sequenced a mouse IL-17 (mCTLA8) genomic clone. The amplified PCR sequence completely matched the nucleotide sequence derived from this mouse genomic clone (data not shown). Further analysis by PCR, using mouse and rat genomic DNAs as templates, or RT–

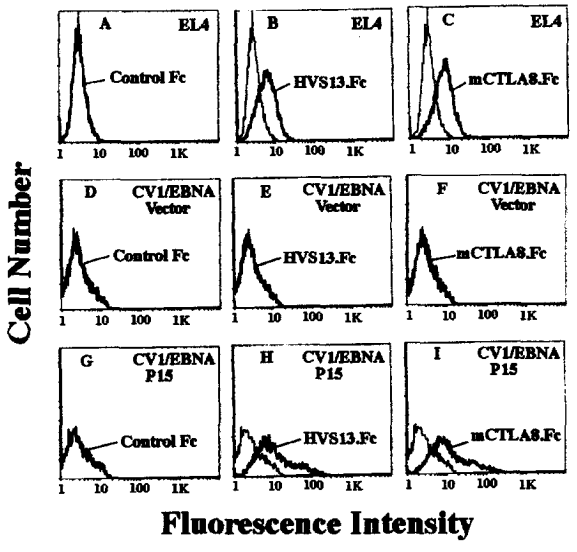

Figure 2. Binding of HVS13.Fc and mCTLA8.Fc to EL4 Cells and CV1/EBNA Cells Transfected with IL-17R cDNA

EL4 cells were analyzed with a control Fc ([A], thick line), HVS13.Fc ([B], thick line), or mCTLA8.Fc ([C], thick line) proteins. Cells stained with secondary reagents alone are shown in thin lines. CV1/EBNA cells transfected with cDNA clone P15 (G–I) or empty vector (D–F) were analyzed with a control Fc (D and G), HVS13.Fc (E and H), or mCTLA8.Fc (F and I) proteins.

PCR, using mouse and rat cell RNAs as templates, strongly suggested that the CTLA8 described by Rouvier et al. (1993) was of rat origin and may have been derived from the rat lymphoma, rather than the mouse cytotoxic T lymphocyte clone as its name suggested. A mCTLA8.Fc was constructed by fusing amino acids 20–150 of mCTLA8 to the Fc region of human IgG1 and expressed and purified in the same way as HVS13.Fc. Both HVS13.Fc and mCTLA8.Fc bound to CV1/EBNA cells transfected with the cloned P15 cDNA, but not to cells transfected with empty vector (Figure 2). Only the result from clone P15-transfected cells is shown. The binding of HVS13.Fc and mCTLA8.Fc with the other IL-17 receptor (IL-17R) cDNA (R11) was essentially identical (data not shown).

Structure of the IL-17R

Sequencing of these cDNA clones revealed that they contained either a 3.3 kb (P15) or a 1.7 kb (R11) insert derived from the same mRNA. Clone P15 contained a single ORF of 2592 bp with a 120 bp 5′ noncoding sequence and a 573 bp 3′ noncoding sequence (Figure 3). The R11 clone is a partial clone containing nucleotides 57–1764. The predicted initiator methionine is preceded by a purine at the −3 position and a purine at the +4 position, making it optimal for translation initiation (Kozak, 1991). The ORF is predicted to encode a type I transmembrane protein of 864 aa. Computer-assisted analysis predicts that this protein has an N-terminal signal peptide with a cleavage site after alanine at amino acid 31. The signal peptide is followed by a 291 aa extracellular domain, a 21 aa transmembrane domain, and a 521 aa cytoplasmic tail (Figure 3).

```
GTCGACTGGAACGAGAGCGACCTGCTGCCGACGAGCGCCAGTCCTCGGCCGGGAAAGCCAT   60
CGCGGGCCCTCGCTGTCGCGCGGAGCCAGCTGCGAGCGCTCCGCGACCGGGCCGAGGGCT  120
ATGGCGATTCGGCGCTGCTGGCCACGGGTCGTCCCCGGGCCCGCGCTGGGATGGCTGCTT  180
M  A  I  R  R  C  W  P  R  V  V  P  G  P  A  L  G  W  L  L
CTGCTGCTGAACGTTCTGGCCCCGGGCCGCGCCTCCCCGCGCCTCCTCGACTTCCCGGCT  240
L  L  L  W  V  L  A  P  G  R  A  S  P  R  L  L  D  F  P  A
CCGGTCTGCGCGGCAGGAGGGGCTGAGCTGCAGAGTCAAGAATAGTACTTGTCTGGATGAC  300
P  V  C  A  Q  E  G  L  S  C  R  V  K  N  S  T  C  L  D  D
AGCTGGATCCACCCCAAAAACCTGACCCCGTCTTCCCCAAAAAACATCTATATCAATCTT  360
S  W  I  H  P  K  N  L  T  P  S  S  P  K  N  I  Y  I  N  L
AGTGTTTCCTCTACCCAGCACGGAGAATTAGTCCCTGTGTTGCATGTTGAGTGGACCCTG  420
S  V  S  S  T  Q  H  G  E  L  V  P  V  L  H  V  E  W  T  L
CAGACAGATGCCAGCATCCTGTACCTCGAGGGTGCAGAGCTGTCCGTCCTGCAGCTGAAC  480
Q  T  D  A  S  I  L  Y  L  E  G  A  E  L  S  V  L  Q  L  N
ACCAATGAGCGGCTGTGTGTCAAGTTCCAGTTTCTGTCCATGCTGCAGCATCACCGTAAG  540
T  N  E  R  L  C  V  K  F  Q  F  L  S  M  L  Q  H  H  R  K
CGGTGGCGGTTTTCCTTCAGCCACTTTGTGGTAGATCCTGGCCAGGAGTATGAAGTGACT  600
R  W  R  F  S  F  S  H  F  V  V  D  P  G  Q  E  Y  E  V  T
GTTCACCACCTGCCGAAGCCCATCCCTGATGGGGACCCAAACCACAAATCCAAGATCATC  660
V  H  H  L  P  K  P  I  P  D  G  D  P  N  H  K  S  K  I  I
TTTGTGCCTGACTGTGAGGACAGCAAGATGAAGATGACTACCTCATGCGTGAGCTCAGGC  720
F  V  P  D  C  E  D  S  K  M  K  M  T  T  S  C  V  S  S  G
AGCCTTTGGGATCCCAACATCACTGTGGAGACCTTGGACACACAGCATCTGCGAGTGGAC  780
S  L  W  D  P  N  I  T  V  E  T  L  D  T  Q  H  L  R  V  D
TTCACCCTGTGGAATGAATCCACCCCCTACCAGGTCCTGCTGGAAAGTTTCTCCGACTCA  840
F  T  L  W  N  E  S  T  P  Y  Q  V  L  L  E  S  F  S  D  S
GAGAACCACAGCTGCTTTGATGTCGTTAAACAAATATTTGCGCCCAGGCAAGAAGAATTC  900
E  N  H  S  C  F  D  V  V  K  Q  I  F  A  P  R  Q  E  E  F
CATCAGCGAGCTAATGTCACATTCACTCTAAGCAAGTTTCACTGGTGCTGCCATCACCAC  960
H  Q  R  A  N  V  T  F  T  L  S  K  F  H  W  C  C  H  H  H
GTGCAGGTCCAGCCCTTCTTCAGCAGCTGCCTAAATGACTGTTTGAGACACGCTGTGACT 1020
V  Q  V  Q  P  F  F  S  S  C  L  N  D  C  L  R  H  A  V  T
GTGCCCTGCCCAGTAATCTCAAATACCACAGTTCCCAAGCCAGTTGCAGACTACATTCCC 1080
V  P  C  P  V  I  S  N  I  T  V  P  K  P  V  A  D  Y  I  P
CTGTGGGTGTATGGCCTCATCACACTCATCGCCATTCTGCTGGTGGGATCTGTCATCGTG 1140
L  W  V  Y  G  L  I  T  L  I  A  I  L  L  V  G  S  V  I  V
CTGATCATCTGTATGACCTGGAGGCTTTCTGGCGCCGATCAAGAGAAACATGGTGATGAC 1200
L  I  I  C  M  T  W  R  L  S  G  A  D  Q  E  K  H  G  D  D
TCCAAAATCAATGGCATCTTGCCCGTAGCAGACCTGACTCCCCCACCCCTGAGGCCCAGG 1260
S  K  I  N  G  I  L  P  V  A  D  L  T  P  P  P  L  R  P  R
AAGGTCTGGATCGTCTACTCGGCCGACCACCCCCTCTATGTGGAGGTGGTCCTAAAGTTC 1320
K  V  W  I  V  Y  S  A  D  H  P  L  Y  V  E  V  V  L  K  F
GCCCAGTTCCTGATCACTGCCTGTGGCACTGAAGTAGCCCTTGACCTCCTGGAAGAGCAG 1380
A  Q  F  L  I  T  A  C  G  T  E  V  A  L  D  L  L  E  E  Q
GTTATCTCTGAGGTGGGGGTCATGACCTGGGTGAGCCGACAGAAGCAGGAGATGGTGGAG 1440
V  I  S  E  V  G  V  M  T  W  V  S  R  Q  K  Q  E  M  V  E
AGCAACTCCAAAATCATCATCCTGTGTTCCCGAGGCACCCAAGCAAAGTGGAAAGCTATC 1500
S  N  S  K  I  I  I  L  C  S  R  G  T  Q  A  K  W  K  A  I
TTGGGTTGGGCTGAGCCTGCTGTCCAGCTACGGTGTGACCACTGGAAGCCTGCTGGGGAC 1560
L  G  W  A  E  P  A  V  Q  L  R  C  D  H  W  K  P  A  G  D
CTTTTCACTGCAGCCATGAACATGATCCTGCCAGACTTCAAGAGGCCAGCCTGCTTCGGC 1620
L  F  T  A  A  M  N  M  I  L  P  D  F  K  R  P  A  C  F  G
ACCTACGTTGTTTGCTACTTCAGTGGCATCTGTAGTGAGAGGGATGTCCCCGACCTCTTC 1680
T  Y  V  V  C  Y  F  S  G  I  C  S  E  R  D  V  P  D  L  F
AACATCACCTCCAGGTACCCACTCATGGACAGATTTGAGGAGGTTTACTTCCGGATCCAG 1740
N  I  T  S  R  Y  P  L  M  D  R  F  E  E  V  Y  F  R  I  Q
GACCTGGAGATGTTTGAACCCGGCCGGATGCACCATGTCAGAGAGCTCACAGGGGACAAT 1800
D  L  E  M  F  E  P  G  R  M  H  H  V  R  E  L  T  G  D  N
TACCTGCAGAGCCCTAGTGGCCGGCAGCTCAAGGAGGCTGTGCTTAGGTTCCAGGAGTGG 1860
Y  L  Q  S  P  S  G  R  Q  L  K  E  A  V  L  R  F  Q  E  W
CAAACCCAGTGCCCCGACTGGTTCGAGCGTGAGAACCTCTGCTTAGCTGATGGCCAAGAT 1920
Q  T  Q  C  P  D  W  F  E  R  E  N  L  C  L  A  D  G  Q  D
CTTCCCTCCCTGGATGAAGAAGTGTTTGAAGACCCACTGCTGCCACCAGGGGGAGGAATT 1980
L  P  S  L  D  E  E  V  F  E  D  P  L  L  P  P  G  G  G  I
GTCAAACAGCAGCCCCTGGTGCGGGAACTCCCATCTGACGGCTGCCTTGTGGTAGATGTC 2040
V  K  Q  Q  P  L  V  R  E  L  P  S  D  G  C  L  V  V  D  V
TGTGTCAGTGAGGAAGAAAGTAGAATGGCAAAGCTGGACCCTCAGCTATGGCCACAGAGA 2100
C  V  S  E  E  E  S  R  M  A  K  L  D  P  Q  L  W  P  Q  R
GAGCTAGTGGCTCACACCCTCCAAAGCATGGTGCTGCCAGCAGAGCAGGTCCCTGCAGCT 2160
E  L  V  A  H  T  L  Q  S  M  V  L  P  A  E  Q  V  P  A  A
CATGTGGTGGAGCCTCTCCATCTCCCAGACGGCAGTGGAGCAGCTGCCCAGCTGCCCATG 2220
H  V  V  E  P  L  H  L  P  D  G  S  G  A  A  A  Q  L  P  M
ACAGAGGACAGCGAGGCTTGCCCGCTGCTGGGGGTCCAGAGGAACAGCATCCTTTGCCTC 2280
T  E  D  S  E  A  C  P  L  L  G  V  Q  R  N  S  I  L  C  L
CCCGTGGACTCAGATGACTTGCCACTCTGTAGCACCCCAATGATGTCACCTGACCACCTC 2340
P  V  D  S  D  D  L  P  L  C  S  T  P  M  M  S  P  D  H  L
CAAGGCGATGCAAGAGAGCAGCTAGAAAGCCTAATGCTCTCGGTGCTGCAGCAGAGCCTG 2400
Q  G  D  A  R  E  Q  L  E  S  L  M  L  S  V  L  Q  Q  S  L
AGTGGACAGCCCCTGGAGAGCTGGCCGAGGCCAGAGGTGGTCCTCGAGGGCTGCACACCC 2460
S  G  Q  P  L  E  S  W  P  R  P  E  V  V  L  E  G  C  T  P
TCTGAGGAGGAGCAGCGGCAGTCGGTGCAGTCGGACCAGGGCTACATCTCCAGGAGCTCC 2520
S  E  E  E  Q  R  Q  S  V  Q  S  D  Q  G  Y  I  S  R  S  S
CCGCAGCCCCCCGAGTGGCTCACGGAGGAGGAAGAGCTAGAACTGGGTGAGCCCGTTGAG 2580
P  Q  P  P  E  W  L  T  E  E  E  E  L  E  L  G  E  P  V  E
TCTCTCTCTGCTGAGGAACTACGGAGCCTGAGGAAGCTCCAGAGGCAGCTTTTCTTCTGG 2640
S  L  S  P  E  E  L  R  S  L  R  K  L  Q  R  Q  L  F  F  W
GAGCTCGAGAAGAACCCTGGCTGGAACAGCTTGGAGCCACGGAGACCCACCCCAGAAGAG 2700
E  L  E  K  N  P  G  W  N  S  L  E  P  R  R  P  T  P  E  E
CAGAATCCCTCCTAGGCCTCCTGAGCCTGCTACTTAAGAGGGTGTATATTGTACTCTGTG 2760
Q  N  P  S  *
TGTGCGTGCGTGTGTGTGTGTGTGTGTGTGTGTGCGTGTGTGTGTGTGTGTGTG 2820
TGTGTGTGTGTAGTGCCCGGCTTAGAAATGTGAACATCTGAATCTGACATAGTGTTGT 2880
ATACCTGAAGTCCCAGCACTTGGGAACTGAGACTTGATGATCTCCTGAAGCCAGGTGTTC 2940
AGGGCCAGTGTGAAAACATAGCAAGACCTCAGAGAAATCAATGCAGACATCTTGGTACTG 3000
ATCCCTAAACACACCCCTTTCCCTGATAACCCGACATGAGCATCTGGTCATCATTGCACA 3060
AGAATCCACAGCCCGTTCCCAGAGCTCATAGCCAAGTGTGTTGCTCATTCCTTGAATATT 3120
TATTCTGTACCTACTATTCATCAGACATTTGGAATTCAAAAACAAGTTACATGACACAGC 3180
CTTAGCCACTAAGAAGCTTAAAATTCGGTAAGGATGTAAAATTAGCCAGGATGAATAGAG 3240
GGCTGCTGCCCTGGCTGCAGAAGAGCAGGTCGTCTCGTTCCAGTCGAC
```

Figure 3. Nucleotide Sequence of a cDNA Encoding the IL-17R

The nucleotide and deduced amino acid sequences of a cDNA encoding the IL-17R are shown. The predicted signal peptide is underlined, the putative transmembrane region doubly underlined, and potential N-linked glycosylation sites are in italics and underlined.

There are eight potential N-linked glycosylation sites in the extracellular domain. The predicted molecular mass for this protein is 97.8 kDa, with an estimated isoelectric point of 4.85. A comparison of both nucleotide and amino acid sequences with the GenBank or European Molecular Biology Laboratory databases revealed no significant homology with known nucleotide and protein sequences.

Cellular and Tissue Distribution of IL-17R mRNA

Poly (A)$^+$ RNA derived from various murine cell lines or tissues was examined by Northern blot analysis using the IL-17R cDNA as a probe. The IL-17R probe hybridized to a single species of mRNA of approximately 3.7 kb in all tissues examined. Among these, strong hybridizing signals were observed in spleen and kidney. Moderate signals were observed in lung and liver, and weaker signals in brain, heart, skeletal muscle, and testes (Figure 4A). Similar-sized mRNAs were detected in the following cells and cell lines: fetal liver epithelial cells (D11), fibroblast (3T3), rat intestinal epithelial cells (1EC6), splenic B cells, muscle cells (BB4), mast cells (H7), triple-negative thymus cells (CD3$^-$CD4$^-$CD8$^-$) (TN), pre-B cells (70Z/3), T cell thymoma (EL4); and T cell clones 7C2 and D10 (Figure 4B). All the cell lines tested were found to express IL-17R mRNA, suggesting a ubiquitous expression of IL-17R message.

Biochemical Characterization of the IL-17R

EL4 cells or CV1/EBNA cells transfected with IL-17R cDNA were surface biotinylated and subsequently immunoprecipitated using HVS13.Fc or mCTLA8.Fc. Both Fc fusion proteins precipitated a protein with a molecular mass of approximately 120 kDa from the CV1/EBNA cells transfected with IL-17R cDNA but not from cells transfected with empty vector (Figure 4C, lanes 1 to 6). A similar-sized protein was precipitated by HVS13.Fc and mCTLA8.Fc proteins from EL4 cells (Figure 4C, lanes 8 and 9). The molecular mass of the IL-17R expressed in EL4 cells and in transfected CV1/EBNA cells is larger than that predicted from its amino acid sequence, suggesting that the N-linked glycosylation sites in the extracellular domain may be utilized.

Mouse Chromosomal Localization and Fine Mapping of IL-17R Gene

A panel of DNA samples from an interspecific cross that had been characterized for over 900 genetic markers throughout the genome was analyzed. The genetic markers included in this map span between 50–80 cM on each mouse autosome and the X chromosome (Chr) (Saunders and Seldin, 1990; Watson et al., 1992). Initially, DNA from the two parental mice (C3H/HeJ–*gld* and [C3H/HeJ–*gld* × Mus spretus] F1) were digested with various restriction endonucleases and hybridized with the IL-17R cDNA probe to determine restriction fragment length variants (RFLVs) to allow haplotype analyses. Informative BglI RFLVs were detected: C3H/HeJ–*gld*, 10.0 kb; Mus spretus, 7.8 kb and 2.2 kb. In each of the backcross mice, either the C3H/HeJ–*gld* parental band or all three bands (both Mus spretus bands and a half-intensity C3H/HeJ–*gld* band) were observed, indicating that a single locus was detected.

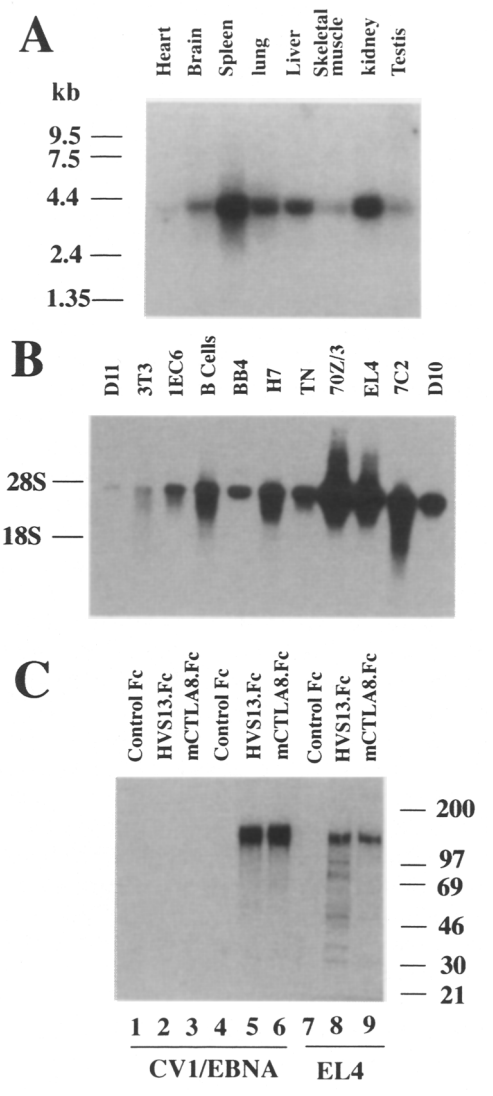

Figure 4. Identification of IL-17R mRNA and Protein

(A and B) Northern blot analysis of IL-17R mRNA. Poly (A)$^+$ RNA from the indicated tissues (A) or from D11, 3T3, 1EC6, splenic B cells, BB4, H7, TN, 70Z/3, EL4, 7C2, and D10 cells (B) were hybridized with radioactive anti-sense riboprobes as described in Experimental Procedures.

(C) Immunoprecipitation of IL-17R with HVS13.Fc and mCTLA8.Fc proteins. CV1/EBNA cells transfected with empty vector (lanes 1–3), or IL-17R cDNA (lanes 4–6), or EL4 cells (lanes 7–9) were surface biotinylated, and cell lysates were precipitated with 5 μg/ml of control Fc (lanes 1, 4, and 7), HVS13.Fc (lanes 2, 5, and 8) or mCTLA8.Fc proteins (lanes 3, 6, and 9) and protein A–sepharose and electrophoresed under reducing conditions on 8%–16% SDS–PAGE. Proteins were blotted onto an ECL membrane and detected using the ECL system after staining with streptavidin-conjugated horseradish peroxidase.

Comparison of the haplotype distribution of the IL-17R RFLVs indicated that this gene cosegregated in 111 of the 114 meiotic events examined with the Raf1 gene locus on mouse Chr 6. The best gene order (Bishop, 1985) ±

SD (Green, 1981) is: (centromere) *Raf1*–2.6 cM ± 1.5 cM – *IL-17R (provisional genetic designation)* – 2.5 cM ± 1.5 cM – *Cd4*.

Expression of IL-17R and Induction of NF-κB Activity by HVS13 and IL-17 (mCTLA8) in Fibroblasts

Murine 3T3 cells were found to express IL-17R by flow cytometry (Figure 5A). We then tested whether engagement of the IL-17R by its ligands would activate the transcription factor, NF-κB. 3T3 cells were incubated with HVS13 or IL-17 (mCTLA8), and electrophoretic mobility shift assays were performed to determine NF-κB activity. Incubation of 3T3 cells with supernatants containing the unmodified form of HVS13 or IL-17 (mCTLA8) or with HVS13.Fc or mCTLA8.Fc fusion proteins for 30 min resulted in the induction of protein–DNA complexes (Figure 5B). The formation of the slowest migrating complex could be inhibited by addition of excess unlabeled NF-κB oligonucleotides containing a wild-type, but not a mutant, NF-κB binding sequence (Figure 5B). To ensure that the stimulatory effects were specific to HVS13, an HVS13-specific MAb was used to neutralize NF-κB induction. The HVS13 MAb completely inhibited HVS13-induced NF-κB activation, while an isotype-matched MAb had no effect (Figure 5B).

To determine the role of IL-17R in HVS13- and IL-17 (mCTLA8)-induced NF-κB activation, 3T3 cells were preincubated with an IL-17R-specific antiserum and then assayed for NF-κB activation by HVS13. Preincubation with the IL-17R antiserum dramatically decreased the NF-κB activity induced by HVS13 as compared with cells preincubated with an irrelevant rat antiserum (Figure 5C). This result indicated that the engagement of the IL-17R by its ligands induced NF-κB activity.

IL-6 Induction by HVS13 and IL-17 (mCTLA8)

IL-6 production was measured from fibroblasts cultured with HVS13 or IL-17 (mCTLA8) containing supernatant or the corresponding Fc fusion proteins. Cells were cultured for 24 hr and conditioned media from these cultures were harvested and assayed for production of IL-6 by enzyme-linked immunoabsorbent assay (ELISA). Both HVS13 and IL-17 (mCTLA8) containing supernatants induced a 20- to 25-fold increase of IL-6 secretion in 3T3 cells (Figure 5D), while a control supernatant did not. Moreover, the induction of IL-6 by HVS13 containing supernatant could be specifically blocked by an HVS13-specific MAb, but not by an isotype-matched irrelevant MAb (Figure 5D). Similarly, HVS13.Fc and mCTLA8.Fc induced IL-6 secretion in 3T3 cells in a dose-dependent manner, while a control Fc protein had no effect. (Figure 5E).

Proliferative Response of T Cells to HVS13 and IL-17 (mCTLA8)

To examine potential biological activity of HVS13 and IL-17 (mCTLA8) on cells of lymphoid origin, HVS13 and IL-17 (mCTLA8) were tested for their ability to stimulate the proliferation of mouse T cells. Purified mouse splenic T cells were cultured with suboptimal concentrations of a costimulus in the presence of titrated amounts of HVS13.Fc or mCTLA8.Fc or supernatants containing the unmodified form of HVS13 or IL-17 (mCTLA8). Proliferation was measured after 3 days of culture. Both HVS13.Fc and mCTLA8.Fc (Figure 6A), as well as their non-Fc counter-

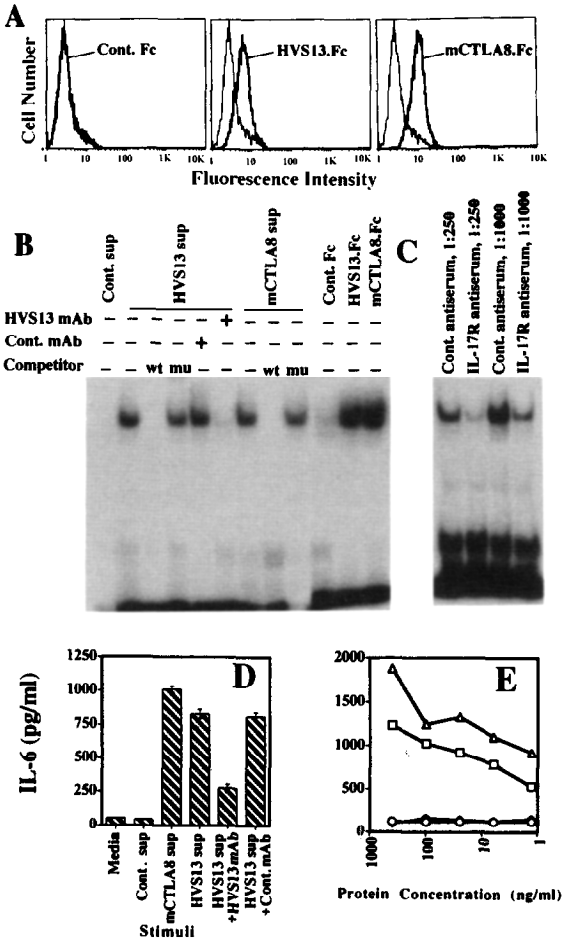

Figure 5. Activation of NF-κB Activity and IL-6 Induction by HVS13 and IL-17 (mCTLA8) in Fibroblasts

(A) Expression of IL-17R on mouse 3T3 cells. 3T3 cells were dislodged from tissue culture flasks and stained with HVS13.Fc, mCTLA8.Fc, or a control Fc (thick lines) as described in Figure 2. Cells stained with secondary antibody only are shown in thin lines.

(B) 3T3 cells were treated with a control supernatant, HVS13, or IL-17 (mCTLA8) containing supernatant, 200 ng/ml of a control Fc, HVS13.Fc, or mCTLA8.Fc proteins for 30 min. Alternatively, HVS13 containing supernatant was preincubated with either a control MAb or an HVS13-specific MAb for 1 hr before adding to 3T3 cells. Nuclear extracts were prepared and, in some cases, reaction mixtures were supplemented with a 100-fold excess of cold oligonucleotides containing either a wild-type or a mutated NF-κB binding site.

(C) Inhibition of HVS13-induced NF-κB activity by an anti-IL-17R serum. 3T3 cells were either treated with 1:250 or 1:1000 dilutions of an IL-17R antiserum or an irrelevant rat anti-serum for 1 hr. NF-κB assays were then performed as described in (B).

(D and E) Induction of IL-6 secretion by HVS13 and IL-17 (mCTLA8). 3T3 cell monolayers were cultured with the indicated stimuli (D) or a titration of HVS13.Fc (open squares), mCTLA8.Fc (open triangles), control Fc (open circles), or media (open diamonds) for 24 hr (E). Conditioned media were collected and assayed for IL-6 by ELISA.

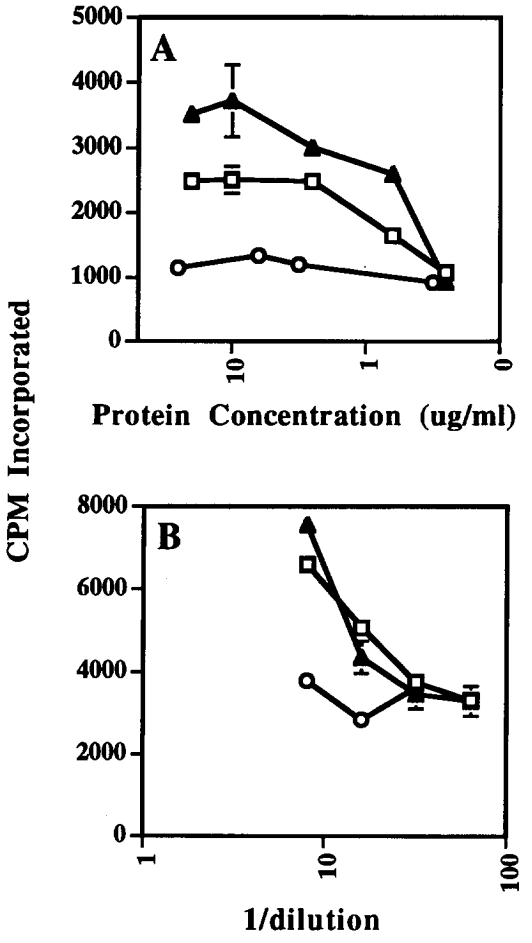

Figure 6. Proliferative Response of T Cells to HVS13 and IL-17 (mCTLA8)

Purified mouse splenic T cells (2 × 10^5/well) were cultured with 1% PHA and a titration of HVS13.Fc (closed triangles), mCTLA8.Fc (open squares), or control Fc (open circles) (A), or serial dilutions of HVS13 (closed triangles), IL-17 (mCTLA8) (open squares) containing supernatant, or a control supernatant (open circles) (B). Results are expressed as mean cpm ± SEM. The cpm for unstimulated cells in (A) was 31; for cells stimulated with PHA, 1163. In (B), cpm for unstimulated cells was 44; for cells stimulated with PHA, 3073.

parts (Figure 6B), enhanced the proliferative response of purified mouse splenic T cells 3- to 4-fold in the presence of suboptimal phytohemagglutinin (PHA). Neither a control Fc protein nor supernatant had any effect on the mouse T cell proliferation (Figures 6A and 6B).

Biological Effect of Soluble IL-17R

A soluble IL-17R.Fc fusion protein was constructed by fusing the extracellular domain (residues 1–323) of IL-17R to the Fc portion of human immunoglobulin IgG1. The effect of the IL-17R.Fc fusion protein on the biological activity of IL-17 (mCTLA8) and HVS13 was examined in a system measuring IL-6 production. 3T3 cells were cultured with either IL-17 (mCTLA8) or HVS13 in the presence of either a soluble IL-17R.Fc or a control Fc protein for 24 hr and IL-6 production was measured by ELISA. When the cells were cultured with IL-17 (mCTLA8) (Figure 7A) or HVS13

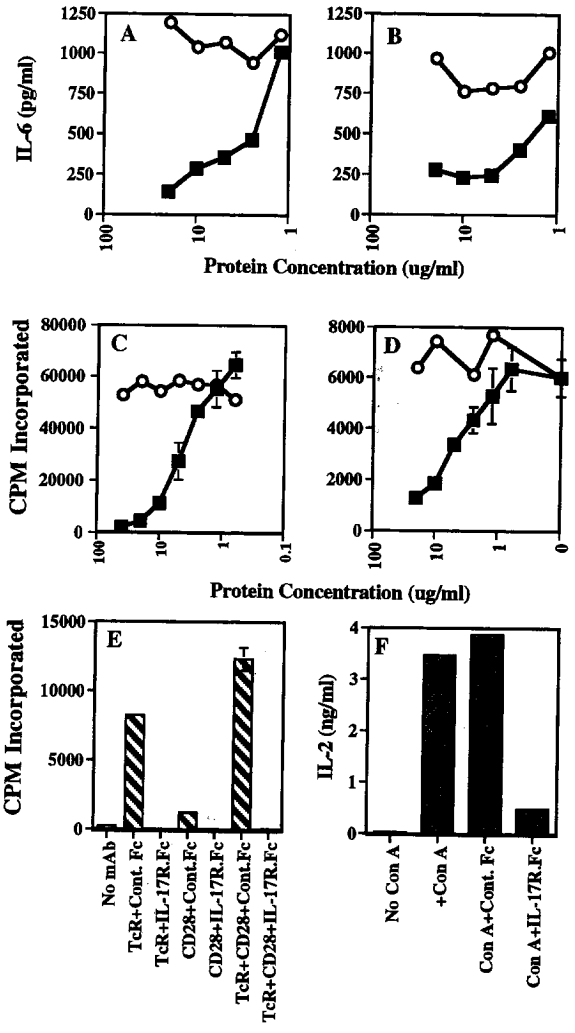

Figure 7. Biological Functions of Soluble IL-17R

(A and B) Effect of soluble IL-17R.Fc protein on HVS13 and IL-17 (mCTLA8) induced IL-6 secretion. 3T3 cells were cultured with 50 ng/ml mCTLA8.Fc (A) or HVS13.Fc (B) in the presence of a titration of IL-17R.Fc (closed square) or a control Fc (open circle) for 24 hr. Conditioned media were collected and IL-6 measured by ELISA.

(C, D, and E) Effect of soluble IL-17R.Fc protein on T cell proliferation induced by conA (C), PHA (D), and immobilized anti-TCR αβ MAb (E). Murine T cells were cultured for 3 days with 1 μg/ml of conA or 2% PHA in the presence of various concentration of IL-17R.Fc (closed square) or a control Fc (open circle). Results are expressed as mean cpm of triplicate cultures ± SEM. These are representative results of eight experiments performed. Alternatively, murine T cells were cultured for 3 days with 1 μg/ml of immobilized anti-TCR αβ MAb or 1 μg/ml anti-CD28 MAb or both in the presence of 20 μg/ml IL-17R.Fc or a control Fc (E). These are representative results of three experiments performed.

(F) Effect of soluble IL-17R.Fc on IL-2 production induced by mitogens. Murine T cells were cultured for 24 hr with 1 μg/ml of conA in the presence or absence of 10 μg/ml of IL-17R.Fc or in the presence of a control Fc. IL-2 production was measured by ELISA and results expressed as ng/ml IL-2 produced. These are representative results of three experiments performed.

(Figure 7B), the addition of increasing amounts of a soluble IL-17R.Fc resulted in increased inhibition of the HVS13- and IL-17 (mCTLA8)-induced IL-6 production. In contrast, the control Fc protein had no effect on HVS13- and IL-17

(mCTLA8)-induced IL-6 secretion. These results demonstrate that the IL-17R.Fc is capable of specifically neutralizing the biological effects of HVS13 and IL-17 (mCTLA8) in an in vitro assay.

To determine whether endogenously produced IL-17 (mCTLA8) plays a role in T cell proliferation, T cells were stimulated with conA or PHA or anti-TCR MAb in the presence of either soluble IL-17R.Fc protein or a control Fc protein. IL-17R.Fc significantly inhibited T cell proliferation induced by conA, PHA, immobilized anti-TCR αβ MAb, and anti-CD28 MAb compared with a control Fc protein (Figures 7C, 7D, and 7E). Analysis of IL-2 production by splenic T cells activated with conA in the presence or absence of IL-17R.Fc in the culture revealed that addition of IL-17R.Fc to the T cell culture inhibited IL-2 production to levels 8- to 9-fold lower than those observed in cultures containing media alone or media plus a control Fc protein (Figure 7F).

Discussion

This paper describes the isolation of a novel murine cytokine receptor using a virus-encoded cytokine as a probe. Recombinant HVS13 and IL-17 (mCTLA8) exhibit classic pleiotropic activities: activation of NF-κB, induction of IL-6 secretion in fibroblasts, and stimulation of T cells to proliferate in the presence of costimuli. These effects are mediated through the interaction between HVS13 and IL-17 (mCTLA8) and a specific cell surface receptor, IL-17R.

IL-17R does not appear to be structurally related to any known cytokine receptor family. Despite the existence of 12 cysteine residues in the extracellular domain, their relative positions are not characteristic of receptors belonging to the immunoglobulin superfamily (Williams and Barclay, 1988) or the TNF receptor family (Smith et al., 1990). The extracellular domain does not contain the WSXWS motif found in hematopoietin receptor family members (Cosman, 1993). The cytoplasmic tail of the IL-17R is rather large in size (521 residues), and an examination of the amino acid sequence indicates no apparent homology with the catalytic domain of any other growth factor receptor known to be a tyrosine kinase (Hanks et al., 1988). There is a relatively large proportion of acidic (16% of aspartic acid and glutamic acid) and proline residues (9%), similar to that observed in other growth factor receptors (Alcover et al., 1987; Hatakeyama et al., 1989). Using a computer-assisted BLOCK search (Henikoff and Henikoff, 1991; Wallace and Henikoff, 1992), a segment (TPPPLR-PRKVW) located proximal to the IL-17R transmembrane domain was found, which is highly conserved among cytokine receptors (Murakami et al., 1991). Furthermore, there are two acidic regions and a serine-rich region in the cytoplasmic domain of IL-17R, similar to that found in the IL-2R β chain (Hatakeyama et al., 1989), IL-4R (Mosley et al., 1989), and G-CSFR (Fukunaga et al., 1990). Whether these regions have any significance in IL-17 signal transduction is under current investigation.

The IL-17R has the ubiquitous tissue distribution seen with several other cytokine receptors including, TNF receptor (Smith et al., 1990), IL-1 receptor (McMahan et al., 1991), and the signal transduction subunit gp130 (Hibi et al., 1990). The wide expression of the receptor is in contrast with the very restricted expression reported for CTLA8 (Rouvier et al., 1993). Our RT–PCR experiments have indicated that IL-17 (mCTLA8) is expressed in both T cells activated by different stimuli and in EL4 cells (data not shown).

The finding that HVS13 activates NF-κB activity is intriguing. NF-κB is a transcription factor known to regulate a large number of gene products involved in cell activation and growth control. NF-κB-induced gene products include molecules involved in immune, inflammatory, or acute phase responses, such as immunoglobulin light chain, major histocompatibility complex (MHC), IL-2R α chain, and cytokines such as IL−1β, IL-6 and TNFα (reviewed by Lenardo and Baltimore, 1989; Siebenlist et al., 1994). NF-κB directly stimulates the HIV enhancer in T cells and can itself be activated by different viral proteins with oncogenic potential, such as the hepatitis B virus HBX protein, EBV LMP1, and HTLV-1 Tax protein (Laherty et al., 1992; Lucito and Schneider, 1992; Hirai et al., 1994). The induction of NF-κB by Tax results in up-regulation of IL-2 and IL-2R and subsequently uncontrolled T cell growth (McGuire et al., 1993). These observations raise interesting questions concerning the role of HVS13 in the etiology of HVS-induced T cell malignancy. Primates, experimentally infected with HVS, die from rapidly progressing neoplasias of the immune system. It is possible that binding of HVS13 to its receptor on the cell surface activates signal transduction pathways that ultimately regulate specific transcription factors involved in oncogenesis. The mitogenic effect of HVS13 may stimulate neighboring uninfected cells into inappropriate proliferation. Alternatively, HVS13 interaction with its receptor may be part of the physiological cytokine network operating within these tumors. Consistent with this hypothesis is the finding that HVS13 is able to induce IL-6 secretion. IL-6 has been shown to be a potent growth factor for myelomas, plasmacytomas, and hybridomas (Kawano et al., 1988; Kishimoto, 1989) and is involved in the growth of Lennert's T lymphoma cells (Shimizu et al., 1988).

The expression of a protein with cytokine activity may be advantageous to HVS during natural infection. HVS13 secreted by HVS-infected cells may bind to neighboring uninfected cells bearing the IL-17R and stimulate their metabolism, enhance their differentiation, cause them to proliferate, or some combination of the three. Activated cells may serve as better targets for infection or more efficiently support virus replication. Human herpesvirus 6 (HHV-6), for example, replicates in activated, but not in quiescent, T lymphocytes (Frenkel et al., 1990). Alternatively, the activation of NF-κB by HVS13 may play a role in the regulation of HVS gene expression. Expression of HIV-1 has been shown to be dependent on tandem κB binding sites in its long terminal repeats (Nabel and Baltimore, 1987). Cytomegalovirus (CMV), another member of the herpesvirus family, contains NF-κB recognition sites in its genome and is transcriptionally stimulated by NF-κB

(Sambucetti et al., 1989; Kowalik et al., 1993). Another possible function of HVS13 might be to induce a subset of cytokines that may control the host immune responses. For example, the viral homolog of IL-10 encoded by EBV inhibits IFNγ synthesis and accessory cell functions of macrophages and thus, inhibits virus-induced T and natural killer cell activation (Hsu et al., 1990; Moore et al., 1993).

Given that different cell types, including fibroblasts, T, and B lineage cells express IL-17R, this ligand–receptor system may play an important role in the interaction of these cell types with one another during an immune response. The fact that IL-17 (mCTLA8) regulates T cell proliferation and is expressed by activated T cells raises the interesting possibility of a role for IL-17 (mCTLA8) as an autocrine factor. The finding that a soluble form of the IL-17R inhibits T cell proliferation induced by polyclonal mitogens and anti-TCR MAb suggests that endogenously produced IL-17 (mCTLA8) and its receptor may be involved in early T cell activation and growth.

Experimental Procedures

Cell Lines and Virus

The murine thymoma cell line EL4 was grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% fetal bovine serum (FBS), 100 μg/ml pyruvate, 50 U/ml penicillin, 50 μg/ml streptomycin, 5×10^{-5} M β-mercaptoethanol. OMK and fibroblast 3T3 cell monolayers were maintained in DMEM containing 10% FBS, supplemented with 10 mM nonessential amino acids, 50 U/ml penicillin, 50 μg/ml streptomycin. HVS was propagated on OMK cell monolayers.

Plasmid Construction and Expression

A soluble type II HVS13.Fc was constructed in the mammalian expression vector pDC406 (McMahan et al., 1991). The CH2 and CH3 domains of human IgG1, which correspond to amino acids 242–470 (Crowe et al., 1992) and a $([Gly_4]Ser)_2$ repeat that serves as a flexible linker domain were inserted downstream of the murine IL-7 leader sequence (amino acids 1–22). This construct was subcloned into pDC406 vector and the resulting plasmid digested with BspEI and NotI. Oligonucleotide primers were used in PCR reactions to amplify an HVS13-specific DNA fragment encoding amino acids 19–151 (full-length excluding signal peptide) using HVS DNA as a template. The primers added a BspEI site at the 5′ end and a NotI site at the 3′ end of the fragment. The PCR product was cut and cloned into the pDC406 carrying the human IgG1.Fc region and flexible linker sequence. An unmodified form of HVS13 was constructed by cloning the complete coding region of HVS13 into a mammalian expression vector pDC409, a derivative of the vector pDC406. The inserts were sequenced in their entirety. To construct a mCTLA8.Fc, two primers were designed based on the published sequence (Rouvier et al., 1993) to amplify a DNA fragment that would encode amino acids 20–150. RNA was prepared from EL4 cells, reverse transcribed with oligo dT primers, and IL-17 (mCTLA8) cDNA was amplified. The PCR fragment was cut and a type II mCTLA8.Fc was constructed in a similar way as described for HVS13.Fc. A soluble unmodified form of IL-17 (mCTLA8) was constructed by cloning IL-17 (mCTLA8) residues 20–150 into a vector with an IL-7 leader sequence. A control Fc was constructed in a similar way by fusing amino acids 19–258 of a vaccinia virus ORF p7.5 to the Fc portion of the human IgG1. A soluble form of IL-17R.Fc fusion protein (type I Fc) was constructed in the mammalian expression vector pDC409 by fusing the extracellular domain of IL-17R (residue 1–322) to the Fc domain of human IgG1.

DEAE transfections were performed in CV1/EBNA cells as described (McMahan et al., 1991). The supernatants from transfected cells were pooled and filtered using a 0.45 μm filter and stored at −70°C before use. Fc fusion purifications were performed as described (Fanslow et al., 1992). Protein concentration was determined by an ELISA specific for the constant domain of human IgG1 and purity confirmed by SDS–PAGE followed by silver stain of the gel. The purified proteins for biological assays were screened for low endotoxin levels (<1 pg/ml at their final concentration) using the Limulus amebocyte lysate assay (Whittaker M. A. Bioproducts, Walkersville, Maryland).

Antisera and MAb

A mouse HVS13 antiserum was prepared by immunizing BALB/c mice with HVS13.Fc fusion protein. Immune sera were tested by ELISA for reactivity to the fusion protein and for their ability to immunoprecipitate the non-Fc form of HVS13 protein. Spleen cells were fused with murine myeloma 8.653 in the presence of selection medium and supernatants tested for binding to HVS13 by ELISA. Hybridomas that produced antibodies positive for binding to HVS13.Fc, but not an irrelevant Fc protein, were cloned by limited dilution methods and further tested by immunoprecipitation. To generate an anti-IL-17R serum, rats were immunized with soluble IL-17R.Fc fusion protein.

Isotopic Labeling and Immunoprecipitation

CV1/EBNA cells were transfected as described above. Cells were labeled 48 hr post-DNA transfection with 100 μCi of L-[^{35}S]methionine (Amersham, Arlington Heights, Illinois) for 3 hr at 37°C. Alternatively, HVS-infected cells were labeled with 300 μCi of L-[^{35}S]methionine for 24 hr. The supernatants and cell lysates were harvested and clarified at 14,000 × g for 30 min. For immunoprecipitation, supernatants or cell lysates were incubated with polyclonal antiserum or preimmune serum followed by the addition of protein A–sepharose beads. After extensive washing with phosphate-buffered saline buffer containing 1% deoxycholate, 1% NP-40, and 0.1% SDS, immunoprecipitates were analyzed on 8%–16% SDS–PAGE.

Flow Cytometric Analysis

Cells (1×10^6) were preincubated on ice for 30 min in 100 μl of FACS buffer (phosphate-buffered saline, 1% FBS, and 0.1% NaN_3) containing 2% normal goat serum and 2% normal rabbit serum to block nonspecific binding. HVS13.Fc, mCTLA8.Fc, or control Fc protein (100 μl) was added at 5 μg/ml and incubated on ice for 30 min. After washing, the cells were stained with phycoerythrin-conjugated anti-human IgG (Fc specific) (Becton Dickinson and Company, Mountain View, California) in 100 μl of FACS buffer. Cells were then washed and a minimum of 5,000 cells were analyzed using a FACScan (Becton Dickinson).

Screening of the Murine EL4 cDNA Expression Library

A murine EL4 cDNA library was constructed and screened as previously described (McMahan et al., 1991; Armitage et al., 1992; Goodwin et al., 1993). In brief, CV1/EBNA cell monolayers on chamber slides were transfected with plasmid DNAs derived from pooled transformants. Cell monolayers were incubated 2 days after transfection with 1 μg/ml of HVS13.Fc, then washed, and incubated with ^{125}I-labeled goat anti-human IgG (New England Nuclear, Cambridge, Massachusetts). After extensive washing, cells were fixed, dipped in photographic emulsion, and then developed. Positive pools of cDNAs were identified and subdivided into smaller cDNA clones until single-positive clones were isolated.

Northern Blot Analysis

Filters containing poly(A)$^+$ RNA (2 μg per lane) from various tissues were purchased from Clontech (Palo Alto, California). Polyadenylated RNA from various cells or cell lines was isolated, fractionated (2 μg per lane) on a 1% agarose formaldehyde gel and blotted onto Hybond nylon membrane (Amersham). Filters were probed with an anti-sense RNA riboprobe corresponding to the coding region of IL-17R cDNA. Hybridization was performed at 63°C followed by three washings in 0.2% × SSC, 0.1% SDS at 68°C. Blots were exposed for 8–48 hr at −70°C.

Cell Surface Biotinylation and Immunoprecipitation

Cell surface protein labeling was performed as described (Meier et al., 1992). In brief, 3×10^7 of EL4 cells or CV1/EBNA cells transfected with IL-17R cDNA in 6-well plates were biotinylated and lysed. Lysates were precleared by centrifugation at 14,000 rpm for 15 min and precipitated with 5 μg/ml of HVS13.Fc or mCTLA8.Fc and run on 8%–16%

SDS–PAGE. The proteins were then blotted onto an enhanced chemiluminescence (ECL) membrane (Amersham). The membrane was incubated with streptavidin–biotinylated horseradish peroxidase complex (Amersham) at 1:100 dilution and developed using the ECL Western blot kit (Amersham) following the instructions of the manufacturer.

Chromosome Localization

C3H/HeJ–*gld* and Mus spretus (Spain) mice and ([C3H/HeJ–*gld* × Mus spretus]F1 × C3H/HeJ–*gld*) interspecific backcross mice were bred and maintained as previously described (Seldin et al., 1988b). DNA isolated from mouse organs was electrophoresed and transferred to Nytran membranes, hybridized at 65°C with radiolabeled IL-17R cDNA probes, and washed under stringent conditions, all as previously described (Watson and Seldin, 1994). Gene linkage was determined by segregation analysis (Green, 1981). Gene order was determined by analyzing all haplotypes and minimizing crossover frequency between all genes that were determined to be within a linkage group. This method resulted in determination of the most likely gene order (Bishop, 1985). The mapping of the following reference loci in this interspecific cross have been previously described: Raf1 and Cd4 (Seldin et al., 1988a; Patel et al., 1995).

Nuclear Extract Preparation and Electrophoretic Mobility Shift Assays

Cells (5×10^6) on a 10 cm dish were stimulated for 30 min with HVS13, IL-17 (mCTLA8) containing supernatant, or a control supernatant. Alternatively, cells were stimulated with 200 ng/ml of control Fc, HVS13.Fc or mCTLA8.Fc. Nuclear extracts were prepared as described (Schütze et al., 1992). Oligonucleotides containing an NF-κB binding site (Ostrowski et al., 1991) were annealed and end-labeled with [γ-^{32}P]ATP using T4 DNA polynucleotide kinase. Mobility shift reactions contained 10 μg of nuclear extract, 4 μg of poly(dI-dC) and 15,000 cpm labeled double-stranded oligonucleotide in the absence or presence of 100-fold cold oligonucleotides containing either a wild-type or a mutated NF-κB binding site in a final volume of 20 μl and incubated at room temperature for 20 min. Resulting protein–DNA complexes were separated on a 6% nondenaturing polyacrylamide gel in 0.25 × Tris–borate–EDTA buffer.

Cytokine Assays

Cells (500 μl at 5×10^6/ml) were distributed into 24-well flat-bottomed microtiter plates (Costar), treated with different culture additions (1:20 dilution of HVS13, IL-17 [mCTLA8] containing supernatants, or purified Fc proteins). After incubation for 24 hr at 37°C, 50 μl of supernatant was removed and assayed for IL-6 using an ELISA assay kit (Genzyme, Boston, Massachusetts). IL-6 levels were calculated by reference to a standard curve constructed with recombinant cytokine.

Proliferation and Cytokine Secretion Assays

Lymphoid organs were harvested aseptically and cell suspensions created as described (Fanslow et al., 1991). Splenic and lymph node T cells were isolated as described (Fanslow et al., 1994). The purity of the resulting splenic T cell preparations was routinely >95% $CD3^+$ and <1% $sIgM^+$. Purified mouse T cells were stimulated with HVS13.Fc, mCTLA8.Fc, or a control Fc protein in culture medium in 96-well plates in the presence or absence of 1% PHA (GIBCO), 1 μg/ml conA (Sigma, St. Louis, Missouri), or 1 μg/ml of immobilized anti-TCR αβ MAb (Pharmingen, San Diego, California). Pulse-labeling and harvesting was as reported previously (Fanslow et al., 1994). Levels of IL-2 in the supernatants of the culture were determined by ELISA.

Acknowledgments

We would like to thank T. Hollingsworth and C.-P. Huang for DNA sequencing. We also wish to thank Dr. D. Cosman for critical reading of the manuscript and helpful suggestions, Drs. D. Williams, K. Mohler, and M. Widmer for critical reading of manuscript, and A. C. Bannister for expert editorial assistance. This work was supported in part by National Institutes of Health grants HG00734 and AR41053 (M. F. S.).

Received August 21, 1995; revised October 2, 1995.

References

Ahuja, S.K., and Murphy, P.M. (1993). Molecular piracy of mammalian interleukin-8 receptor type B by herpesvirus saimiri. J. Biol. Chem. *268*, 20691–20694.

Albrecht, J.-C., Nicholas, J., Biller, D., Cameron, K.R., Biesinger, B., Newman, C., Wittmann, S., Craxton, M.A., Coleman, H., Fleckenstein, B., and Honess, R.W. (1992). Primary structure of the herpesvirus saimiri genome. J. Virol. *66*, 5047–5058.

Alcamí, A., and Smith, G.L. (1992). A soluble receptor for interleukin-1β encoded by vaccinia virus: a novel mechanism of virus modulation of the host response to infection. Cell *71*, 153–167.

Alcover, A., Ramarli, D., Richardson, N.E., Chang, H.C., and Reinherz, E.L. (1987). Functional and molecular aspects of human T lymphocyte activation via T3-Ti and T11 pathways. Immunol. Rev. *95*, 5–36.

Arai, K.-I., Lee, F., Miyajima, A., Miyatake, S., Arai, N., and Yokota, T. (1990). Cytokines: coordinators of immune and inflammatory responses. Annu. Rev. Biochem. *59*, 783–836.

Armitage, R.J., Fanslow, W.C., Strockbine, L., Sato, T.A., Clifford, K.N., Macduff, B.M., Anderson, D.M., Gimpel, S.D., Davis-Smith, T., Maliszewski, C.R., Clark, E.A., Smith, C.A., Grabstein, K.H., Cosman, D., and Spriggs, M.K. (1992). Molecular and biological characterization of a murine ligand for CD40. Nature *357*, 80–82.

Biesinger, B., Müller-Fleckenstein, I., Simmer, B., Lang, G., Wittmann, S., Platzer, E., Desrosiers, R.C., and Fleckenstein, B. (1992). Stable growth transformation of human T lymphocytes by Herpesvirus saimiri. Proc. Natl. Acad. Sci. USA *89*, 3116–3119.

Bishop, D.T. (1985). The information content of phase-known matings for ordering genetic loci. Genet. Epidemiol. *2*, 349–361.

Colamonici, O.R., Domanski, P., Sweitzer, S.M., Larner, A., and Buller, R.M.L. (1995). Vaccinia virus B18R gene encodes a type I interferon-binding protein that blocks interferon a transmembrane signaling. J. Biol. Chem. *270*, 15974–15978.

Cosman, D. (1993). The hematopoietin receptor superfamily. Cytokine *5*, 95–106.

Crowe, J.S., Hall, V.S., Smith, M.A., Cooper, H.J., and Tite, J.P. (1992). Humanized monoclonal antibody CAMPATH-1H: myeloma cell expression of genomic constructs, nucleotide sequence of cDNA constructs and comparison of effector mechanisms of myeloma and Chinese hamster ovary cell-derived material. Clin. Exp. Immunol. *87*, 105–110.

Fanslow, W.C., Clifford, K.N., Park, L.S., Rubin, A.S., Voice, R.F., Beckmann, M.P., and Widmer, M.B. (1991). Regulation of alloreactivity in vivo by IL-4 and the soluble IL-4 receptor. J. Immunol. *147*, 535–540.

Fanslow, W.C., Anderson, D., Grabstein, K.H., Clark, E.A., Cosman, D., and Armitage, R.J. (1992). Soluble forms of CD40 inhibit biologic responses of human B cells. J. Immunol. *149*, 655–660.

Fanslow, W.C., Clifford, K.N., Seaman, M., Alderson, M.R., Spriggs, M.K., Armitage, R.J., and Ramsdell, F. (1994). Recombinant CD40 ligand exerts potent biologic effects on T cells. J. Immunol. *152*, 4262–4269.

Fleckenstein, B., and Desrosiers, R.C. (1982). Herpesvirus saimiri and herpesvirus ateles. In The Herpesviruses, I.B. Roizman, ed. (New York: Plenum Publishing Press), pp. 253–332.

Frenkel, N., Schirmer, E.C., Katsafanas, G., and June, C.H. (1990). T cell activation is required for efficient replication of human herpesvirus 6. J. Virol. *64*, 4598–4602.

Fukunaga, R., Ishizaka-Ikeda, E., Seto, Y., and Nagata, S. (1990). Expression cloning of a receptor for murine granulocyte colony-stimulating factor. Cell *61*, 341–350.

Gao, J.-L., and Murphy, P.M. (1994). Human cytomegalovirus open reading frame *US28* encodes a functional β chemokine receptor. J. Biol. Chem. *269*, 28539–28542.

Gooding, L.R. (1992). Virus proteins that counteract host immune defenses. Cell *71*, 5–7.

Goodwin, R.G., Alderson, M.R., Smith, C.A., Armitage, R.J., VandenBos, T., Jerzy, R., Tough, T.W., Schoenborn, M.A., Davis-Smith, T., Hennen, K., Falk, B., Cosman, D., Baker, E., Sutherland, G.R., Grabstein, K.H., Farrah, T., Giri, J.G., and Beckmann, M.P. (1993). Molecular and biological characterization of a ligand for CD27 defines a new family of cytokines with homology to tumor necrosis factor. Cell *73*, 447–456.

Green, E.L. (1981). Linkage, Recombination, and Mapping (New York: Oxford University Press), pp. 77–113.

Greene, W.C., Böhnlein, E., and Ballard, D.W. (1989). HIV-1, HTLV-1 and normal T cell growth: transcriptional strategies and surprises. Immunol. Today *10*, 272–278.

Hanks, S.K., Quinn, A.M., and Hunter, T. (1988). The protein kinase family: conserved features and deduced phylogeny of the catalytic domains. Science *241*, 42–52.

Hatakeyama, M., Mori, H., Doi, T., and Taniguchi, T. (1989). A restricted cytoplasmic region of IL-2 receptor β chain is essential for growth signal transduction but not for ligand binding and internalization. Cell *59*, 837–845.

Henikoff, S., and Henikoff, J.G. (1991). Automated assembly of protein blocks for database searching. Nucl. Acids Res. *19*, 6565–6572.

Hibi, M., Murakami, M., Saito, M., Hirano, T., Taga, T., and Kishimoto, T. (1990). Molecular cloning and expression of an IL-6 signal transducer, gp130. Cell *63*, 1149–1157.

Hirai, H., Suzuki, T., Fujisawa, J., Inoue, J., and Yoshida, M. (1994). Tax protein of human T cell leukemia virus type I binds to the ankyrin motifs of inhibitory factor κB and induces nuclear translocation of transcription factor NF-κB proteins for transcriptional activation. Proc. Natl. Acad. Sci. USA *91*, 3584–3588.

Hsu, D.-H., de Waal Malefyt, R., Fiorentino, D.F., Dang, M.-N., Vieira, P., deVries, J., Spits, H., Mosmann, T.R., and Moore, K.W. (1990). Expression of interleukin-10 activity by Epstein–Barr virus protein BCRF1. Science *250*, 830–832.

Kawano, M., Hirano, T., Matsuda, T., Taga, T., Horii, Y., Iwato, K., Asaoku, H., Tang, B., Tanabe, O., Tanaka, H., Kuramoto, A., and Kishimoto, T. (1988). Autocrine generation and requirement of BSF-2/IL-6 for human multiple myelomas. Nature *332*, 83–85.

Kishimoto, T. (1989). The biology of interleukin-6. Blood *74*, 1–10.

Kishimoto, T., Taga, T., and Akira, S. (1994). Cytokine signal transduction. Cell *76*, 253–262.

Kowalik, T.F., Wing, B., Haskill, J.S., Azizkhan, J.C., Baldwin, A.S., Jr., and Huang, E.-S. (1993). Multiple mechanisms are implicated in the regulation of NF-κB activity during human cytomegalovirus infection. Proc. Natl. Acad. Sci. USA *90*, 1107–1111.

Kozak, M. (1991). An analysis of vertebrate mRNA sequences: intimations of translational control. J. Cell. Biol. *115*, 887–903.

Laherty, C.D., Hu, H.M., Opipari, A.W., Wang, F., and Dixit, V.M. (1992). The Epstein–Barr virus LMP1 gene product induces A20 zinc finger protein expression by activating nuclear factor κB. J. Biol. Chem. *267*, 24157–24160.

Lenardo, M.J., and Baltimore, D. (1989). NF-κB: a pleiotropic mediator of inducible and tissue-specific gene control. Cell *58*, 227–229.

Lucito, R., and Schneider, R.J. (1992). Hepatitis B virus X protein activates transcription factor NF-κB without a requirement for protein kinase C. J. Virol. *66*, 983–991.

McGuire, K.L., Curtiss, V.E., Larson, E.L., and Haseltine, W.A. (1993). Influence of human T cell leukemia virus type I *tax* and *rex* on interleukin-2 gene expression. J. Virol. *67*, 1590–1599.

McMahan, C.J., Slack, J.L., Mosley, B., Cosman, D., Lupton, S.D., Brunton, L.L., Grubin, C.E., Wignall, J.M., Jenkins, N.A., Brannan, C.I., Copeland, N.G., Huebner, K., Croce, C.M., Cannizzarro, L.A., Benjamin, D., Dower, S.K., Spriggs, M.K., and Sims, J.E. (1991). A novel IL-1 receptor, cloned from B cells by mammalian expression, is expressed in many cell types. EMBO J. *10*, 2821–2832.

Meier, T., Arni, S., Malarkannan, S., Poincelet, M., and Hoessli, D. (1992). Immunodetection of biotinylated lymphocyte-surface proteins by enhanced chemiluminescence: a nonradioactive method for cell-surface protein analysis. Anal. Biochem. *204*, 220–226.

Moore, K.W., O'Garra, A., de Waal Malefyt, R., Vieira, P., and Mosmann, T. R. (1993). Interleukin-10. Annu. Rev. Immunol. *11*, 165–190.

Mosley, B., Beckmann, M.P., March, C.J., Idzerda, R.L., Gimpel, S.D., VandenBos, T., Friend, D., Alpert, A., Anderson, D., Jackson, J., Wignall, J.M., Smith, C., Gallis, B., Sims, J.E., Urdal, D., Widmer, M.B., Cosman, D., and Park, L.S. (1989). The murine interleukin-4 receptor: molecular cloning and characterization of secreted and membrane bound forms. Cell *59*, 335–348.

Murakami, M., Narazaki, M., Hibi, M., Yawata, H., Yasukawa, K., Hamaguchi, M., Taga, T., and Kishimoto, T. (1991). Critical cytoplasmic region of the interleukin 6 signal transducer gp130 is conserved in the cytokine receptor family. Proc. Natl. Acad. Sci. USA *88*, 11349–11353.

Nabel, G., and Baltimore, D. (1987). An inducible transcription factor activates expression of human immunodeficiency virus in T cells. Nature *326*, 711–713.

Nicholas, J., Cameron, K.R., and Honess, R.W. (1992). Herpesvirus saimiri encodes homologues of G protein–coupled receptors and cyclins. Nature *355*, 362–365.

Ostrowski, J., Sims, J.E., Sibley, C.H., Valentine, M.A., Dower, S.K., Meier, K.E., and Bomsztyk, K. (1991). A serine/threonine kinase activity is closely associated with a 65-kDa phosphoprotein specifically recognized by the κB enhancer element. J. Biol. Chem. *266*, 12722–12733.

Patel, A., Rochelle, J.M., Jones, J.M., Sumegi, J., Uhl, G.R., Seldin, M.F., Meisler, M.H., and Gregor, P. (1995). Mapping of the taurine transporter gene to mouse chromosome 6 and to the short arm of human chromosome 3. Genomics *25*, 314–317.

Paul, W.E., and Seder, R.A. (1994). Lymphocyte responses and cytokines. Cell *76*, 241–251.

Pickup, D.J. (1994). Poxviral modifiers of cytokine responses to infection. Infect. Agents Dis. *3*, 116–127.

Poli, G., Bressler, P., Kinter, A., Duh, E., Timmer, W.C., Rabson, A., Justement, J.S., Stanley, S., and Fauci, A.S. (1990). Interleukin 6 induces human immunodeficiency virus expression in infected monocytic cells alone and in synergy with tumor necrosis factor α by transcriptional and post-transcriptional mechanisms. J. Exp. Med. *172*, 151–158.

Roizman, B. (1992). The family herpesviridae: general description, taxonomy, and classification. In The Herpesviruses, B. Roizman, ed. (New York: Plenum Press), pp. 1–23.

Rouvier, E., Luciani, M.-F., Mattéi, M.G., Denizot, F., and Golstein, P. (1993). CTLA-8, cloned from an activated T cell, bearing AU-rich messenger RNA instability sequences, and homologous to a herpesvirus saimiri gene. J. Immunol. *150*, 5445–5456.

Sambucetti, L.C., Cherrington, J.M., Wilkinson, G.W., and Mocarski, E.S. (1989). NF-κB activation of the cytomegalovirus enhancer is mediated by a viral transactivator and by T cell stimulation. EMBO J. *8*, 4251–4258.

Saunders, A.M., and Seldin, M.F. (1990). A molecular genetic linkage map of mouse chromosome 7. Genomics *8*, 525–535.

Schall, T.J., Stein, B., Gorgone, G., and Bacon, K.B. (1995). Cytomegalovirus encodes a functional receptor for C–C chemokines. In Viroceptors, Virokines and Related Immune Modulators Encoded by DNA Viruses, G. McFadden, ed. (Austin, Texas: R. G. Landes Company), pp. 201–214.

Schütze, S., Potthoff, K., Machleidt, T., Berkovic, D., Wiegmann, K., and Krönke, M. (1992). TNF activates NF-κB by phosphatidylcholine-specific phospholipase C-induced "acidic" sphingomyelin breakdown. Cell *71*, 765–776.

Seldin, M.F., D'Hoostelaere, L.A., Huppi, K., Mock, B.A., Steinberg, A.D., Parnes, J.R., and Morse, H.C., III (1988a). Mapping of the Ly-4 (L3T4) T cell differentiation antigen on mouse chromosome 6 by the use of RFLPs in an interspecific cross. Immunogenetics *27*, 396–398.

Seldin, M.F., Morse, H.C., III, Reeves, J.P., Scribner, C.L., LeBoeuf, R.C., and Steinberg, A.D. (1988b). Genetic analysis of autoimmune *gld* mice. I. Identification of a restriction fragment length polymorphism closely linked to the *gld* mutation within a conserved linkage group.

J. Exp. Med. *167*, 688–693.

Shaw, G., and Kamen, R. (1986). A conserved AU sequence from the 3′ untranslated region of GM-CSF mRNA mediates selective mRNA degradation. Cell *46*, 659–667.

Shimizu, S., Hirano, T., Yoshioka, R., Sugai, S., Matsuda, T., Taga, T., Kishimoto, T., and Konda, S. (1988). Interleukin-6 (B-cell stimulatory factor 2)-dependent growth of a Lennert's lymphoma-derived T cell line (KT-3). Blood *72*, 1826–1828.

Siebenlist, U., Franzoso, G., and Brown, K. (1994). Structure, regulation and function of NF-κB. Annu. Rev. Cell Biol. *10*, 405–455.

Smith, C.A., Davis, T., Anderson, D., Solam, L., Beckmann, M.P., Jerzy, R., Dower, S.K., Cosman, D., and Goodwin, R.G. (1990). A receptor for tumor necrosis factor defines an unusual family of cellular and viral proteins. Science *248*, 1019–1023.

Smith, C.A., Davis, T., Wignall, J.M., Din, W.S., Farrah, T., Upton, C., McFadden, G., and Goodwin, R.G. (1991). T2 open reading frame from the shope fibroma virus encodes a soluble form of the TNF receptor. Biochem. Biophys. Res. Comm. *176*, 335–342.

Spriggs, M.K. (1994). Cytokine and cytokine receptor genes 'captured' by viruses. Curr. Opin. Immunol. *6*, 526–529.

Spriggs, M.K., Hruby, D.E., Maliszewski, C.R., Pickup, D.J., Sims, J.E., Buller, R.M.L., and VanSlyke, J. (1992). Vaccinia and cowpox viruses encode a novel secreted interleukin-1-binding protein. Cell *71*, 145–152.

Symons, J.A., Alcamí, A., and Smith, G.L. (1995). Vaccinia virus encodes a soluble type I interferon receptor of novel structure and broad species specificity. Cell *81*, 551–560.

Upton, C., Mossman, K., and McFadden, G. (1992). Encoding of a homolog of the IFNγ receptor by myxoma virus. Science *258*, 1369–1372.

Wallace, J.C., and Henikoff, S. (1992). PATMAT: a searching and extraction program for sequence, pattern and block queries and databases. Comput. Appl. Biosci. *8*, 249–254.

Watson, M.L., and Seldin, M.F. (1994). Application of mouse crosses towards defining the genetics of disease phenotypes. Meth. Mol. Genet. *5*, 369–387.

Watson, M.L., Eustachio, P.D., Mock, B.A., Steinberg, A.D., Morse, H.C., Oakley, R.J., Howard, T.A., Rochelle, J.M., and Seldin, M.F. (1992). A linkage map of chromosome 1 using an interspecific cross segregating for the *gld* autoimmunity mutation. Mamm. Genome *2*, 158–171.

Williams, A.F., and Barclay, A.N. (1988). The immunoglobulin superfamily: domains for cell surface recognition. Annu. Rev. Immunol. *6*, 381–405.

GenBank Acession Number

The nucleotide sequence of IL-17R has been deposited in GenBank under accession number U31993.