Immunity, Vol. 10, 127–136, January, 1999, Copyright ©1999 by Cell Press

Interleukin-18 Binding Protein: A Novel Modulator of the Th1 Cytokine Response

Daniela Novick,*§ Soo-Hyun Kim,*§
Giamila Fantuzzi,† Leonid L. Reznikov,†
Charles A. Dinarello,† and Menachem Rubinstein*‡
*Department of Molecular Genetics
Weizmann Institute of Science
Rehovot 76100
Israel
†Division of Infectious Diseases
University of Colorado Health Sciences Center
Denver, Colorado 80262

Summary

An interleukin-18 binding protein (IL-18BP) was purified from urine by chromatography on IL-18 beads, sequenced, cloned, and expressed in COS7 cells. IL-18BP abolished IL-18 induction of interferon-γ (IFNγ), IL-8, and activation of NF-κB in vitro. Administration of IL-18BP to mice abrogated circulating IFNγ following LPS. Thus, IL-18BP functions as an inhibitor of the early Th1 cytokine response. IL-18BP is constitutively expressed in the spleen, belongs to the immunoglobulin superfamily, and has limited homology to the IL-1 type II receptor. Its gene was localized on human chromosome 11q13, and no exon coding for a transmembrane domain was found in an 8.3 kb genomic sequence. Several Poxviruses encode putative proteins highly homologous to IL-18BP, suggesting that viral products may attenuate IL-18 and interfere with the cytotoxic T cell response.

Introduction

The cytokine interleukin (IL)-18, initially described as an interferon-γ (IFNγ)-inducing factor (Nakamura et al., 1989), is an early signal in the development of T-lymphocyte helper type 1 (Th1) responses. IL-18 acts together with IL-12, IL-2, antigens, mitogens, and possibly IFNα (Sareneva et al., 1998) to induce the production of IFNγ (Okamura et al., 1998). IL-18 also enhances the production of GM-CSF and IL-2, potentiates anti-CD3-induced T cell proliferation, and increases Fas-mediated killing of natural killer cells (Micallef et al., 1996; Tsutsui et al., 1996). The obligatory role for IL-18 in IFNγ production was first demonstrated by using passive immunization with antibodies to murine (mu) IL-18 (Okamura et al., 1995). The secondary structure of IL-18 associates it with the IL-1 family of cytokines (Bazan et al., 1996), and similarly to IL-1, mature IL-18 is produced from its precursor by the IL-1β converting enzyme (ICE, caspase-1). Indeed, the lack of IFNγ production following endotoxin in mice deficient in caspase-1 was attributed to absence of mature IL-18 in these mice (Ghayur et al., 1997; Gu et al., 1997; Fantuzzi et al., 1998). Similarly, induction of IFNγ in mice deficient in IL-18 (IL-18$^{-/-}$ mice) in response to endotoxin is markedly reduced, despite normal production of IL-12 (Takeda et al., 1998). Natural killer (NK) cells from these IL-18$^{-/-}$ mice also exhibit an impaired cytotoxic response in vitro.

The IL-18 receptor consists of at least two components, cooperating in ligand binding. High- and low-affinity binding sites for IL-18 were found in murine IL-12-stimulated T cells (Yoshimoto et al., 1998), suggesting a multiple chain receptor complex. A ligand-binding component of this receptor complex has been isolated from membrane proteins by affinity purification with a neutralizing monoclonal antibody directed against the IL-18 receptor. The sequence of this receptor matched that of the previously identified IL-1 receptor related protein (IL-1Rrp; Torigoe et al., 1997). A second receptor subunit, termed accessory protein-like (AcPL), essential for signaling, was recently cloned (Born et al., 1998). Both receptor subunits belong to the IL-1 receptor family (Parnet et al., 1996).

IL-18 shares with IL-1 and TNF family members some of their signaling mechanisms. Following exposure to IL-18, IL-1Rrp recruits the IL-1 receptor activating kinase (IRAK), which in turn associates with the downstream signaling molecule TRAF6 (Croston et al., 1995; Robinson et al., 1997; Kojima et al., 1998). The latter was found to associate with the NF-κB-inducing kinase (NIK; Malinin et al., 1997), which activates a signaling cascade involving two IκB kinases, IKKα and IKKβ. IκB, the specific substrate of IKKs, is constitutively associated with NF-κB. Upon phosphorylation of IκBα by IKK, cytoplasmic NF-κB is released and translocates to the nucleus, where its dimeric forms act as transcription factors (DiDonato et al., 1997; Regnier et al., 1997).

IL-1Rrp exhibits a rather low affinity (*Kd* of 25–45 nM) for IL-18. Previously, it was demonstrated that soluble receptors corresponding to ligand-binding domains of cytokine receptors may be conveniently isolated from urine and then used for cloning of the cell-surface receptor (Engelmann et al., 1989, 1990; Novick et al., 1989, 1994). In each case, the sequence of the isolated soluble receptor was identical to the extracellular ligand-binding domain of a corresponding cell surface receptor.

Here, we present the isolation of an IL-18 binding protein (IL-18BP) from human urine and cloning of its human and mouse cDNA and the human gene. Unlike previously isolated urinary proteins, IL-18BP is not a variant of a cell-surface receptor. The urinary IL-18BP, as well as IL-18BP expressed in COS7 cells, specifically bound IL-18 and neutralized its biological activity in vitro. Furthermore, recombinant IL-18BP inhibited lipopolysaccharide (LPS) induction of IFNγ in mice. IL-18BP belongs to a novel family of secreted proteins, which include several Poxvirus-encoded proteins, suggesting that the latter may also act as inhibitors of IL-18 activity.

Results

Isolation of an IL-18BP from Human Urine

In an effort to isolate a soluble IL-18 receptor, we passed a preparation of proteins concentrated from 500 liters

‡To whom correspondence should be addressed (e-mail: lvrub@weizmann.weizmann.ac.il).
§These authors contributed equally to this work.

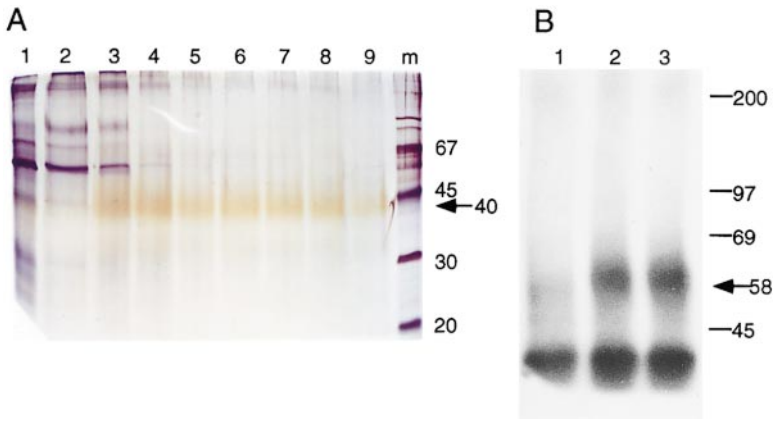

Figure 1. Affinity Purification of IL-18BP and Its Cross-Linking with IL-18

(A) Crude urinary proteins from the affinity purification process were analyzed by SDS-PAGE (10% acrylamide, nonreducing conditions) and stained with silver. Lane 1, crude urinary proteins (1.5 μg); lanes 2–9 depict 50 μl samples of progressively eluted fractions. The yellow protein band indicated by an arrow is IL-18BP. Molecular weight markers are indicated on the right side.

(B) Aliquots of fractions from the affinity purification process cross-linked to ^{125}I-IL-18 were analyzed by SDS-PAGE (7.5% acrylamide) under reducing conditions. Lane 1, a fraction containing unbound crude urinary proteins. Lane 2, a partially purified IL-18BP (elution fraction 2, Figure 1A, lane 3). Lane 3, a purified IL-18BP (elution fraction 3, Figure 1A, lane 4). The arrow indicates the cross-linked 58 kDa product. The lower band is unbound ^{125}I-IL-18.

of normal human urine on an IL-18-agarose column. Following washing, bound proteins were eluted at a low pH. As shown in Figure 1A, SDS-PAGE and silver staining revealed an ~40 kDa IL-18BP in the eluted fractions, exhibiting a distinct yellow color. Upon SDS-PAGE of an N-glycanase-treated IL-18BP, the ~40 kDa band disappeared and a 20 kDa band was observed, suggesting that the ~40 kDa IL-18BP is highly glycosylated (data not shown).

To determine the specificity of IL-18 binding to IL-18BP, aliquots of the ~40 kDa IL-18 from the affinity column were cross-linked with ^{125}I-IL-18 and the resulting products were assessed by SDS-PAGE and autoradiography. As shown in Figure 1B, a 58 kDa cross-linked product, corresponding in size to a 1:1 complex of the 18–19 kDa IL-18 plus the ~40 kDa IL-18BP, was observed (Figure 1B, lanes 2 and 3). Cross-linking of IL-18 with a wash fraction of the affinity column, consisting of urinary proteins not binding to the IL-18 affinity column, did not result in any cross-linked product, thereby demonstrating that the ~40 kDa IL-18BP binds IL-18 in a specific manner (Figure 1B, lane 1).

Urinary IL-18BP Inhibits the IFNγ-Inducing Activity of IL-18

IL-18 induces IFNγ when added together with low concentration of LPS, IL-12, or TNFα. The activity of IL-18BP was tested in murine splenocytes, in the human KG-1 cell line, and in human peripheral blood mononuclear cells (PBMC). As shown in Figure 2A, IL-18BP blocked the activity of human (hu)-IL-18 in murine splenocytes in a dose-dependent manner. The activity of recombinant murine (mu)-IL-18 was similarly inhibited by the huIL-18BP. In contrast, as a control, soluble interferon-α/β receptor (sIFNAR2; Novick et al., 1994) had no effect. High concentrations of LPS (10 μg/ml) induce endogenous IL-18 in murine splenocytes, as determined by production of IFNγ. Indeed, the urinary IL-18BP inhibited the production of IFNγ by high dose LPS (Figure 2B). Concanavalin A (Con A) activates T cells to produce IFNγ in the absence of IL-18 (Fantuzzi et al., 1998). As expected, IL-18BP did not inhibit the induction of IFNγ by Con A, even at high concentrations of the binding protein (Figure 2B). This observation demonstrated that IL-18BP was a specific inhibitor of IL-18 bioactivity rather than a nonspecific inhibitor of IFNγ production. IL-18BP also inhibited the activity of huIL-18 in human cells. Induction of IFNγ in KG-1 cells by a combination of IL-18 and TNFα was inhibited by the urinary IL-18BP (Figure 2C). Similarly, induction of IFNγ in PBMC by a combination of IL-18 and IL-12 (Micallef et al., 1996) was inhibited by the urinary IL-18BP (data not shown).

The above data demonstrate that urinary IL-18BP inhibits human as well as muIL-18 activity. The concentration of IL-18BP that abolished IL-18 activity was comparable to that of IL-18 itself, suggesting a high-affinity interaction between these two proteins. Thus, IL-18BP appears to act as a soluble decoy receptor.

Protein Sequencing of the Urinary IL-18BP

Initial attempts to determine the N-terminal amino acid sequence of a PVDF-blotted ~40 kDa urinary IL-18BP band from nonreducing SDS-PAGE yielded a mixed sequence of two polypeptides, one corresponding to a fragment of human defensin and another one starting with amino acids Thr-Pro-Val. To obtain a single and longer protein sequence, a reduced and alkylated sample of the affinity-purified IL-18BP was subjected to protein microsequencing and treated with o-phtalaldehyde (OPA) after the first sequencing cycle. OPA blocks all N-terminal amino acids except that of proline. Indeed, using OPA, a single sequence of 40 amino acids residues was obtained (Figure 3A, dot-underlined section). This sequence was not found in GenBank; however, a search in the human cDNA database of The Institute of Genomic Research (TIGR) resulted in an almost perfect match with the cDNA clone THC123801, derived from a human Jurkat T cell library and coding for a protein of unknown function.

Cloning of the Human and Mouse IL-18BP cDNA and Their Homology with a Family of Putative Poxvirus Proteins

The TIGR cDNA sequence appeared to be incomplete and had several mismatches when compared with the 40

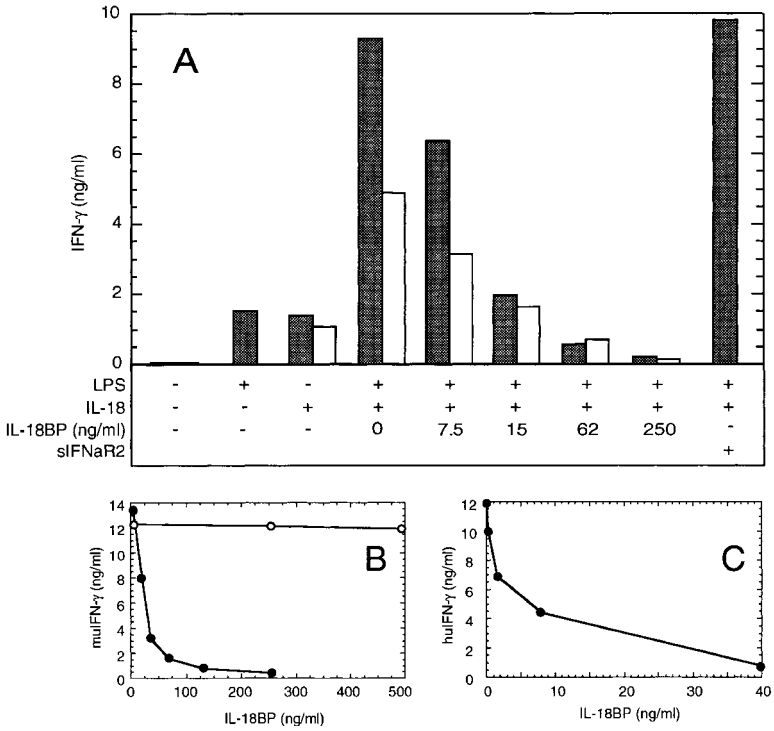

Figure 2. Inhibition of IL-18-Induced Production of IFNγ by IL-18BP

(A) Mouse splenocytes were stimulated with the indicated combinations of LPS and huIL-18 (black bars), added either in RPMI or after premixing with urinary IL-18BP in RPMI. Similarly, mouse splenocytes were incubated with LPS and muIL-18 (white bars), premixed with increasing concentrations of huIL-18BP.

(B) Mouse splenocytes were incubated with LPS (10 μg/ml, black circles, to induce endogenous IL-18) and increasing concentrations of huIL-18BP. Alternatively, mouse splenocytes were incubated with Con A (1 μg/ml, white circles) and increasing concentrations of huIL-18BP.

(C) Human KG-1 cells were stimulated with TNFα (20 ng/ml) and huIL-18, premixed with increasing concentrations of urinary IL-18BP.

amino acid sequence of the urinary IL-18BP. Therefore, several cDNA libraries were screened in order to identify a complete IL-18BP cDNA and possible splice variants. A probe for screening cDNA libraries was prepared by reverse-transcription-polymerase chain reaction (RT-PCR) of Jurkat cell RNA with primers from the TIGR sequence. Four human cDNA libraries, derived from Jurkat cells, peripheral blood monocytes, human PBMC, and from human spleen were screened. Altogether, we have obtained and characterized 106 independent cDNA clones, corresponding to three IL-18BP splice variants. All splice variants coded for putative soluble secreted proteins. The most abundant one, designated IL-18BPa (accession number AF110799), had an open reading frame of 192 codons. It coded for a signal peptide of 28 amino acid residues followed by a mature putative IL-18BPa, whose first 40 residues matched perfectly with the N-terminal protein sequence of the urinary IL-18BP (Figure 3A). The amino acid sequences around cysteines 83 and 148 and tryptophan 98 suggested that this polypeptide belongs to the immunoglobulin (Ig) super-family (Williams and Barclay, 1988). Interestingly, each of the four Gln residues within mature IL-18BPa was a potential N-glycosylation site. The two other splice variants of IL-18BP were significantly less abundant (see below).

Database searching of murine cDNA libraries for homologs of human IL-18BP revealed a partially sequenced (447 bp) cDNA clone of unknown function (GenBank accession number AA498857). Its partial open reading frame of 74 codons was 65.7% identical at the amino acid level with the huIL-18BP. This level of homology suggested that clone AA498857 is the cDNA of muIL-18BP. A probe was then prepared by RT-PCR of RNA from mouse spleen with primers from the partial murine cDNA sequence. Screening of a mouse spleen cDNA library with this probe yielded 54 independent cDNA clones. A group of 15 independent cDNA clones of 2.6–4 kb (designated muIL-18BPc, accession number AF110802) coded for a soluble, secreted protein having a signal peptide of 26 amino acid residues and a mature IL-18BPc of 166 amino acid residues. The open reading frame of the murine IL-18BPc exhibited 66.7% identity with huIL-18BPc at the protein level (Figure 3B). Another splice variant, designated muIL-18BPd (accession number AF110803), also coded for a soluble protein. No cDNA coding for a TM IL-18BP was identified in the human and mouse cDNA libraries.

Homology searches of IL-18BP did not reveal an obvious similarity at the primary structure to any cytokine receptor or to any other known eukaryotic protein. No significant homology was found between IL-18BP and IL-1Rrp. Also, the hydropathy profile of the IL-18BP variants did not match that of any known cytokine receptor (data not shown). Yet, the Ig domain of IL-18BP is homologous to the third Ig domain of the decoy receptor IL-1R type II. Furthermore, IL-18BP was significantly homologous to a family of putative proteins encoded by several Poxviruses. These viral proteins also belong to the Ig superfamily. Each has a signal peptide and therefore appears to be secreted. Two genes of these proteins were found in the genome of Molluscum contagiosum virus (MCV) subtype 1, whereas others were found in Swinepox, Cowpox, Variola, and Ectromelia viruses. The highest homology (47% identity in a sequence of 82 amino acid residues) was obtained with the putative protein encoded by open reading frame (ORF) 54L of the MCV genome. Each of these viral proteins showed homology to the Ig domain (codons 82–163) of the huIL-18BPa (Figure 3B).

A

```
                                                                GAGAA      5
GAGGACGTTGTCACAGATAAAGAGCCAGGCTCACCAGCTCCTGACGCATGCATCATGACC     65
ATGAGACACAACTGGACACCAGACCTCAGCCCTTTGTGGGTCCTGCTCCTGTGTGCCCAC    125
M  R  H  N  W  T  P  D  L  S  P  L  W  V  L  L  L  C  A  H     20

GTCGTCACTCTCCTGGTCAGAGCCACACCTGTCTCGCAGACCACCACAGCTGCCACTGCC    185
V  V  T  L  L  V  R  A  T  P  V  S  Q  T  T  T  A  A  T  A     40

TCAGTTAGAAGCACAAAGGACCCCTGCCCCTCCCAGCCCCCAGTGTTCCCAGCAGCTAAG    245
S  V  R  S  T  K  D  P  C  P  S  Q  P  P  V  F  P  A  A  K     60

CAGTGTCCAGCATTGGAAGTGACCTGGCCAGAGGTGGAAGTGCCACTGAATGGAACGCTG    305
Q  C  P  A  L  E  V  T  W  P  E  V  E  V  P  L  N* G  T  L     80

AGCTTATCCTGTGTGGCCTGCAGCCGCTTCCCCAACTTCAGCATCCTCTACTGGCTGGGC    365
S  L  S  C  V  A  C  S  R  F  P  N* F  S  I  L  Y  W  L  G    100

AATGGTTCCTTCATTGAGCACCTCCCAGGCCGACTGTGGGAGGGGAGCACCAGCCGGGAA    425
N* G  S  F  I  E  H  L  P  G  R  L  W  E  G  S  T  S  R  E    120

CGTGGGAGCACAGGTACGCAGCTGTGCAAGGCCTTGGTGCTGGAGCAGCTGACCCCTGCC    485
R  G  S  T  G  T  Q  L  C  K  A  L  V  L  E  Q  L  T  P  A    140

CTGCACAGCACCAACTTCTCCTGTGTGCTCGTGGACCCTGAACAGGTTGTCCAGCGTCAC    545
L  H  S  T  N* F  S  C  V  L  V  D  P  E  Q  V  V  Q  R  H    160

GTCGTCCTGGCCCAGCTCTGGGCTGGGCTGAGGGCAACCTTGCCCCCCACCCAAGAAGCC    605
V  V  L  A  Q  L  W  A  G  L  R  A  T  L  P  P  T  Q  E  A    180

CTGCCCTCCAGCCACAGCAGTCCACAGCAGCAGGGTTAAGACTCAGCACAGGGCCAGCAG    665
L  P  S  S  H  S  S  P  Q  Q  Q  G                            192

CAGCACAACCTTGACCAGAGCTTGGGTCCTACCTGTCTACCTGGAGTGAACAGTCCCTGA    725
CTGCCTGTAGGCTGCGTGGATGCGCAACACACCCCCTCCTTCTCTGCTTTGGGTCCCTTC    785
TCTCACCAAATTCAAACTCCATTCCCACCTACCTAGAAAATCACAGCCTCCTTATAATGC    845
CTCCTCCTCCTGCCATTCTCTCTCCACCTATCCATTAGCCTTCCTAACGTCCTACTCCTC    905
ACACTGCTCTACTGCTCAGAAACCACCAAGACTGTTGATGCCTTAGCCTTGCACTCCAGG    965
GCCCTACCTGCATTTCCCACATGACTTTCTGGAAGCCTCCCAACTATTCTTGCTTTTCCC   1025
AGACAGCTCCCACTCCCATGTCTCTGCTCATTTAGTCCCGTCTTCCTCACCGCCCCAGCA   1085
GGGGAACGCTCAAGCCTGGTTGAAATGCTGCCTCTTCAGTGAAGTCATCCTCTTTCAGCT   1145
CTGGCCGCATTCTGCAGACTTCCTATCTTCGTGCTGTATGTTTTTTTTTCCCCCTTCAC   1205
TCTAATGGACTGTTCCAGGGAAGGGATGGGGGCACCAGCTGCTTCGGATCCACACTGTAT   1265
CTGTGTCATCCCCACATGGGTCCTCATAAAGGATTATTCAATGGAAAAAAAAAAAAAAAA   1325
```

B

```
huIL18BPa    82  LSCVAC-SRFPNFSILYWLGNGS--FIEHLPGRLWEGSTSRER-G 122
muIL18BPc    77  LSCTAC-SRFPYFSILYWLGNGS--FLEHLPGRLKEGHTSREH-R 117
mcu60315_54  52  LTCLGC-TNHTHVSLIYWIVNES--FPEQLDSSLSEGRTHKHKFP  93
mcu60315_53  55  VSCVAC-SHTPLFSRLYWIKNGS--HAEH--------QGSTWITYTP  90
u94848_4     38  -HCSGCVEHMPEFSYMYWLAKDT-KFIEHLGDGIKEDETVRTTDS  80
swphlsb_12   38  VKCNG-YTNS-TYSILYWMVGNNNTFVEQLNSDHYKEKKYNSTEK  80

huIL18BPa   123  STGTQLCKA-LVLEQLTPALHSTNFSCVLVDPEQVVQRHVVL     163
muIL18BPc   118  NTSTWLHRA-LVLEELSPTLRSTNFSCLFVDPGQVAQYHIIL     158
mcu60315_54  94  NQSLTEIST-NLTVGPDVATHSTNFSCVLVDPEQVVQRHLALT    135
mcu60315_53  91  NGTRLSRQL-HLGADFTLT---QNLTCRLADGEHVQEKSIVL     116
u94848_4     81  GITTLRKVL-HVTD--TNKFAHYRFTCVLTTIDGVSKKNIWLK    120
swphlsb_12   81  NEHMYKLRTDLIIYNITSEMEMTKLTCVLSDIYTPIKASIIL     122
```

Figure 3. The Sequence of huIL-18BPa cDNA and Protein

(A) Nucleotide and amino acid sequence of huIL-18BPa. The signal peptide is underlined and the region corresponding to the N-terminal protein sequence of the urinary IL-18BP is indicated by a dotted line. Putative N-glycosylation sites are indicated by asterisks and the polyadenylation signal is underlined. (B) Homology among IL-18BP family members. The immunoglobulin domains of human and muIL-18BP were compared with those of putative viral proteins whose GenPept file number is shown on the left. Regions of high homology are boxed and highly conserved residues are shown in bold. The Variola virus protein VVU18340_6 is also expressed in cowpox virus (GenPept file CV41KBPL_14) and in Ectromelia virus (GenPept file U01161_3).

IL-18BP Expressed in COS7 Cells Is Biologically Active

A vector coding for human IL-18BPa with a His_6 tag at its C terminus was constructed and used for transient expression of IL-18BPa in monkey COS7 cells. The structure and biological activity of purified recombinant IL-18BPa was compared with that of the urinary IL-18BP. The recombinant protein gave a single ~40 kDa band upon SDS-PAGE and silver staining under reducing and nonreducing conditions (data not shown). Protein sequence analysis of recombinant IL-18BPa revealed the same N-terminal sequence as that of the urinary IL-18BP, indicating that the latter was not degraded at its N terminus. Recombinant and urinary IL-18BP had the same molecular mass and were immunologically cross-reactive as determined by immunoblotting with antibodies to the urinary IL-18BP (Figure 4A). Therefore, IL-18BPa corresponds structurally to the urinary IL-18BP.

The recombinant IL-18BPa was then tested for its ability to inhibit the biological activity of IL-18 in vitro. IL-18 activates NF-κB in human KG-1 cells as determined by electrophoretic mobility shift assay with cell extracts and a radiolabeled oligonucleotide of the NF-kB consensus sequence. IL-18 induced the formation of the p50 NF-kB homodimer. We found that urinary and recombinant IL-18BP blocked the IL-18-induced formation of the p50 NF-kB homodimer (Figure 4B). Induction of the p65/p50 NF-kB heterodimer by IL-18 was

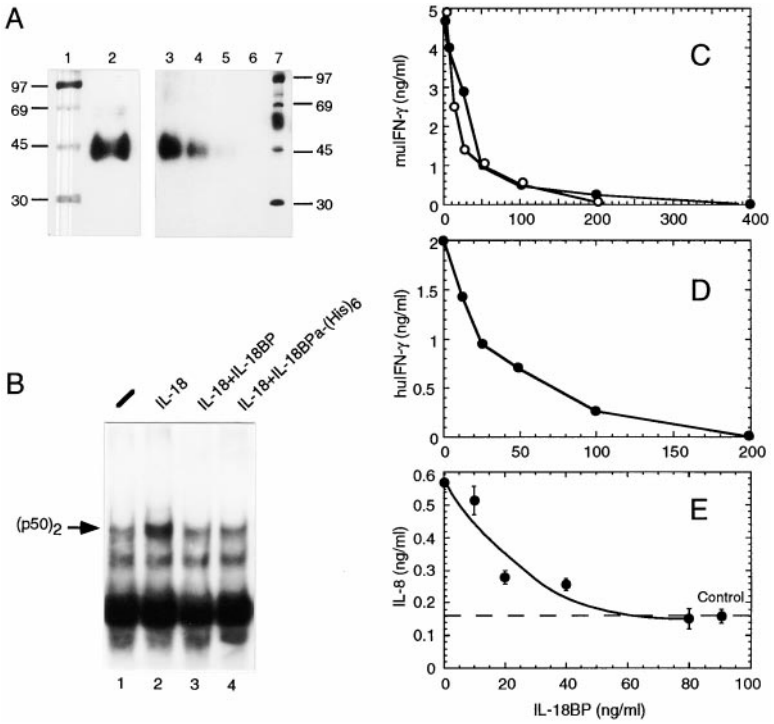

Figure 4. Recombinant IL-18BPa-His_6 Is Immunologically Cross-Reactive with Urinary IL-18BP and Inhibits Human and Murine IL-18 Activity In Vitro

(A) IL-18BP was subjected to SDS-PAGE under nonreducing conditions and immunoblotting with a rabbit antiserum to urinary IL-18BP. Lanes 1 and 7, molecular mass markers; lane 2, urinary IL-18BP; lanes 3–6, recombinant IL-18BPa-His_6, produced in COS7 cells and successively eluted in four fractions from a metal chelate column.

(B) KG-1 cells were stimulated with huIL-18, added either in RPMI or after premixing with IL-18BP in RPMI. Cell extracts were mixed with a labeled ds DNA corresponding to the NF-κB binding element and subjected to electrophoretic mobility shift assay. The lanes are: 1, control; 2, IL-18; 3, IL-18 preincubated with urinary IL-18BP; and 4, IL-18 preincubated with IL-18BPa-His_6.

(C) Mouse splenocytes were stimulated with the indicated combinations of LPS and huIL-18 (black circles), premixed with the indicated concentrations of IL-18BPa-His_6. Similarly, mouse splenocytes were incubated with LPS and muIL-18 (white circles) premixed with increasing concentrations of human IL-18BPa-His_6. IFNγ level was determined in the cultures.

(D) Human KG-1 cells were stimulated with TNFα (20 ng/ml) and huIL-18 (10 ng/ml), premixed with the indicated concentrations of IL-18BPa-His_6. The level of huIFNγ in the culture was then determined.

(E) Human PBMC were stimulated with IL-12 (10 ng/ml) and huIL-18 (25 ng/ml), pre-mixed with the indicated concentrations of IL-18BPa-His_6. The level of IL-8 in the cultures was then determined. Control and the dashed line represent basal IL-8 level in noninduced cultures of PBMC ($n = 3$).

seen only upon a longer exposure (data not shown) and was also inhibited by IL-18BP.

Recombinant IL-18BPa inhibited in a dose-dependent manner the IFNγ-inducing activity of human and murine IL-18 in murine splenocytes. Thus, at an ~10-fold molar excess, IL-18BP abolished the activity of human and muIL-18 (Figure 4C). Similar results were obtained when human KG-1 cells were induced with huIL-18 (Figure 4D). Induction of IFNγ by IL-18 in PBMC was also inhibited by IL-18BP (data not shown). Similarly, induction of the proinflammatory chemokine IL-8 by huIL-18 in PBMC was inhibited by recombinant IL-18BPa in a dose-dependent manner, bringing it to basal levels (Figure 4E). Because IL-18 and IL-1 are structurally related (Bazan et al., 1996), we tested the ability of IL-18BP to interact with IL-1. IL-1β (10 ng/ml) was preincubated either with control medium or with medium containing IL-18BP (250 ng/ml). The mixtures were added to cultures of PBMC and the level of IL-1-induced IL-8 was determined after 48 hr. We found that IL-18BP did not inhibit the activity of IL-1β ($n = 3$; data not shown).

The results of the bioassays as well as the NF-κB mobility shift demonstrate that inhibition of IL-18 activity is an intrinsic property of the cloned human IL-18BP and not that of any accompanying impurities in urinary IL-18BP, such as the coeluting fragment of defensin. Furthermore, IL-18BP is a specific inhibitor of IL-18.

Administration of Recombinant IL-18BP Inhibits LPS-Induced IFNγ In Vivo

To test the effects of IL-18BP in vivo, mice were injected intraperitoneally with LPS, LPS plus recombinant IL-18BP, or IL-18BP alone. Sera, splenocytes, as well as mRNA from spleen cells were obtained 6 hr post injection. RNA blot analysis revealed that IFNγ mRNA was nondetectable in splenocytes of mice injected with LPS plus IL-18BP compared to LPS only (Figure 5A). ELISA of serum IFNγ revealed that IL-18BP inhibited LPS-induced circulating IFNγ by >90% ($P < 0.001$, Figure 5B). IFNγ was not detected in sera of mice injected with IL-18BP alone. We also observed a significant reduction in spontaneous IFNγ production in splenocyte cultures from mice injected with LPS plus IL-18BP compared to LPS only (0.047 ± 0.03 ng/ml, $n = 3$ versus 0.57 ± 0.17 ng/ml, $n = 3$, respectively, $P < 0.05$). In contrast, IL-1β mRNA was unaffected in mice injected with LPS plus IL-18BP compared to LPS only (data not shown). Given the multiple cytokine cascade triggered by LPS in vivo, coadministration of IL-18BP selectively and effectively inhibits IFNγ gene expression, synthesis, and release in vivo.

Genomic Organization and Chromosomal Localization of the IL-18BP Gene

To study the possible existence of membrane-associated forms of IL-18BP, we screened a human genomic

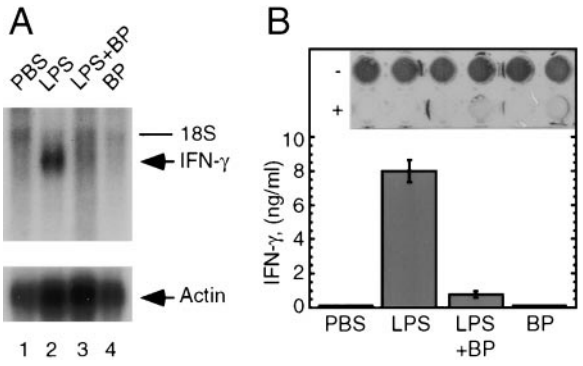

Figure 5. Recombinant IL-18BP Inhibits LPS-Induced IFNγ In Vivo

(A) Four groups of three C57Bl6 mice were injected with PBS (lane 1), LPS (100 μg/mouse, lane 2), LPS plus recombinant huIL-18BPa (lane 3), or IL-18BPa alone (lane 4). After 6 hr, total RNA was isolated from mouse splenocytes and aliquots (7 μg) were blotted with a cDNA probe corresponding to muIFNγ. An actin probe was used for normalization. BP denotes IL-18BPa.

(B) IFNγ levels in sera of the various groups of mice (see A), 6 hr post injection. Inset: two rows of the microtiter plate showing the amount of IFNγ as detected in the ELISA wells, containing sera of 3 mice, each one nondiluted and 2-fold diluted. Upper row (-): IFNγ from mice injected with LPS alone. Lower row (+): IFNγ from mice injected with LPS plus IL-18BP.

library with a probe corresponding to full-length IL-18BPa cDNA and identified five genomic clones. These clones were subcloned and then subjected to DNA sequencing. Altogether, an 8.3 kb sequence was assembled (accession number AF110798; Figure 6). No exon, or combination of two adjacent exons, coding for a TM domain was identified within any one of the three reading frames of the 8.3 kb sequence.

Exon skipping (in genomic nucleotides 2373–2956) and in-exon splicing (in genomic nucleotides 2724–2956) gave rise to at least three splice variants. Each of them shared a common ATG translation start site and coded for the same signal peptide of 28 amino acid residues. All variants coded for a soluble mature protein, varying somewhat in its size and in its C-terminal sequence. The abundant IL-18BPa cDNA (96 independent clones) consisted of five exons, coding for a mature protein of 164 amino acid residues. A shorter rare splice variant (1 kb, designated IL-18BPb, accession number AF110800), represented by three independent cDNA clones, coded for a mature protein of 85 amino acid residues. A third variant, IL-18BPc (accession number AF110801), represented by seven independent cDNA clones, was significantly longer (a 7 kb sequence was constructed from two partial cDNA clones), but also coded for a soluble protein of 169 amino acid residues. The 5′ untranslated region (UTR) of IL-18BPc was longer than that of the other two splice variants, suggesting that its expression may be differently regulated.

The IL-18BP locus contains an additional gene, coding for the nuclear mitotic apparatus protein 1 (NuMA1), positioned at the minus strand (accession number AF110460). This finding localizes the IL-18BP gene to human chromosome 11q13 (Sparks et al., 1993).

IL-18BP Is Expressed Constitutively Mainly in the Spleen

RNA blot analysis of IL-18BP showed a major 1.8 kb transcript in resting human spleen cells. Other tissues, including thymus and resting PBMC, revealed the same transcript but at a lower intensity. This transcript corresponded in size and abundance to the huIL-18BPa cDNA clone. Longer transcripts were seen as well, but their intensities were weaker compared with the major 1.8 kb band found in the spleen (Figure 7A). One of the minor transcripts was ≥ 8 kb. Its size and relatively low abundance corresponded to the cloned IL-18BPc. RNA

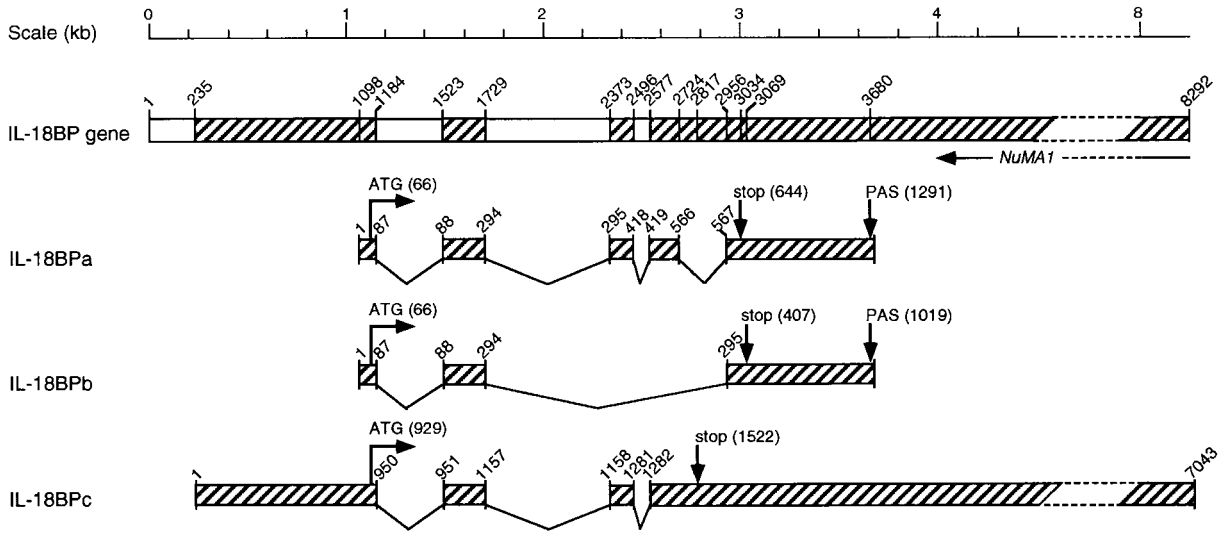

Figure 6. Exon-Intron Organization of the huIL-18BP Gene

The sequence of 5 huIL-18BP genomic clones (8.3 kb) is aligned with that of the three IL-18BP cDNA clones. Exons are striped and exon usage is shown in the three splice variants. Translation start sites (ATG), stop codons, polyadenylation signals (PAS), and the position of the NuMA1 gene on the minus strand are indicated.

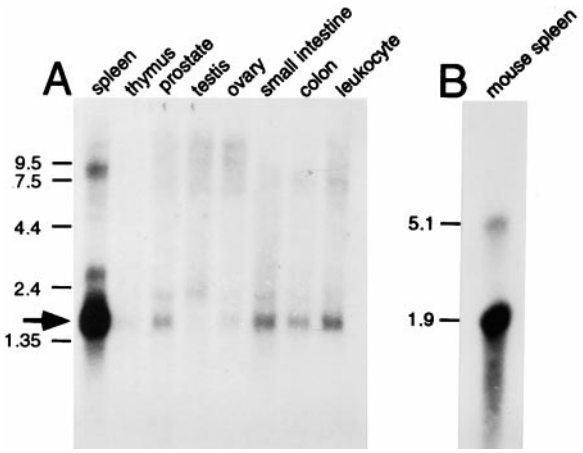

Figure 7. Expression of IL-18BP mRNA in Human Tissues and Mouse Spleen

(A) Human MTN blot II, containing equal amounts of poly(A)$^+$ RNA from the indicated tissues, was hybridized with a probe corresponding to the cDNA clone of huIL-18BPa. A major 1.8 kb band (indicated by an arrow) was obtained in the spleen and to a lesser extent in other tissues. A weaker ≥8 kb band, corresponding in size to the huIL-18BPc cDNA clone, was seen as well.

(B) Poly(A)$^+$ RNA from mouse splenocytes was subjected to RNA blot analysis with a probe corresponding to muIL-18BPc cDNA. A major 1.9 kb band and a minor 5.1 kb band were observed.

blot analysis of resting mouse splenocytes with a muIL-18BP cDNA probe revealed a major band of 1.9 kb and a minor band of 5.1 kb (Figure 7B). The cloned muIL-18BPc cDNA probably corresponded in size to the 5.1 kb transcript.

Discussion

In this study, we describe the isolation and cloning of a novel secreted protein, IL-18BP, which binds IL-18 and blocks its biological activity. IL-18 is an early signal leading to Th1 cytokine responses that are essential for the cytotoxic T cell response. Therefore, IL-18BP may modulate one of the earliest phases of the Th1 immune response. The dramatic (>90%) attenuation of IFNγ production in mice given recombinant IL-18BP demonstrated in our study was also observed in mice with the IL-18-gene disruption after LPS (Takeda et al., 1998). Splenic mRNA level of IL-1β was unaffected by IL-18BP administration, demonstrating the specificity of IL-18BP as a suppressor of IFNγ production in vivo. The inability of IL-BP to inhibit IL-1β, a structurally related homolog of IL-18, demonstrates the high specificity of the interaction between IL-18 and IL-18BP.

IL-18BP is a member of a novel family of soluble proteins, which also includes several Poxvirus-encoded putative proteins. Poxviruses encode decoy receptors of many cytokines and these receptors are instrumental in viral evasion of immune responses, as demonstrated by reduced virulence following their deletion (Spriggs, 1996; Ploegh, 1998). The putative protein encoded by ORF 54L of the MCV genome is the one most similar to IL-18BP (Figure 3B). A remarkable feature of MCV infection is that it produces small skin lesions, containing a large number of virus particles but lacking inflammatory cell infiltrates. Since IL-18 induces the chemokine IL-8 (Puren et al., 1998), inhibition of IL-18 by an MCV-encoded protein may attenuate the inflammatory antiviral Th1 response.

At high molar ratios, some soluble receptors, e.g., IL-1, IL-4, TNFα, and IFNα/β, inhibit their corresponding cytokines (Engelmann et al., 1989, 1990; Mosley et al., 1989; Novick et al., 1994; Sims et al., 1994) and have been used as immunosuppressors in patients (Moreland et al., 1997). However, at low concentrations, these soluble receptors may function as stabilizers of cytokines and enhance their activity, as was reported for IL-4 and TNFα (Mosley et al., 1989; Aderka et al., 1992). IL-18BP is highly effective as an inhibitor of IL-18 in vitro, and when added at lower concentrations, enhancement of IL-18 activity was not observed. Therefore, IL-18BP probably functions in vivo as an inhibitor rather than a stabilizer of IL-18.

A close association of IL-18 expression with autoimmune responses was reported in the pancreatic islets of nonobese diabetic (NOD) mice. IL-18 mRNA was observed during the spontaneous development of autoimmune insulitis and diabetes, preceding the rise in IFNγ mRNA and subsequent diabetes (Rothe et al., 1997a, 1997b). Endogenous IL-18 also appears to account for IFNγ production in *P. acnes* and LPS-mediated lethality (Okamura et al., 1995). Therefore, IL-18BP may be beneficial as an inhibitor of various Th1-mediated diseases. It is also possible that circulating IL-18BP serves to inhibit basal levels of IL-18. Unlike the precursors of IL-1β or TNFα, that of IL-18 is constitutively expressed in the spleens of normal mice and in freshly obtained peripheral blood monocytes from healthy humans (Puren et al., 1998). Therefore, it is likely that the net balance of the relative levels of IL-18 and IL-18BP affects immune responses such as the cytotoxic T cell response to viral diseases.

Our studies have shown that human and mouse IL-18BP is expressed as a soluble protein, lacking a TM domain. Furthermore, none of the IL-18BP splice variants that we have identified coded for a protein that has the signature of a GPI-anchor at its C terminus (Moran and Caras, 1991; Moran et al., 1991). In addition, an antibody generated against the urinary IL-18BP did not block the biological activity of IL-18 in human PBMC and in KG-1 cells (data not shown). Therefore, IL-18BP appears to exist only as a soluble circulating protein. Since IL-18 is expressed as a leaderless precursor, it is possible that an IL-18 precursor is also inserted into the cell membrane of activated macrophages as a biologically active "membrane cytokine" similar to IL-1α (Bailly et al., 1990). IL-18BP may block such a cytokine.

The families of the IL-1 and IL-18 receptors are remarkably similar, not only in structure but also in function. Type I IL-1 receptor (IL-1R1) and IL-1Rrp are the major ligand-binding subunits of IL-1 and IL-18, respectively. The IL-1 receptor accessory protein (IL-1RAcP) and the recently cloned AcPL function as accessory subunits of IL-1R and IL-18R, respectively. Thus, IL-1Rrp and AcPL may now be termed IL-18Rα and IL-18Rβ, respectively. IL-18BP is a soluble decoy receptor, structurally and functionally similar to the membrane-associated IL-1 receptor type II (IL1R2). It is possible

that IL-18BP evolved from a primordial cell-surface protein that lost its membrane-anchoring domain. IL-18BP, osteoprotegerin, and the recently cloned cytokine-like factor-1 belong to a group of receptor-like proteins that exist only in a soluble form (Anderson et al., 1997; Simonet et al., 1997; Elson et al., 1998; Yasuda et al., 1998).

In conclusion, we have identified a constitutively expressed circulating inhibitor of IL-18 that is a member of a novel family of soluble receptors. Because IL-18 is an early stimulant of Th1 cells, IL-18BP probably plays an important role in regulation of the immune response.

Experimental Procedures

Cells and Reagents

KG-1 (CCL 246), COS7 (CRL 1651), and Jurkat (CRL 8163) cells were from the American Type Culture Collection. Recombinant huIL-18 was from Peprotech Inc. (Rocky Hill, NJ), Vertex Pharmaceuticals (Cambridge, MA), and R&D Systems (Minneapolis, MN). Recombinant huIL-12, huTNFα, and muIL-18 were also from Peprotech. HuIL-18 (7×10^6 cpm/μg) was radiolabeled by the Bolton and Hunter's reagent (NEN Life Science products, Boston, MA), according to the manufacturer's procedure. Crude urinary proteins were kindly provided by C. Serafini, Istituto di Ricerca Cesare Serono (Rome). Endotoxin concentration was below 10 pg/ml in the various culture media.

Purification of the Urinary IL-18-Binding Protein

Recombinant huIL-18 (2.5 mg) was coupled to 0.5 ml Affigel-10 (BioRad, Richmond, CA). Crude urinary proteins (500 ml of a 1000-fold concentrate) were passed on the IL-18 column. The column was washed with 250 ml phosphate-buffered 0.65 M NaCl (pH 7.4). Bound proteins were then eluted in 1 ml fractions with 25 mM citric acid (pH 2.2) and benzamidine (1 mM) and immediately neutralized. The fractions were analyzed by SDS PAGE under nonreducing conditions and silver staining.

Immunization, RIA, and Immunoblotting

A rabbit was injected subcutaneously with affinity-purified urinary IL-18BP (~5 μg) in complete Freund's adjuvant and then boosted six times with IL-18BP (~5 μg per boost). Urinary IL-18BP (2.5 μg) was labeled with ^{125}I by the chloramine T procedure to 10^6 cpm/μg. An antibody binding titer of 1:1000 was obtained as determined by immunoprecipitation of ^{125}I-IL-18BP with protein G beads (data not shown). RIA of IL-18BP and immunological cross-reactivity were similarly done by competition with ^{125}I-IL-18BP using protein G beads for immunoprecipitation. Immunoblotting was done with antiserum to IL-18BP (1:200) on preparations containing IL-18BP that were resolved by SDS-PAGE (10% acrylamide) under nonreducing conditions. Immunoblotting of IL-18BP was done under nonreducing conditions, using ^{125}I-protein A and autoradiography.

Cross-Linking of Radiolabeled IL-18 to IL-18BP

IL-18BP (40 μl from eluted fractions of the affinity purification step) and ^{125}I-IL-18 (5×10^6 cpm, 7×10^6 cpm/μg) were cross-linked with disuccinimidyl suberate (Pierce, Rockford, IL) as previously described (Novick et al., 1994). Products were analyzed by SDS-PAGE (7.5% acrylamide) under reducing conditions (25 mM DTT) followed by autoradiography.

Assay of IL-18 and Its Blocking by IL-18BP In Vitro

IL-18BP activity was determined by its ability to block the production of IFNγ and IL-8 in the following systems.

Murine splenocytes

Freshly isolated splenocytes of C57Bl6 mice in RPMI-10% fetal bovine serum (RPMI-10, 2.5×10^6 cells/ml) were stimulated (24 hr, 37°C) with preformed mixtures (20 min) of LPS (1 μg/ml), IL-18 (10 ng/ml), and the indicated amounts of IL-18BP in RPMI-10. Clarified supernatants of 3× frozen cultures were assayed for muIFNγ by a sandwich-type ELISA (Fantuzzi et al., 1998).

Human KG-1 cells

Cells in RPMI-10 (250 μl, 1.2×10^6/ml) were stimulated (24 hr, 37°C) with TNFα (20 ng/ml) together with either huIL-18 (10 ng/ml) or IL-18 premixed with IL-18BP. Clarified supernatants of 3× frozen cultures were assayed for huIFNγ by ELISA with monoclonal antibodies Nos. 3.3 and biotin-labeled-166.5, (Novick et al., 1983; Konishi et al., 1997).

Human PBMC

PBMC were isolated by centrifugation of heparinized blood over Ficoll-Hypaque gradients and washed with pyrogen-free saline. Cells (5×10^6 cells/ml) in RPMI containing 1% heat-inactivated human AB serum were stimulated (48 hr, 37°C) as above. Clarified supernatants of 3× frozen cultures were assayed for IFNγ and IL-8 by an electro-chemiluminisence assay (Igen, Inc., Gaithersburg, MD; Puren et al., 1998).

Protein Sequence Analysis

Protein bands blotted onto a PVDF membrane were subjected to protein microsequencing (Model 491 HT-Procise, Applied Biosystems, Foster City, CA) as described (Matsudaira, 1987). Proteins in solution were sequenced following reduction (2 hr, 100 mM DTT, 6 M guanidine HCl [pH 8]), and alkylation by 4-vinyl pyridine (2 hr, 150 mM). N-terminal amino acid residues other than proline were blocked with o-phtalaldehyde in the reaction chamber of the protein sequencer as described (Brauer et al., 1983).

Construction of Probes and Screening of Libraries

Total RNA from Jurkat cells was reverse-transcribed with SuperScript RNase H$^-$ reverse transcriptase (GIBCO-BRL) and random primers (Promega, Madison WI). The resulting cDNA was amplified by PCR (30 cycles of annealing at 55°C, 2 min, and extension at 70°C, 1 min) using Taq DNA polymerase and primers corresponding to TIGR clone THC123801 nucleotides 24–44 (sense) and 500–481 (reverse). The PCR products were cloned into pGEM-Teasy TA cloning vector (Promega). DNA from individual clones was sequenced with T7 and SP6 primers.

The resulting 477 bp fragment was ^{32}P-labeled by random priming and used for screening the following human cDNA libraries: a human monocyte cDNA library, constructed in λpCEV9 cloning vector (Gutkind et al., 1991), kindly provided by T. Miki; a human Jurkat leukemic T cell cDNA library; a human peripheral blood leukocyte cDNA library, and a human spleen cDNA library, all from Clontech (Palo Alto, CA). A mouse spleen λ Zap II cDNA library and a human placenta genomic library in λ FIX II vector were from Stratagene (La Jolla, CA). Plasmids were excised from the λpCEV9 clones and self-ligated. cDNA clones from other libraries were isolated according to the manufacturer's instructions. Standard protocols were used for these cloning procedures (Sambrook et al., 1989). Automated DNA sequence analysis was performed on the isolated clones.

RNA Blotting

Poly(A$^+$) RNA was isolated from the total mouse spleen RNA by the PolyATract kit (Promega). Poly(A$^+$) RNA (1-2 μg) was resolved by agarose (1.5%) gel electrophoresis in MOPS-formaldehyde, transferred to a nylon membrane (Hybond N, Amersham) in 10× SSC, and baked (80°C, 2 hr in vacuum). Membranes were probed with [^{32}P]-dCTP-labeled DNA probes, washed, and autoradiographed (Sambrook et al., 1989). A membrane containing resolved human Poly(A$^+$) RNA of different tissues (Human MTN Blot II, Clontech) was similarly probed according to the manufacturer's instructions.

Expression of IL-18BP-His$_6$ in COS7 Cells

The coding region of the human IL-18BPa cDNA was amplified by PCR with the sense primer 5′TATATCTAGAGCCACCATGAGACACA ACTGGACACCA and the reverse primer 5′ATATCTAGATTAATGATG ATGATGATGACCCTGCTGCTGTGGACTGC.

The reverse primer includes a cassette coding for a C-terminal His$_6$ tag. The resulting PCR product was cut with XbaI and cloned into the XbaI site of the pEF-BOS expression vector (Mizushima and Nagata, 1990), kindly provided by S. Nagata, to yield pEF-BOS-IL-18BPa.

Batches of 6×10^7 COS7 cells suspended in 1.4 ml TD buffer, containing plasmid pEF-BOS-IL-18BPa (10 μg) and DEAE-dextran

(1.2 mg), were incubated for 30 min at room temperature (Sompayrac and Danna, 1981). The cells were washed with DMEM-10% FBS, plated for 4 hr, washed, and incubated 3 days in a serum-free DMEM. Culture medium was concentrated 15-fold by ultrafiltration (10 kDa cutoff), and the IL-18BPa-His_6 was isolated on a Talon column (Clontech) according to the manufacturer's instructions.

Electrophoretic Mobility Shift Assays

KG-1 cells (4×10^6 in 1 ml RPMI) were stimulated (20 min, 37°C) with either huIL-18 (10 ng/ml) or huIL-18 premixed with IL-18BP. The cells were then washed 3× with ice-cold phosphate-buffered saline (PBS) and immediately frozen in liquid nitrogen. The pellets were resuspended in 3× the packed cell volume of buffer A (20 mM Tris [pH 7.6], 0.4 M NaCl, 0.2 mM EDTA, 20% glycerol, 1.5 mM $MgCl_2$, 2 mM DTT, 0.4 mM PMSF, 1 mM Na_3VO_4, and 2 μg/ml each of leupeptin, pepstatin, and aprotinin). Aliquots of the clarified (15,000 × g, 15 min) extracts (10 μg protein) were incubated (15 min, room temperature) with a [^{32}P]γATP-labeled probe (3×10^4 cpm), corresponding to the NF-κB binding element (Promega), together with poly dI.dC (500 ng, Pharmacia, Uppsala, Sweden) and denatured salmon sperm DNA (100 ng, Sigma) in 20 μl HEPES buffer (pH 7.5, 10 mM), 60 mM KCl, 1 mM $MgCl_2$, 2 mM EDTA, 1 mM DTT, and 5% glycerol. The mixtures were then loaded onto 5% nondenaturing polyacrylamide gels and electrophoresed at 185 V in 0.5× TBE (40 mM Tris HCl, 45 mM boric acid, and 2.5 mM EDTA), vacuum dried, and autoradiographed.

Inhibition of LPS-Induced Responses in Mice by IL-18BP

Four groups of three C57Bl6 female mice were injected intraperitoneally with LPS (100 μg/mouse), LPS plus COS7-expressed human IL-18BP (300 μg/mouse), IL-18BP alone, or PBS. Mice were bled 6 hr after injection, and the level of serum IFNγ was determined by ELISA. Splenocytes of these mice were obtained at 6 hr, cultured for 24 hr in media without additional stimulants, and IFNγ production was then determined. Total RNA was isolated from the remaining splenocytes and subjected to RNA blotting with probes corresponding to murine IFNγ and actin.

Homology Studies and Database Searches

GenBank DNA and protein databases were searched by the BLAST program at the National Center for Biotechnology Information (NCBI), NIH. Protein homologies were identified by the Smith-Watermann algorithm with the aid of a Biocellerator (Compugen, Israel). Multiple sequence alignments were generated by the Pileup program (Genetic Computer Group Inc.) and further processed by the SeqVu program. Prediction of exon-intron boundaries was done with the Genie program (Reese et al., 1997).

Acknowledgments

We thank Y. Stabinsky (Peprotech) for the generous supply of huIL-18. We are also grateful to M. Su and Y. Gu (Vertex) and M. Tsang (R&D Systems) for their gifts of huIL-18. We thank S. Barak for excellent technical help, N. Tal for protein sequence analysis, M. Balass for biotinylation of antibody 166.5, and Y. Groner, D. Givol, O. Reiner, C. Kahana, D. Levanon, and M. Shuchman for advice and discussion. This work was supported by the Ares-Serono Group of companies (to M. R.) and by National Institutes of Health Grants AI15614 and AI2532359 (to C. A. D). M. R. is Edna and Maurice Weiss Professor of Cytokine Research.

Received October 7, 1998; revised December 7, 1998.

References

Aderka, D., Engelmann, H., Maor, Y., Brakebusch, C., and Wallach, D. (1992). Stabilization of the bioactivity of tumor necrosis factor by its soluble receptors. J. Exp. Med. *175*, 323–329.

Anderson, D.M., Maraskovsky, E., Billingsley, W.L., Dougall, W.C., Tometsko, M.E., Roux, E.R., Teepe, M.C., DuBose, R.F., Cosman, D., and Galibert, L. (1997). A homologue of the TNF receptor and its ligand enhance T-cell growth and dendritic-cell function. Nature *390*, 175–179.

Bailly, S., Ferrua, B., Fay, M., and Gougerot-Pocidalo, M.-A. (1990). Paraformaldehyde fixation of LPS-stimulated human monocytes: technical parameters permitting the study of membrane IL-1 activity. Eur. Cytokine Net. *1*, 47–51.

Bazan, J.F., Timans, J.C., and Kaselein, R.A. (1996). A newly defined interleukin-1? Nature *379*, 591.

Born, T.L., Thomassen, E., Bird, T.A., and Sims, J.E. (1998). Cloning of a novel receptor subunit, AcPL, required for interleukin-18 signaling. J. Biol. Chem. *273*, 29445–29450.

Brauer, A.W., Oman, C.L., and Margolies, M.N. (1983). Use of o-phtalaldehyde to reduce background during automated edman degradation. Anal. Biochem. *137*, 134–142.

Croston, G.E., Cao, Z., and Goeddel, D.V. (1995). NFκB activation by interleukin-1 requires an IL-1 receptor-associated protein kinase activity. J. Biol. Chem. *270*, 16514–16517.

DiDonato, J.A., Hayakawa, M., Rothwarf, D.M., Zandi, E., and Karin, M. (1997). A cytokine-responsive IκB kinase that activates the transcription factor NF-κB. Nature *388*, 548–554.

Elson, G.C.A., Graber, P., Losberger, C., Herren, S., Gretener, D., Menoud, L.N., Wells, T.N.C., Kosco-Vilbois, M.H., and Gauchat, J.-F. (1998). Cytokine-like factor-1, a novel soluble protein, shares homology with members of the cytokine type I receptor family. J. Immunol. *161*, 1371–1379.

Engelmann, H., Aderka, D., Rubinstein, M., Rotman, D., and Wallach, D. (1989). A tumor necrosis factor-binding protein purified to homogeneity from human urine protects cells from tumor necrosis factor toxicity. J. Biol. Chem. *264*, 11974–11980.

Engelmann, H., Novick, D., and Wallach, D. (1990). Two tumor necrosis factor-binding proteins purified from human urine. Evidence for immunological cross-reactivity with cell surface tumor necrosis factor receptors. J. Biol. Chem. *265*, 1531–1536.

Fantuzzi, G., Puren, A.J., Harding, M.W., Livingston, D.J., and Dinarello, C.A. (1998). IL-18 regulation of IFN-γ production and cell proliferation as revealed in interleukin-1β converting enzyme-deficient mice. Blood *91*, 2118–2125.

Ghayur, T., Banerjee, S., Hugunin, M., Butler, D., Herzog, L., Carter, A., Quintal, L., Sekut, L., Talanian, R., Paskind, M., et al. (1997). Caspase-1 processes IFN-γ-inducing factor and regulates LPS-induced IFN-γ production. Nature *386*, 619–623.

Gu, Y., Kuida, K., Tsutsui, H., Ku, G., Hsiao, K., Fleming, M.A., Hayashi, N., Higashino, K., Okamura, H., Nakanishi, K., et al. (1997). Activation of interferon-γ inducing factor mediated by interleukin-1β converting enzyme. Science *275*, 206–209.

Gutkind, J.S., Link, D.C., Katamine, S., Lacal, P., Miki, T., Ley, T.J., and Robbins, K.C. (1991). A novel c-*fgr* exon utilized in Epstein-Barr virus-infected B lymphocytes but not in normal monocytes. Mol. Cell. Biol. *11*, 1500–1507.

Kojima, H., Takeuchi, M., Ohta, T., Nishida, Y., Arai, N., Ikeda, M., Ikegami, H., and Kurimoto, M. (1998). Interleukin-18 activates the IRAK-TRAF6 pathway in mouse EL-4 Cells. Biochem. Biophys. Res. Commun. *244*, 183–186.

Konishi, K., Tanabe, F., Taniguchi, M., Yamauchi, H., Tanimoto, T., Ikeda, M., Orita, K., and Kurimoto, M. (1997). A simple and sensitive bioassay for the detection of human interleukin-18/interferon-γ inducing factor using human myelomonocytic KG-1 cells. J. Immunol. Methods *209*, 187–191.

Malinin, N.L., Boldin, M.P., Kovalenko, A.V., and Wallach, D. (1997). MAP3K-related kinase involved in NF-κB induction by TNF, CD95 and IL-1. Nature *385*, 540–544.

Matsudaira, P. (1987). Sequence from picomole quantities of proteins electroblotted onto polyvinylidene difluoride membranes. J. Biol. Chem. *262*, 10035–10038.

Micallef, M.J., Ohtsuki, T., Kohno, K., Tanabe, F., Ushio, S., Namba, M., Tanimoto, T., Torigoe, K., Fujii, M., Ikeda, M., et al. (1996). Interferon-γ-inducing factor enhances T helper 1 cytokine production by stimulated human T cells: synergism with interleukin-12 for interferon-γ production. Eur. J. Immunol. *26*, 1647–1651.

Mizushima, S., and Nagata, S. (1990). pEF-BOS, a powerful mammalian expression vector. Nucleic Acid Res. *18*, 5322.

Moran, P., and Caras, I.W. (1991). Fusion of sequence elements

from non-anchored proteins to generate a fully functional signal for glycophosphatidylinositol membrane anchor attachment. J. Cell. Biol. *115*, 1595–1600.

Moran, P., Raab, H., Kohr, W.J., and Caras, I.W. (1991). Glycophospholipid membrane anchor attachment. Molecular analysis of the cleavage/attachment site. J. Biol. Chem. *266*, 1250–1257.

Moreland, L.W., Baumgartner, S.W., Schiff, M.H., Tindall, E.A., Fleischmann, R.M., Weaver, A.L., Ettlinger, R.E., Cohen, S., Koopman, W.J., Mohler, K., et al. (1997). Treatment of rheumatoid arthritis with a recombinant human tumor necrosis factor receptor (p75)-Fc fusion protein. N. Engl. J. Med. *337*, 141–147.

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

Nakamura, K., Okamura, H., Wada, M., Nagata, K., and Tamura, T. (1989). Endotoxin-induced serum factor that stimulates gamma interferon production. Infect. Immun. *57*, 590–595.

Novick, D., Eshhar, Z., Fischer, D.G., Friedlander, J., and Rubinstein, M. (1983). Monoclonal antibodies to human interferon-γ: production, affinity purification and radioimmunoassay. EMBO J. *2*, 1527–1530.

Novick, D., Engelmann, H., Wallach, D., and Rubinstein, M. (1989). Soluble cytokine receptors are present in normal human urine. J. Exp. Med. *170*, 1409–1414.

Novick, D., Cohen, B., and Rubinstein, M. (1994). The human interferon α/β receptor—characterization and molecular cloning. Cell *77*, 391–400.

Okamura, H., Tsutsui, H., Komatsu, T., Yutsudo, M., Hakura, A., Tanimoto, T., Torigoe, K., Okura, T., Nukada, Y., Hattori, K., et al. (1995). Cloning of a new cytokine that induces IFN-γ production by T cells. Nature *378*, 88–91.

Okamura, H., Kashiwamura, S., Tsutsui, H., Yoshimoto, T., and Nakanishi, K. (1998). Regulation of interferon-γ production by IL-12 and IL-18. Curr. Opin. Immunol. *10*, 259–264.

Parnet, P., Garka, K.E., Bonnert, T.P., Dower, S.K., and Sims, J.E. (1996). IL-1Rrp is a novel receptor-like molecule similar to the type I interleukin-1 receptor and its homologues T1/ST2 and IL-1R AcP. J. Biol. Chem. *271*, 3967–3970.

Ploegh, H.L. (1998). Viral strategies of immune evasion. Science *280*, 248–253.

Puren, A.J., Fantuzzi, G., Gu, Y., Su, M.S.-S., and Dinarello, C.A. (1998). Interleukin-18 (IFN-γ-inducing factor) induces IL-8 and IL-1β via TNF-α production from non-CD14+ human blood mononuclear cells. J. Clin. Invest. *101*, 711–721.

Reese, M.G., Eeckman, F.H., Kulp, D., and Haussler, D. (1997). Improved splice site detection in Genie. In Proc. 1st Annu. Int. Conference Computational Molec. Biol. (Santa Fe, NM: ACM Press, New York).

Regnier, C.H., Song, H.Y., Gao, X., Goeddel, D.V., Cao, Z., and Rothe, M. (1997). Identification and characterization of an IκB kinase. Cell *90*, 373–383.

Robinson, D., Shibuya, K., Mui, A., Zonin, F., Murphy, E., Sana, T., Hartley, S.B., Menon, S., Kastelein, R., Bazan, F., et al. (1997). IGIF does not drive Th1 development but synergizes with IL-12 for interferon-γ production and activates IRAK and NF-κB. Immunity *7*, 571–581.

Rothe, H., Hibino, T., Itoh, Y., Kolb, H., and Martin, S. (1997a). Systemic production of interferon-γ inducing factor (IGIF) versus local IFN-γ expression involved in the development of Th1 insulitis in NOD mice. J. Autoimmun. *10*, 251–256.

Rothe, H., Jenkins, N.A., Copeland, N.G., and Kolb, H. (1997b). Active stage of autoimmune diabetes is associated with the expression of a novel cytokine, IGIF, which is located near Idd2. J. Clin. Invest. *99*, 469–474.

Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual, Second Edition (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press).

Sareneva, T., Matikainen, S., Kurimoto, M., and Julkunen, I. (1998). Influenza A virus-induced IFN-α/β and IL-18 synergistically enhance IFN-γ gene expression in human T cells. J. Immunol. *160*, 6032–6038.

Simonet, W.S., Lacey, D.L., Dunstan, C.R., Kelley, M., Chang, M.S., Luthy, R., Nguyen, H.Q., Wooden, S., Bennett, L., Boone, T., et al. (1997). Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. Cell *89*, 309–319.

Sims, J.E., Giri, J.G., and Dower, S.K. (1994). The two interleukin-1 receptors play different roles in IL-1 activities. Clin. Immunol. Immunopathol. *72*, 9–14.

Sompayrac, L.H., and Danna, K.L. (1981). Efficient infection of monkey cells with DNA of simian virus 40. Proc. Natl. Acad. Sci. USA *78*, 7575–7578.

Sparks, C.A., Bangs, P.L., McNeil, G.P., Lawrence, J.B., and Fey, E.G. (1993). Assignment of the nuclear mitotic apparatus protein NuMA gene to human chromosome 11q13. Genomics *17*, 222–224.

Spriggs, M.K. (1996). One step ahead of the game: viral immunomodulatory molecules. Annu. Rev. Immunol. *14*, 101–130.

Takeda, K., Tsutsui, H., Yoshimoto, T., Adachi, O., Yoshida, N., Kishimoto, T., Okamura, H., Nakanishi, K., and Akira, S. (1998). Defective NK cell activity and Th1 response in IL-18-deficient mice. Immunity *8*, 383–390.

Torigoe, K., Ushio, S., Okura, T., Kobayashi, S., Taniai, M., Kunikate, T., Murakami, T., Sanou, O., Kojima, H., Fuji, M., et al. (1997). Purification and characterization of the human interleukin-18 receptor. J. Biol. Chem. *272*, 25737–25742.

Tsutsui, H., Nakanishi, K., Matsui, K., Higashino, K., Okamura, H., Miyazawa, Y., and Kaneda, K. (1996). IFN-γ-inducing factor up-regulates Fas ligand-mediated cytotoxic activity of murine natural killer cell clones. J. Immunol. *157*, 3967–3973.

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

Yasuda, H., Shima, N., Nakagawa, N., Mochizuki, S.I., Yano, K., Fujise, N., Sato, Y., Goto, M., Yamaguchi, K., Kuriyama, M., et al. (1998). Identity of osteoclastogenesis inhibitory factor (OCIF) and osteoprotegerin (OPG): a mechanism by which OPG/OCIF inhibits osteoclastogenesis in vitro. Endocrinology *139*, 1329–1337.

Yoshimoto, T., Takeda, K., Tanaka, T., Ohkusu, K., Kashiwamura, S., Okamura, H., Akira, S., and Nakanishi, K. (1998). IL-12 upregulates IL-18 receptor expression on T cells, Th1 cells and B cells: synergism with IL-18 for IFN-γ production. J. Immunol. *161*, 3400–3407.

GenBank Accession Number

The GenBank accession numbers for the sequences reported in this paper are AF110798, AF110799, AF110800, AF110801, AF110802, AF110803, and AF110460.