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# Identification of a Critical Ig-Like Domain in IL-18 Receptor $\alpha$ and Characterization of a Functional IL-18 Receptor Complex[1]

**Tania Azam,* Daniela Novick,† Philip Bufler,* Do-Young Yoon,‡ Menachem Rubinstein,† Charles A. Dinarello,* and Soo Hyun Kim[2]*§**

**Steady state mRNA levels in various human tissues reveal that the proinflammatory cytokine IL-18 is constitutively and ubiquitously expressed. However, limited IL-18R $\alpha$-chain (IL-18R$\alpha$) expression in tissues may restrict ligand-acting sites and contribute to a specific response for IL-18. To study the IL-18R complex, [$^{125}$I]IL-18 was studied for binding to the cell surface receptors of IL-18-responsive NK and macrophagic KG-1 cells. After cross-linking, [$^{125}$I]IL-18 formed three IL-18R complexes with sizes of approximately 93, 160, and 220 kDa. In KG-1 cells, Scatchard analysis revealed the presence of 135 binding sites/cell, with an apparent dissociation constant ($K_d$) of 250 pM; in NK cells, there were 350 binding sites per cell with an apparent $K_d$ of 146 pM. Each domain of extracellular IL-18R$\alpha$ was cloned and individually expressed in *Escherichia coli*. An mAb specifically recognized the membrane-proximal third domain; this mAb blocked IL-18-induced IFN-$\gamma$ production in NK cells. Furthermore, deletion of the membrane-proximal third domain of IL-18R$\alpha$ prevented the formation of IL-18R ternary complex with IL-18R $\beta$-chain. The present studies demonstrate that the biologically active IL-18R complex requires the membrane-proximal third Ig-like domain in IL-18R$\alpha$ for the formation of IL-18R ternary complex as well as for signal transduction involved in IL-18-induced IFN-$\gamma$ in NK cells.** *The Journal of Immunology,* **2003, 171: 6574–6580.**

Interleukin-18 is a member of the IL-1 family and shares the common structure of IL-1 family members (1–3). IL-18 also shares cell signaling with IL-1 family members, recruiting IL-1R-associated kinases, the formation of the IL-1R-associated kinase complexes with the TNF receptor-associated factor-6, and activation of the cascade of I$\kappa$B$\alpha$/NF-$\kappa$B (4–6). Like most members of the IL-1 family, IL-18 lacks a signal peptide. Recently, several new members of the IL-1 family were identified by sequence homology, some of which have a determined biological function, whereas others remain without a known activity (7–9). One of the members, IL-1H4, was found to bind IL-18R$\alpha$, but its biological function has yet to be determined (7, 10, 11).

Most cytokines are transiently expressed shortly after exposure of the host to pathogens or injury. IL-18, unlike other cytokines, is constitutively expressed and pre-exists as a precursor molecule before becoming an active molecule. Both caspase-1-dependent (12–15) and caspase-1-independent (processing through Fas ligand stimulation) (16) result in activating intrinsic IL-18 pathways.

In general, cytokines have at least two receptor chains that collaborate during ligand-induced signaling. IL-18 has two known receptor chains, a ligand-binding IL-18R $\alpha$-chain and a signal-transducing IL-18R $\beta$-chain. Both chains of the IL-18R belong to the IL-1R family and consist of three Ig-like domains in the extracellular region (17–19). IL-18-binding protein (IL-18BP)[3] is not a part of the IL-18 signaling complex, but, rather, antagonizes IL-18 activity (2, 20). It is a unique, secreted receptor-like molecule and consists of a single Ig-like domain. IL-18BP shares a significant homology with poxvirus proteins and a limited homology with the IL-1R family. Viral IL-18BP, like mammalian IL-18BP, neutralizes the biological activities of human IL-18 (21). Human IL-18BP is secreted constitutively in healthy subjects, with circulating concentrations of 2–5 ng/ml (22). IFN-$\gamma$ increases gene expression for IL-18BP in renal mesangial cells, which functions as a feedback loop and reduces IL-18-induced IFN-$\gamma$ (23).

An IL-18R$\alpha$ polymorphism has been identified (24). This polymorphism is generated by alternative splicing of the coding region of IL-18R$\alpha$ rather than by a genomic mutation. The deletion of three bases at position +950 to +952 (CAG) in the IL-18R$\alpha$ cDNA results in the deletion of alanine 317 in the membrane-proximal third domain of IL-18R$\alpha$. The deletion of alanine 317 reduces IL-18-induced IFN-$\gamma$ production in PBMC of affected individuals (24). Xu et al. (25) reported that an Ab directed against the peptide corresponding to residues 247–266 of murine IL-18R$\alpha$ inhibited IL-18-induced IFN-$\gamma$ in Th1 cells, and this Ab also reduced LPS-induced mortality in mice.

Although IL-18 is studied to reveal its broad biological effects, regulation, and structure, little is known about its functional receptor complex. The present study identifies the critical domain of IL-18R$\alpha$ for IL-18-induced IFN-$\gamma$ as well as characterizes the biologically active IL-18R complex of IL-18-responsive cells.

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[3] Abbreviations used in this paper: IL-18BP, IL-18-binding protein; DSS, disuccinimidyl suberate; ECD, extracellular domain; His$^6$, six histidine; ICE, IL-1$\beta$-converting enzyme; IL-1Ra, IL-1R antagonist; IL-18R$\alpha$-ECD-$\Delta$D3, soluble IL-18R$\alpha$-deleted membrane-proximal third domain; IL-18R$\alpha$-$\Delta$D3, transmembrane IL-18R$\alpha$-deleted membrane-proximal third domain.



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## Materials and Methods

### *Reagents and cytokines*

RPMI 1640 culture medium and Lipofectamine 2000 were purchased from Invitrogen (Carlsbad, CA) and were supplemented with 10 mM L-glutamine, 24 mM $NaHCO_3$, 10 mM HEPES, 100 U/ml penicillin, and 100 $\mu$g/ml streptomycin from Cellgro (Waukesha, WI). FBS was obtained from Invitrogen. Recombinant human IL-18 and IL-18BPa isoform were gifts from Serono Pharmaceutical Research Institute (Geneva, Switzerland). Recombinant human IL-12, IL-15, and TNF-$\alpha$ were obtained from PeproTech (Rocky Hill, NJ). All restriction endonucleases, reverse transcriptase (SuperScript II), and *Taq* DNA polymerase were purchased from Invitrogen. Talon affinity columns were obtained from Clontech Laboratories (Palo Alto, CA) and were used as recommended. The mAb anti-six histidine ($His^6$) tag, biotinylated affinity-purified goat anti-human IL-18 for immunoprecipitation, and mAb anti-human IL-18R$\alpha$ for neutralization and Western blot were purchased from R&D Systems (Minneapolis, MN). The secondary Abs and goat anti-mouse peroxidase-conjugated Abs were purchased from Jackson ImmunoResearch Laboratories (West Grove, PA) and were developed using ECL from NEN Life Science Products (Boston, MA). For chemical cross-linking, disuccinimidyl suberate (DSS) was purchased from Pierce (Rockford, IL).

### *Construction of probe and Northern blotting*

The cDNA of both IL-18 and IL-18R$\alpha$ were cloned as previously described (26, 27). The cDNA of actin probe for Northern blot normalization was prepared as follows; the sense primer (5′-ACCGCGAGAAGATGAC CCAG) and the reverse primer (5′-CCATCTCGTTCTCGAAGTCCA) were used for RT-PCR with the total RNA from human A549 cell line. The PCR product was purified with GeneClean II kit (Q-BIO gene, Carlsbad, CA). All three cDNA probes were [$^{32}$P]dCTP-labeled (NEN Life Science Products) by random priming and using Klenow (New England Biolabs, Beverly, MA). A membrane containing electrophoresed human poly(A)$^+$ RNA from different tissues (Human MTN Blot II; Clontech Laboratories) was probed with cDNA probe and autoradiographed.

### *Construction of vectors for* Escherichia coli *expression*

For the expression of soluble IL-18R$\alpha$-extracellular domain (ECD) and its separate domains, $His^6$ tag was fused at the N terminus without signal peptide. Human IL-18R$\alpha$ cDNA was cloned by direct PCR using a human T-B lymphoblast cDNA library (Stratagene, La Jolla, CA) as previously described (26). The following primers were used: primer 1 (sense), 5′-ATATGAATTCGAATCTTGTACTTCACG; primer 2 (reverse), 5′-TAT ATCTAGATTATCTTGTGAAGACGTGGC; primer 3 (reverse), 5′-TAT ATCTAGATTATACTTGTCTTTCAGTGAA; primer 4 (sense), 5′-ATA TGAATTCAAACACAGCTGTTTCACT; primer 5 (reverse), 5′-TATAT CTAGATTAATTACTGCGATCTTCCA; and primer 6 (sense), 5′-ATATGAATTCATAGTTCCGGTTCTTCT.

For the whole IL-18R$\alpha$-ECD (domains 1–3), the sense primer was designed with an *Eco*RI site before mature IL-18R$\alpha$ cDNA (primer 1), and the reverse primer was designed with a stop codon just before the transmembrane domain (glycine 330) of IL-18R$\alpha$ cDNA, followed by an *Xba*I site (primer 2). The separate domains were generated using the following primers in the same manner: primers 1 and 3 were used for domain 1, primers 4 and 5 were used for domain 2, primers 6 and 2 were used for domain 3, primers 1 and 5 were used for domains 1 and 2, and primers 4 and 2 were used for domains 2 and 3.

Each of the six cDNA separate domains of IL-18R$\alpha$ was ligated into Bluescript for sequence confirmation and then ligated into pPROEX HTa for expression of the protein in *E. coli*. The pPROEX HTa vector has a $His^6$ tag fused at the N terminus in each of the separate domains to facilitate protein purification.

### *Construction of vectors for mammalian expression*

For the expressions of soluble IL-18R$\alpha$ with a third-domain deletion (IL-18R$\alpha$-ECD-$\Delta$D3) and IL-18R$\alpha$-ECD, the same cDNA of IL-18R$\alpha$ was used. Unlike construction for *E. coli* expression, these constructs have the signal peptide at the N terminus for transient transfection of COS-7 cells, and a $His^6$ tag was fused at the C terminus of each construct to facilitate protein purification. The following primers were used: primer 1 (sense), 5′-ATATCTCGAGCCACCATGAATTGTAGAGAATTACC; primer 2 (reverse), 5′-TATAGCGGCCGCTAATGATGATGATGATGATTA CTGCGATCTTCC; primer 3 (reverse), 5′-TATAGCGGCCGCTAAT GATGATGATGATGTCTTGTGAAGACGTGG; primer 4 (sense), 5′-GTGGAAGATCGCAGTAATGGAATGATCATAGCTGTT; primer 5 (reverse), 5′-AACAGCTATGATCATTCCATTACTGCGATCTTCCAC; and primer 6 (reverse), 5′-TATAGCGGCCGCTTAAGACTCGGAAA GAACAG.

For IL-18R$\alpha$-ECD-$\Delta$D3 and IL-18R$\alpha$-ECD, the sense primer (primer 1) was designed with an *Xho*I site and Kozak consensus sequence before the IL-18R$\alpha$-coding region. The reverse primers contain a $His^6$ tag, a stop codon, and a *Not*I site. IL-18R$\alpha$-ECD-$\Delta$D3 and IL-18R$\alpha$-ECD were generated using the following primers in the same manner: primers 1 and 2 were used for IL-18R$\alpha$-ECD-$\Delta$D3, and primers 1 and 3 were used for IL-18R$\alpha$-ECD.

For the expression of transmembrane IL-18R$\alpha$ with a third-domain deletion (IL-18R$\alpha$-$\Delta$D3), two-step PCR was performed to remove the third domain of IL-18R$\alpha$. The first step is to generate an N-terminal PCR fragment with primers 1 and 5 and a C-terminal PCR fragment with primers 4 and 6. The N-terminal fragment contains the 212 amino acids of the first and second domains with the additional six amino acids of transmembrane domain, from glycine 330 to valine 315. The C-terminal fragment contains six amino acids of the end of the second domain, from valine 207 to asparagine 212, including the transmembrane and cytoplasmic domains of IL-18R$\alpha$. The second-step PCR was performed in the presence of both purified N- and C-terminal PCR fragments using primers 1 and 6. This second-step PCR removed the second domain of IL-18R$\alpha$, then ligated into pGEMT-easy vector.

IL-18R$\beta$ cDNA was cloned as previously described (26). The construction of vector for the soluble extracellular IL-18R$\beta$ was described as IL-18R$\alpha$ above. The following primers were used: primer 1 (sense), 5′-ATATCTCGAGGCCACCATGCTCTGTTTGGG; and primer 2 (reverse), 5′-TATAGCGGCCGCTAATGATGATGATGATGTCCTCTCTTTT CTTTC. Each of the four constructs was ligated into pGEMT-easy for sequence confirmation and then ligated into pTARGET for transient expression in COS-7 cells or A549 human lung carcinoma cells.

### *Cell lines and bioassay*

The NK is a subclone of human NK92 and was provided by T. Hoshino (National Cancer Institute-Frederick Cancer Research and Development Center, Frederick, MD) (28, 29). NK cells were maintained in supplemented RPMI 1640 medium containing 10% FBS, 50 pg/ml IL-2, and 200 pg/ml IL-15. The human A549 lung carcinoma cell line and monkey kidney COS-7 cell line were obtained from American Type Culture Collection (Manassas, VA) and maintained according to instructions provided by American Type Culture Collection.

Bioassay was performed in 96-well microtiter plate. Briefly, NK cells ($5 \times 10^5$/ml; 0.2 ml) in RPMI 1640 medium were preincubated with increasing concentrations of anti-IL-18R$\alpha$ mAbs. The cells were then stimulated with 0.5 ng/ml IL-12 and 20 ng/ml IL-18. After 16–20 h at 37°C in humidified air with 5% $CO_2$, the culture supernatant was collected for IFN-$\gamma$ measurement. For IL-8 assay, the A549 cell line supernatant was collected and measured after transient transfection of various constructs, then stimulation with IL-18 as described in figures and legends.

### *Protein expression and purification in* E. coli

The six pPROEX HTa-IL-18R$\alpha$ separate domain plasmids were transformed into the competent *E. coli* strain DH5$\alpha$ (Invitrogen) and expressed as described previously (27). An overnight culture of 5 ml was added to 200 ml of Luria-Bertani medium containing 100 $\mu$g/ml ampicillin and grown to a density of 0.6–1 $OD_{600}$. Protein expression was induced by the addition of isopropylthiogalactoside (0.3 mM), and the cells continued to shake in a 37°C incubator for 3 h. The six separate IL-18R-ECD of recombinant proteins were found in inclusion bodies. The bacterial cells were harvested by centrifugation (5000 $\times$ *g* for 15 min at 4°C), and the pellet was suspended in 8 M urea (20 ml) in Talon buffer (50 mM $NaH_2PO_4$, 20 mM Tris-HCl, and 100 mM NaCl, pH 8) for 1 h on ice. The supernatant obtained after centrifugation (6000 $\times$ *g* for 30 min at 4°C) was applied to a 2-ml column of Talon. The Talon column was then washed with 30 bed volumes of Talon buffer and eluted with 6 ml of 100 mM imidazole in Talon buffer. The six separate IL-18R$\alpha$ were resolved by 10% SDS-PAGE under reducing conditions, and bands were visualized by Coomassie Blue staining.

### *Transient transfection into COS-7 cells and purification of soluble IL-18Rs*

Batches of $6 \times 10^7$ COS-7 cells were transfected with either an empty plasmid pTARGET (Promega, Madison, WI) or a plasmid encoding different constructs of IL-18Rs in pTARGET vector as described above. Ten micrograms of DNA and DEAE-dextran (1.2 mg in 140 mM NaCl, 5 mM KCl, 0.7 mM $K_2HPO_4$, and 25 mM Tris-HCl, pH 7.4) were incubated for 30 min at room temperature as previously described (20). The cells were

then washed with DMEM/10% FBS, plated in DMEM/10% FBS for 4 h, washed with serum-free DMEM, and incubated for 4 days in serum-free DMEM. Culture supernatants were concentrated 20-fold in boiled dialysis bags submerged in saturated polyethylene glycol (8 kDa), followed by dialysis in Talon buffer. The concentrated supernatants were applied to a Talon column, and the $His^6$ tag recombinant proteins were eluted with imidazole buffer according to the manufacturer's instructions. The different IL-18R recombinant proteins were resolved by 10% reducing SDS-PAGE, followed by Western analysis.

### *Luciferase assay*

The transmembrane IL-18Rα or IL-18Rα-ΔD3 was transiently transfected into A549 cells in the presence of IL-18Rβ and pTK-NFκB-Luc (26) with Lipofectamine 2000 (Invitrogen, Carlsbad, CA) according to the manufacturer's instruction. The transiently transfected cells were stimulated with IL-18 (50 ng/ml) for 18 h, then supernatant and cells were harvested separately for IL-8 and luciferase assays, respectively. The cell pellet was lysed with the cell lysis buffer from luciferase report kit (Promega), then samples were read with Luminescence (monolight 2010 Luminometer; Analytical Luminescence Laboratories, San Diego, CA).

### *RT-PCR*

The transiently transfected A549 cells were examined for different IL-18R constructs expression. Total RNA was isolated with Tri-Reagent (Sigma-Aldrich, St. Louis, MO), and the cells were treated as described for the luciferase and IL-8 assays. The first-strand cDNA was synthesized with SuperScript II (Invitrogen), then PCR was performed with following primers: T7 (sense) for IL-18Rα and IL-18Rβ, TAATACGACTCACTATAG GGCGAATTC; IL-18Rα (reverse), TAGGACAATGATTAGTCTTCGGC; IL-18Rβ (reverse), TCCTGAATGATGTGAGGATAGC; GAPDH (sense), ACCACAGTCCATGCCATCAC; and GAPDH (reverse), TCCACCAC CCTGTTGCTGTA. PCR reaction was performed at 94°C for 45 s, 70°C for 2 min, and 59°C for 1 min for 30 cycles.

### *Analysis of cytokines*

The liquid phase electrochemiluminescence method was used to measure IFN-γ and IL-8 (30) in cell culture medium. The amount of electrochemiluminescence was determined using an Origen analyzer (Igen, Gaithersburg, MD).

### *Radioiodination of human IL-18*

Mature human IL-18 (10 μg) in borate buffer was radiolabeled with $^{125}$I-labeled Bolton-Hunter reagent (NEN Life Science Products) according to the manufacturer's recommendations. The reaction was stopped with an excess of glycine. Unbound $^{125}$I was removed by chromatography on Sephadex G-25 equilibrated in PBS containing 0.25% gelatin and 0.02% sodium azide. Each fraction was counted, and the peak fractions were pooled. The pooled radiolabeled [$^{125}$I]IL-18 contained ~$4.2 \times 10^7$ cpm/μg and was used for competition binding assays and cell surface receptor cross-linking.

### *Binding of [$^{125}$I]IL-18 to human KG-1 and NK cells*

Human KG-1 and NK cells ($1 \times 10^6$, 0.25 ml) were suspended in binding solution (cell culture medium containing 2% FBS and 0.02% sodium azide) containing increasing concentrations of [$^{125}$I]IL-18. Eppendorf tubes were mixed occasionally for 1 h at 4°C. The mixtures were then washed three times at 4°C with the binding solution, and cell-bound [$^{125}$I]IL-18 was determined using a gamma counter. The affinity constants and the number of receptors were calculated using Scatchard analysis (31).

### *Cross-linking and immunoprecipitation of intrinsic IL-18R complexes with NK cells*

For chemical cross-linking, cells ($0.6–2 \times 10^7$) were incubated with [$^{125}$I]IL-18 ($4.5 \times 10^6$ cpm/125 ng) for 1 h at 4°C in the presence or the absence of a 100-fold molar excess of IL-18 or IL-18BP, respectively. DSS was added for a final concentration of 1 mM and placed on ice for 20 min. After chemical cross-linking, the cells were washed twice in PBS, and then membrane proteins were prepared. Briefly, 0.5 ml of PBS was added to each cell pellet and then homogenized on ice. The homogenates were centrifuged for 10 min at $500 \times g$. The supernatants were transferred into new Eppendorf tubes and centrifuged for 15 min at 14,000 rpm. For immunoprecipitation of IL-18R complexes, [$^{125}$I]IL-18 was cross-linked and processed in same manner as described above. The membrane pellet was lysed with 1% Triton X-100 for 30 min, then incubated with biotinylated goat anti-IL-18 (3 μg/ml) for 2 h at 4°C. Immunoprecipitation was performed with avidin-coated magnetic beads (Igen) by centrifugation. The resulting pellets containing membrane proteins and immunoprecipitated IL-18R complex were analyzed by 10% SDS-PAGE under reducing conditions and autoradiographed.

### *Cross-linking of different IL-18Rα domains with IL-18Rb*

For chemical cross-linking, different domains of IL-18Rα-ECDs expressed in COS-7 were preincubated alone, in the presence of IL-18, or in the presence of IL-18 plus IL-18Rβ-ECD for 1 h at room temperature. Then DSS was added for the final concentration of 1 mM, followed by 20 min of incubation at room temperature. The individual IL-18R-ECDs (150 ng in each lane) were analyzed by10% SDS-PAGE under reducing conditions, followed by Western blotting with anti-$His^6$ tag mAb.

### *Western blot analysis of* E. coli *IL-18Rα individual domains*

Each of the six individual IL-18Rα domains was resolved by 10% SDS-PAGE under reducing conditions. Gels were transferred to nitrocellulose membranes and then probed with the primary mAb anti-$His^6$ tag. After 24 h, goat anti-mouse IgG peroxidase was added, and the blot was developed using ECL.

## Results

### *Expression of IL-18 and IL-18Rα in different human tissues*

The expression of human IL-18 and IL-18Rα was determined using MTN Blot II (Clontech, Palo Alto, CA) containing resolved poly(A)$^+$ RNA from different human tissues. Both IL-18 and IL-18Rα mRNA were highly expressed in the spleen, similar to IL-18BP expression patterns (20). Analysis of the IL-18 Northern blot showed a major 1.3-kb transcript band in several human tissues, whereas high molecular mass transcript bands between 4.4–7.5 kb were observed only in the spleen (Fig. 1*A*, *lane 1*). A moderate level of IL-18 mRNA expression was observed in prostate, small intestine, colon, as well as leukocytes; however, thymus, testis, and ovary showed only weak expression (Fig. 1*A*). In contrast, a high intensity 4.2-kb mRNA transcript for IL-18Rα was seen primarily in spleen and leukocytes. The 4.2-kb mRNA transcript was barely detectable in other tissues (Fig. 1*B*). A high molecular mass transcript of IL-18Rα mRNA (>7.5 kb) was seen only in leukocytes

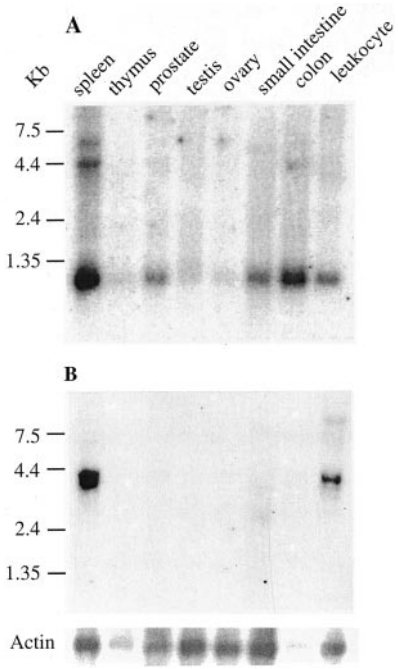


**FIGURE 1.** Expression of IL-18 and IL-18Rα mRNA in human tissues. Equal amounts of poly(A)$^+$ RNA containing human MTN blot II was probed with $^{32}$P-radiolabeled human IL-18 and IL-18Rα cDNA. Each lane is labeled for different tissues. The numbers on the *leftmost* lane indicate the size of mRNA. *A*, IL-18; *B*, IL-18Rα.

(Fig. 1*B*). The actin probe revealed that the different degrees of IL-18 and IL-18R$\alpha$ expression are not due to the amount of mRNA in each lane.

### *Scatchard analysis of [$^{125}$I]IL-18 binding to both KG-1 and NK cells*

IL-18R complex was studied in KG-1 and NK cell lines. Table I depicts the results of Scatchard analysis in KG-1 and NK cells. KG-1 cells showed specific binding and a dissociation constant ($K_d$) of $2.5 \times 10^{-10}$ (250 pM) with 135 binding sites/cell. The binding of [$^{125}$I]IL-18 to NK cells yielded a $K_d$ of $1.46 \times 10^{-10}$ (146 pM) with 350 binding sites/cell (Table I).

### *Characterization of a functional IL-18R complex in NK cells*

A functional IL-18R complex was characterized in NK cells with the aid of chemical cross-linking. The [$^{125}$I]IL-18 was bound to NK cells ($5 \times 10^6$), followed by cross-linking with the divalent lysine-directed cross-linker, DSS. Cross-linking products were subjected to SDS-PAGE. The membrane protein fraction yielded complexes with molecular masses of ~93 (R3), 160 (R2), and 220 kDa (R1; Fig. 2*A*). The R3 complex may consist of [$^{125}$I]IL-18 and IL-18R$\alpha$, and the R2 complex probably contains [$^{125}$I]IL-18, IL-18R$\alpha$, and IL-18R$\beta$, which is the functional complex. The R1 band most likely consists of a multicomplex consisting of more than one ternary complex or participation of an unknown IL-18R component.

We next examined the specificity of the three bands using a 100-fold molar excess of nonradiolabeled IL-18 or IL-18BP added before incubation with [$^{125}$I]IL-18. These bands were completely abolished in the presence of the nonradiolabeled IL-18 (Fig. 2*A*, *lane 3*) or IL-18BP (Fig. 2*A*, *lane 4*). The unbound [$^{125}$I]IL-18, as found in the cell-free supernatant, was examined in the next three lanes. The [$^{125}$I]IL-18 appears to be a dimer rather than a monomer, and this dimer (Fig. 2*A*, *lanes 5* and *6*) was absent when cross-linking was performed in the presence of the nonradiolabeled IL-18BP. [$^{125}$I]IL-18 and nonradiolabeled IL-18BP form a complex between 45–69 kDa (Fig. 2*A*, *lane 7*).

We next studied the IL-18R complex using an anti-IL-18 Ab. In the immunoprecipitation of IL-18R complex, anti-IL-18 Ab precipitated R1 and R3 complexes, but not R2. This may be due to the possibility that IL-18 epitopes are masked in the complex containing both IL-18Rs (Fig. 2*B*).

### *The third domain of IL-18R$\alpha$ is important for IL-18 activity*

IL-18R$\alpha$-ECD was divided into three domains according to homology between IL-1RI and IL-18R$\alpha$. Determination of the three domains of IL-1RI-ECD was identified by x-ray crystallography (17, 18). In the present study individual IL-18R$\alpha$-ECDs were expressed separately in *E. coli* as described in *Materials and Methods* (Fig. 3*A*). An equal amount of each IL-18R$\alpha$-ECD was resolved on SDS-PAGE under reducing conditions and probed with an anti-IL-18R$\alpha$ mAb (Fig. 3*B*). The anti-IL-18R$\alpha$ mAb specifically recognized the third domain of IL-18R$\alpha$-ECD (Fig. 3*B*, *lanes 3*, *5*, and *6*). The same anti-IL-18R$\alpha$ mAb was used in the biological assay of IL-18 on NK cells. The third domain-specific anti-IL-18R$\alpha$ mAb neutralized IL-18-induced IFN-$\gamma$ in a dose-dependent manner (Fig. 4).

Table I. *Scatchard analysis of specific binding of IL-18 to KG-1 and NK cells*[a]

| Cell Lines | $K_d$ | Affinity (pM) | Binding Sites/ Cell |
|---|---|---|---|
| KG-1 | $2.5 \times 10^{-10}$ | 250 | 135 |
| NK | $1.46 \times 10^{-10}$ | 146 | 350 |

[a] KG-1 and NK cells were incubated for 1 h at 4°C with increasing concentrations of [$^{125}$I]IL-18 as described in *Materials and Methods*.

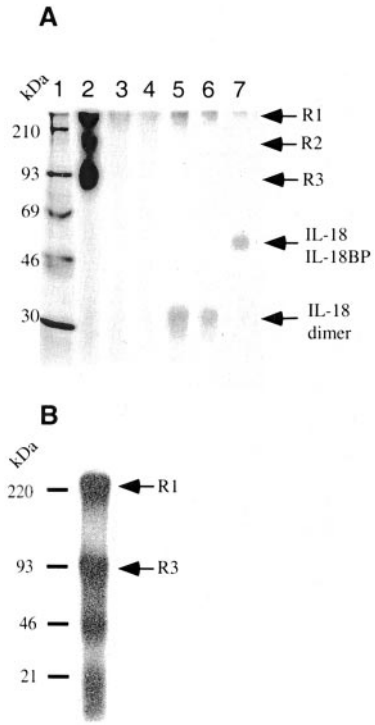


**FIGURE 2.** Analysis of IL-18R complex with NK cells. *A*, A 100-fold molar excess of nonradiolabeled IL-18 or IL-18BP was preincubated for 20 min before adding [$^{125}$I]IL-18 (2 nM). *Lane 1*, Molecular mass makers; *lane 2*, [$^{125}$I]IL-18 was cross-linked to membrane proteins; *lane 3*, [$^{125}$I]IL-18 was cross-linked to membrane proteins in the presence of a 100-fold molar excess of nonradiolabeled IL-18; *lane 4*, [$^{125}$I]IL-18 was cross-linked to membrane proteins in the presence of a 100-fold molar excess of nonradiolabeled IL-18BP; *lane 5*, the supernatant (10 $\mu$l) of [$^{125}$I]IL-18 after cross-linking; *lane 6*, the supernatant (10 $\mu$l) of [$^{125}$I]IL-18 after cross-linking in the presence of a 100-fold molar excess of nonradiolabeled IL-18; *lane 7*, the supernatant (10 $\mu$l) of [$^{125}$I]IL-18 after cross-linking in the presence of a 100-fold molar excess of nonradiolabeled IL-18BP. *B*, The immunoprecipitation of IL-18R complexes by anti-IL-18 as described in *Materials and Methods*. Samples were directly subjected to 10% SDS-PAGE under reducing conditions, and the gel was dried and autoradiographed.

### *Lack of the third Ig-like domain in IL-18R$\alpha$ prevents formation of the ternary complex of IL-18 with its receptors*

IL-18R$\alpha$ and IL-18R$\beta$ require glycosylation in the extracellular segment, and this glycosylation is important for IL-18 binding to its receptors, whereas *E. coli*-expressed IL-18R is not appropriate for IL-18 binding (data not shown). Therefore, the entire IL-18R$\alpha$-ECD, a third-domain deletion (IL-18R$\alpha$-ECD-$\Delta$D3), and the entire IL-18R$\beta$-ECD were transiently expressed in COS-7 cells for glycosylation of IL-18R-ECDs. Each IL-18R-ECD was fused with His$^6$ tag at the C terminus. An anti-His$^6$ tag mAb detected the expression of glycosylated IL-18R-ECDs (Fig. 5). Chemical cross-linking and Western blot showed COS-7 IL-18R-ECDs in the presence of DSS only (Fig. 6, *lanes 1–3*). IL-18 binds to IL-18R$\alpha$-ECD, but not to IL-18R$\alpha$-ECD-$\Delta$D3 or IL-18R$\beta$-ECD (*lanes 4–6*). A ternary complex was only observed in the presence of the complete IL-18R$\alpha$-ECD and IL-18R$\beta$-ECD (*lane 8*), but not in the presence of IL-18R$\alpha$-ECD-$\Delta$D3 and IL-18R$\beta$-ECD (*lane 7*).

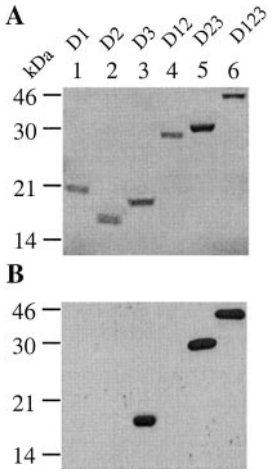


**FIGURE 3.** Affinity-purified separate IL-18Rα-ECDs. *A*, Coomassie Blue stain; *B*, Western blot with a neutralizing anti-IL-18 mAb. The numbers in the *left lane* indicate molecular masses. Each lane is indicated by domain 1 (D1), domain 2 (D2), domain 3 (D3), domains 1 and 2 (D12), domains 2 and 3 (D23), and the entire ECD (D123) in both *A* and *B*.

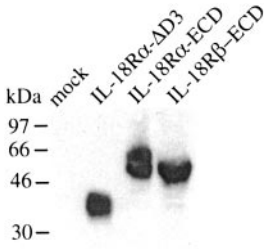


**FIGURE 5.** Western blot of separate domains of glycosylated IL-18Rs. Individual domains of IL-18R-ECDs were expressed and purified over a Talon column and then subjected to 10% SDS-PAGE under reducing conditions and transferred to nitrocellulose. The membrane was probed with anti-His$^6$ tag mAb.

*Deletion of the third Ig-like domain in transmembrane IL-18Rα loses its function*

The third Ig-like domain-deleted transmembrane IL-18Rα (IL-18Rα-ΔD3) was created by two-step PCR as described in *Materials and Methods*. The RT-PCR data exhibit transient expression of IL-18Rα with IL-18Rβ or IL-18Rα-ΔD3 with IL-18Rβ, but not in mock transfection (Fig. 7*A*). The difference between IL-18Rα and IL-18Rα-ΔD3 was shown in the *upper panel* that is 350 bp for the 117 aa of the third domain of IL-18Rα. The same transfectants were stimulated with human IL-18 and measured for NF-κB-Luc or IL-8 induction. As shown in Fig. 7, *B* and *C*, IL-18Rα with IL-18Rβ induced NFκB-Luc and IL-8 at 3-fold that of the mock transfection with NFκB-Luc alone. IL-18Rα-ΔD3 with IL-18Rβ failed to induce NFκB-Luc and IL-8, although IL-18Rα-ΔD3 expression was as great as with IL-18Rα.

## Discussion

These studies characterize the functional receptor complex for IL-18 activity using cell lines responding to IL-18. We also identified a critical domain of IL-18Rα for IL-18-induced IFN-γ and compared the expression of steady state IL-18 and IL-18Rα mRNA in different human tissues. The limited expression of IL-18Rα presumably controls the activity of constitutively expressed IL-18. Another participant in the control of IL-18 activity is IL-18BP (20, 22). Unlike other cytokines, cells contain pre-existing mRNA and/or protein levels of the precursor IL-18. IL-18 exists in a pro form awaiting cleavage for activation and therefore is likely to participate in the immediate host defense against infection or bodily injury. IL-18 mRNA is expressed in moderate levels in various tissues (Fig. 1*A*). However, IL-18Rα mRNA expression is limited in spleen and leukocytes, distinct from IL-18 mRNA expression patterns (Fig. 1*B*).

Most cytokine receptors are heterodimeric with ligand-binding and signal- transducing chains that cooperate in ligand-induced signal transduction. This is unlike growth factors, which have a homodimeric conformation, or the TNF receptor, which has a trimeric conformation. To date, IL-18 has the typical cytokine receptor components; IL-18Rα is the ligand-binding chain (32), and IL-18Rβ is the signal-transducing chain (33). Usually cytokine receptors are present at low levels on cell surfaces, although these same cells are highly responsive to the ligand, as is the case for IL-1RI for IL-1β (34). A biological response occurs when only 2–3% of IL-1RI is occupied by the ligand (34, 35). IL-18 shares this common characteristic of cytokine receptor biology. The Scatchard analysis revealed that KG-1 cells possess 135 receptors/cell and that NK cells possess 350 receptors/cell (Table I). The affinity of IL-18-responsive cell lines (146–250 pM) is comparable to that found for IL-18BP (2), and both are 100-fold higher than the affinity of L428 cells, which may have a single receptor component, IL-18Rα (32).

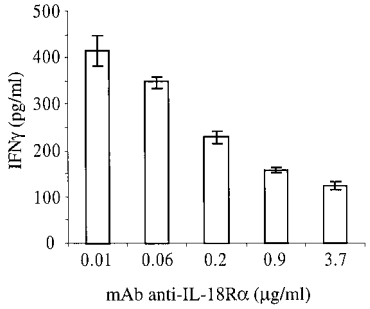


**FIGURE 4.** The effect of mAb anti-IL-18Rα on IL-18-induced IFN-γ in human NK cells. The mAb anti-IL-18Rα neutralizes IL-18 (20 ng/ml)-induced IFN-γ in the presence of IL-12 (0.5 ng/ml) on human NK cells. The horizontal axis indicates the concentration of mAb anti-IL-18Rα. The data represent the mean ± SEM of three separate experiments.

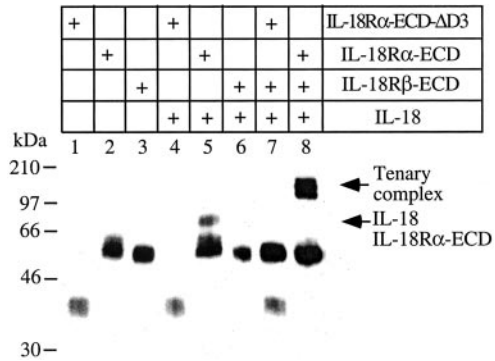


**FIGURE 6.** Western blot of IL-18R ternary complex. SDS-PAGE of IL-18 cross-linked to different receptor domains probed with mAb to anti-His$^6$ tag. *Lanes 1–3* contain the different domains alone. *Lanes 4–6* are the different domains in the presence of IL-18. *Lanes 7* and *8* are the different domains in the presence of both IL-18 and the IL-18Rβ-ECD.

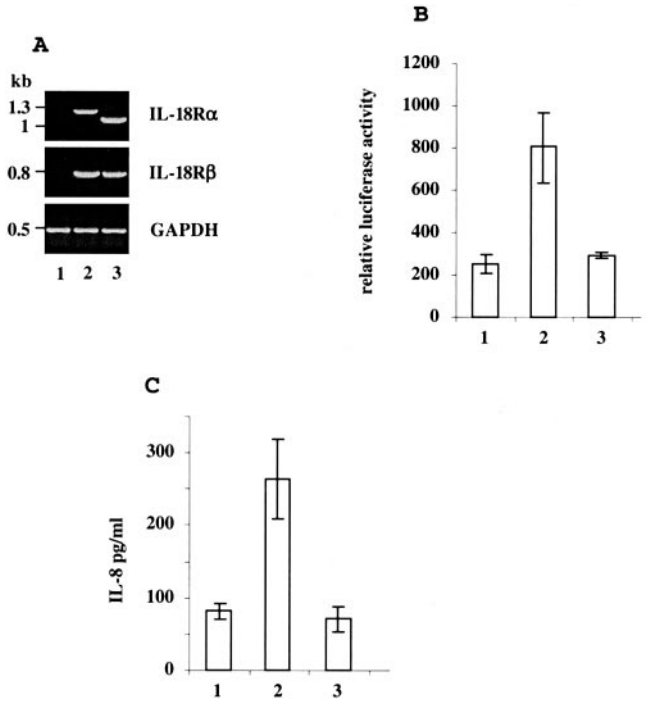


**FIGURE 7.** Deletion of the third Ig-like domain in transmembrane IL-18R$\alpha$. A549 human lung carcinoma cells were transiently transfected with various constructs. *Lane 1*, pTK-NF$\kappa$B-Luc with pTARGET (Mock); *lane 2*, pTARGET-IL-18R$\alpha$ with pTARGET-IL-18R$\beta$ in the presence of pTK-NF$\kappa$B-Luc; *lane 3*, pTARGET-IL-18R$\alpha$-$\Delta$D3 with pTARGET-IL-18R$\beta$ in the presence of pTK-NF$\kappa$B-Luc. *A*, RT-PCR analysis of transiently transfected A549 cell line. The data represent one of three separate experiments. *B*, The relative induction of NF$\kappa$B-Luc after stimulation with IL-18 (50 ng/ml) for 18 h. *C*, The induction of IL-8 in cell culture supernatant. The data in *B* and *C* represent the mean $\pm$ SEM of three separate experiments.

Chemical cross-linking of [$^{125}$I]IL-18 using the biologically responsive NK cells revealed IL-18R complexes of the whole membrane. These complexes were also revealed using immunoprecipitation with anti-IL-18 Ab. The major complex band was 93 kDa (R3) and consisted of [$^{125}$I]IL-18 and IL-18R$\alpha$. The band at 160 kDa (R2) is probably the functional complex comprised of [$^{125}$I]IL-18, IL-18R$\alpha$, and IL-18R$\beta$. This 160-kDa (R2) complex was not observed in COS cells (32), and an affinity-purified polyclonal anti-IL-18 failed to precipitate R2 complex. The failure of R2 complex immunoprecipitation may due to masking IL-18 epitopes in the complex containing both IL-18Rs. Although most cell lines will bind the IL-18 to form the 95-kDa (R3) complex, they do not respond to IL-18 because they lack the IL-18R$\beta$ required for a ternary complex (160 kDa; R2). The finding of a molecular mass complex of 220 kDa (R1) may represent a multimeric formation with more than one of each IL-18R component, or possibly this complex contains an unknown receptor component.

The determination of the interaction of IL-1$\beta$ with the three Ig-like domains in IL-1RI-ECD was resolved by x-ray crystallography (17, 18). The x-ray crystallography of the complex of IL-1$\beta$ or IL-1Ra with IL-1RI-ECD illustrated the differences in their interactions. The crystal structure revealed that IL-1RI-ECD consists of three Ig-like domains, which wrap around IL-1$\beta$ in a manner similar to those of other cytokine receptor complexes. There are two receptor binding regions on IL-1$\beta$ identified by site-directed mutagenesis, both making contact with the receptor: one binds to the first two domains of the receptor, whereas the other binds exclusively to the third domain (18). Unlike IL-1$\beta$, IL-1Ra interacts solely with the first and second domains of IL-1RI, but not with the third domain.

In the present study the third domain-specific anti-IL-18R$\alpha$ neutralized IL-18-induced IFN-$\gamma$ in a dose-dependent manner. This third Ig-like domain in IL-18R$\alpha$ is essential for IL-18 binding and formation of the ternary complex. In addition, deletion of third Ig-like domain in soluble IL-18R$\alpha$ abrogated IL-18 binding to IL-18R$\alpha$, and the formation of ternary complex (Fig. 6) as well as deletion of third Ig-like domain in transmembrane IL-18R$\alpha$ (IL-18R$\alpha$-$\Delta$D3) caused loss of function (Fig. 7). Other investigators have reported data that support our findings. Xu et al. (25) have shown that a rabbit anti-mouse IL-18R$\alpha_{247–266}$, a peptide residing in the third domain of mouse IL-18R$\alpha$, specifically neutralizes mouse IL-18 biological activity in vivo. Thus, the x-ray crystallography of IL-1/IL-1RI complex as well as the human and mouse anti-IL-18R$\alpha$ third-domain-specific Abs support the role of the third domain in the IL-1R family. It is important to note that anti-cytokine receptor Abs can bind to receptors, but do not interrupt the biological activity of cytokines (36, 37). This excludes the possibility of stereographic interruption by epitope-specific Abs.

The consistency of IL-1$\beta$- and IL-18-induced signal transduction with a role for the third Ig-like domain in the IL-1R family is an important for understanding the biology of the growing family of IL-1 ligands and receptors.

## References


1. Bazan, J. F., J. C. Timans, and R. A. Kastelein. 1996. A newly defined interleukin-1? *Nature 379:591*.
2. Kim, S. H., M. Eisenstein, L. Reznikov, G. Fantuzzi, D. Novick, M. Rubinstein, and C. A. Dinarello. 2000. Structural requirements of six naturally occurring isoforms of the IL-18 binding protein to inhibit IL-18. *Proc. Natl. Acad. Sci. USA 97:1190*.
3. Lin, H., A. S. Ho, D. Haley-Vicente, J. Zhang, J. Bernal-Fussell, A. M. Pace, D. Hansen, K. Schweighofer, N. K. Mize, and J. E. Ford. 2001. Cloning and characterization of IL-1HY2, a novel interleukin-1 family member. *J. Biol. Chem. 276:20597*.
4. Kojima, H., M. Takeuchi, T. Ohta, Y. Nishida, N. Arai, M. Ikeda, H. Ikegami, and M. Kurimoto. 1998. Interleukin-18 activates the IRAK-TRAF6 pathway in mouse EL-4 cells. *Biochem. Biophys. Res. Commun. 244:183*.
5. Matsumoto, S., K. Tsuji-Takayama, Y. Aizawa, K. Koide, M. Takeuchi, T. Ohta, and M. Kurimoto. 1997. Interleukin-18 activates NF-$\kappa$B in murine T helper type 1 cells. *Biochem. Biophys. Res. Commun. 234:454*.
6. Robinson, D., K. Shibuya, A. Mui, F. Zonin, E. Murphy, T. Sana, S. B. Hartley, S. Menon, R. Kastelein, F. Bazan, et al. 1997. IGIF does not drive Th1 development but synergizes with IL-12 for interferon-$\gamma$ production and activates IRAK and NF$\kappa$B. *Immunity 7:571*.
7. Kumar, S., P. C. McDonnell, R. Lehr, L. Tierney, M. N. Tzimas, D. E. Griswold, E. A. Capper, R. Tal-Singer, G. I. Wells, M. L. Doyle, et al. 2000. Identification and initial characterization of four novel members of the interleukin-1 family. *J. Biol. Chem. 275:10308*.
8. Sims, J. E., Y. Pan, D. E. Smith, M. J. Nicklin, J. L. Barton, J. F. Bazan, R. A. Kastelein, S. J. Busfield, J. E. Ford, H. Lin, et al. 2001. A new nomenclature for IL-1-family genes. *Trends Immunol. 22:536*.
9. Smith, D. E., B. R. Renshaw, R. R. Ketchem, M. Kubin, K. E. Garka, and J. E. Sims. 2000. Four new members expand the interleukin-1 superfamily. *J. Biol. Chem. 275:1169*.
10. Kumar, S., C. R. Hanning, M. R. Brigham-Burke, D. J. Rieman, R. Lehr, S. Khandekar, R. B. Kirkpatrick, G. F. Scott, J. C. Lee, F. J. Lynch, et al. 2002. Interleukin-1F7B (IL-1H4/IL-1F7) is processed by caspase-1 and mature IL-1F7B binds to the IL-18 receptor but does not induce IFN-$\gamma$ production. *Cytokine 18:61*.
11. Pan, G., P. Risser, W. Mao, D. T. Baldwin, A. W. Zhong, E. Filvaroff, D. Yansura, L. Lewis, C. Eigenbrot, W. J. Henzel, et al. 2001. IL-1H, an interleukin 1-related protein that binds IL-18 receptor/IL-1Rrp. *Cytokine 13:1*.
12. Ghayur, T., S. Banerjee, M. Hugunin, D. Butler, L. Herzog, A. Carter, L. Quintal, L. Sekut, R. Talanian, M. Paskind, et al. 1997. Caspase-1 processes IFN-$\gamma$-inducing factor and regulates LPS-induced IFN-$\gamma$ production. *Nature 386:619*.
13. Gu, Y., K. Kuida, H. Tsutsui, G. Ku, K. Hsiao, M. A. Fleming, N. Hayashi, K. Higashino, H. Okamura, K. Nakanishi, et al. 1997. Activation of interferon-$\gamma$ inducing factor mediated by interleukin-1$\beta$ converting enzyme. *Science 275:206*.
14. Pirhonen, J., T. Sareneva, M. Kurimoto, I. Julkunen, and S. Matikainen. 1999. Virus infection activates IL-1$\beta$ and IL-18 production in human macrophages by a caspase-1-dependent pathway. *J. Immunol. 162:7322*.
15. Schumann, R. R., C. Belka, D. Reuter, N. Lamping, C. J. Kirschning, J. R. Weber, and D. Pfeil. 1998. Lipopolysaccharide activates caspase-1 (interleukin-1-converting enzyme) in cultured monocytic and endothelial cells. *Blood 91:577*.

16. Tsutsui, H., N. Kayagaki, K. Kuida, H. Nakano, N. Hayashi, K. Takeda, K. Matsui, S. Kashiwamura, T. Hada, S. Akira, et al. 1999. Caspase-1-independent, Fas/Fas ligand-mediated IL-18 secretion from macrophages causes acute liver injury in mice. *Immunity 11:359*.
17. Schreuder, H., C. Tardif, S. Trump-Kallmeyer, A. Soffientini, E. Sarubbi, A. Akeson, T. Bowlin, S. Yanofsky, and R. W. Barrett. 1997. A new cytokine-receptor binding mode revealed by the crystal structure of the IL-1 receptor with an antagonist. *Nature 386:194*.
18. Vigers, G. P., L. J. Anderson, P. Caffes, and B. J. Brandhuber. 1997. Crystal structure of the type-I interleukin-1 receptor complexed with interleukin-1β. *Nature 386:190*.
19. Vigers, G. P., D. J. Dripps, C. K. Edwards, and B. J. Brandhuber. 2000. X-ray crystal structure of a small antagonist peptide bound to interleukin-1 receptor type 1. *J. Biol. Chem. 275:36927*.
20. Novick, D., S. H. Kim, G. Fantuzzi, L. L. Reznikov, C. A. Dinarello, and M. Rubinstein. 1999. Interleukin-18 binding protein: a novel modulator of the Th1 cytokine response. *Immunity 10:127*.
21. Xiang, Y., and B. Moss. 1999. IL-18 binding and inhibition of interferon γ induction by human poxvirus-encoded proteins. *Proc. Natl. Acad. Sci. USA 96:11537*.
22. Novick, D., B. Schwartsburd, R. Pinkus, D. Suissa, I. Belzer, Z. Sthoeger, W. F. Keane, Y. Chvatchko, S. H. Kim, G. Fantuzzi, et al. 2001. A novel IL-18BP ELISA shows elevated serum IL-18BP in sepsis and extensive decrease of free IL-18. *Cytokine 14:334*.
23. Muhl, H., H. Kampfer, M. Bosmann, S. Frank, H. Radeke, and J. Pfeilschifter. 2000. Interferon-γ mediates gene expression of IL-18 binding protein in nonleukocytic cells. *Biochem. Biophys. Res. Commun. 267:960*.
24. Watanabe, M., H. Kaneko, H. Shikano, M. Aoki, H. Sakaguchi, E. Matsui, R. Inoue, Z. Kato, K. Kasahara, O. Fukutomi, et al. 2002. Predominant expression of 950delCAG of IL-18R α chain cDNA is associated with reduced IFN-γ production and high serum IgE levels in atopic Japanese children. *J. Allergy Clin. Immunol. 109:669*.
25. Xu, D., W. L. Chan, B. P. Leung, D. Hunter, K. Schulz, R. W. Carter, I. B. McInnes, J. H. Robinson, and F. Y. Liew. 1998. Selective expression and functions of interleukin 18 receptor on T helper (Th) type 1 but not Th2 cells. *J. Exp. Med. 188:1485*.
26. Kim, S. H., L. L. Reznikov, R. J. Stuyt, C. H. Selzman, G. Fantuzzi, T. Hoshino, H. A. Young, and C. A. Dinarello. 2001. Functional reconstitution and regulation of IL-18 activity by the IL-18R β chain. *J. Immunol. 166:148*.
27. Kim, S. H., T. Azam, D. Y. Yoon, L. L. Reznikov, D. Novick, M. Rubinstein, and C. A. Dinarello. 2001. Site-specific mutations in the mature form of human IL-18 with enhanced biological activity and decreased neutralization by IL-18 binding protein. *Proc. Natl. Acad. Sci. USA 98:3304*.
28. Hoshino, T., R. T. Winkler-Pickett, A. T. Mason, J. R. Ortaldo, and H. A. Young. IL-13 production by NK cells: IL-13-producing NK and T cells are present in vivo in the absence of IFN-γ. *J. Immunol. 162:51, 1999a*.
29. Hoshino, T., R. H. Wiltrout, and H. A. Young. IL-18 is a potent coinducer of IL-13 in NK and T cells: a new potential role for IL-18 in modulating the immune response. *J. Immunol. 162:5070, 1999b*.
30. Puren, A. J., P. Razeghi, G. Fantuzzi, and C. A. Dinarello. 1998a. Interleukin-18 enhances lipopolysaccharide-induced interferon-γ production in human whole blood cultures. *J. Infect. Dis. 178:1830*.
31. Scatchard, G. 1949. The attraction of proteins for small molecules and ions. *Ann. NY Acad. Sci. 51:660*.
32. Torigoe, K., S. Ushio, T. Okura, S. Kobayashi, M. Taniai, T. Kunikata, T. Murakami, O. Sanou, H. Kojima, M. Fujii, et al. 1997. Purification and characterization of the human interleukin-18 receptor. *J. Biol. Chem. 272:25737*.
33. Debets, R., J. C. Timans, T. Churakowa, S. Zurawski, R. de Waal Malefyt, K. W. Moore, J. S. Abrams, A. O'Garra, J. F. Bazan, and R. A. Kastelein. 2000. IL-18 receptors, their role in ligand binding and function: anti-IL-1RAcPL antibody, a potent antagonist of IL-18. *J. Immunol. 165:4950*.
34. Ye, K., K. C. Koch, B. D. Clark, and C. A. Dinarello. 1992. Interleukin-1 downregulates gene and surface expression of interleukin-1 receptor type I by destabilizing its mRNA whereas interleukin-2 increases its expression. *Immunology 75:427*.
35. Gallis, B., K. S. Prickett, J. Jackson, J. Slack, K. Schooley, J. E. Sims, and S. K. Dower. 1989. IL-1 induces rapid phosphorylation of the IL-1 receptor. *J. Immunol. 143:3235*.
36. Yoon, D. Y., and C. A. Dinarello. 1998. Antibodies to domains II and III of the IL-1 receptor accessory protein inhibit IL-1β activity but not binding: regulation of IL-1 responses is via type I receptor, not the accessory protein. *J. Immunol. 160:3170*.
37. Clark, B. D., T. Ikejima, J. Mancilla, S. F. Orencole, S. P. Sirko, N. Ishii, K. Okuda, and C. A. Dinarello. 1996. An antibody to a 17 amino acid synthetic peptide of the type I interleukin-1 receptor preferentially blocks interleukin-1β binding. *J Interferon Cytokine Res. 16:1079*.