Purification and Characterization of the Human Interleukin-18 Receptor*

(Received for publication, April 28, 1997, and in revised form, July 14, 1997)

Kakui Torigoe, Shimpei Ushio, Takanori Okura, Susumu Kobayashi, Madoka Tanai, Toshio Kunikata, Tadatoshi Murakami, Osamu Sanou, Hirotada Kojima, Mitsukiyo Fujii, Tsunetaka Ohta, Masao Ikeda, Hakuo Ikegami, and Masashi Kurimoto

From the Fujisaki Institute, Hayashibara Biochemical Laboratories, Inc., 675-1 Fujisaki, Okayama, 702 Japan

Interleukin (IL)-18 was identified as a molecule that induces IFN-γ production and enhances NK cell cytotoxicity. In this paper, we report upon the purification and characterization of human IL-18 receptor (hIL-18R). We selected the Hodgkin's disease cell line, L428, as the most strongly hIL-18R-expressing cell line based on the results of binding assays. This binding was inhibited by IL-18 but not by IL-1β. The dissociation constant (Kd) of 125I-IL-18 binding to L428 cells was about 18.5 nM, with 18,000 binding sites/cell. After immunizing mice with L428 cells and cloning, a single monoclonal antibody (mAb) against hIL-18R was obtained (mAb 117-10C). Sequentially, hIL-18R was purified from 3-[3-cholamidopropyl]dimethylammonio]-1-propanesulfonic acid (CHAPS)-extracted L428 cells by wheat germ lectin-Sepharose 4B chromatography and mAb 117-10C-Sepharose chromatography. The internal amino acid sequences of hIL-18R all matched those of human IL-1 receptor-related protein (IL-1Rrp), the ligand of which was unknown to date. When expressed in COS-1 cells, the cDNA of IL-1Rrp conferred IL-18 binding properties on the cells and the capacity for signal transduction. From these results, we conclude that a functional IL-18 receptor component is IL-1Rrp.

Murine interleukin-18 (mIL-18) was identified as the liver of mice sequentially injected with heat-killed Propionibacterium acnes and with lipopolysaccharide (1). Murine IL-18 cDNA was cloned from murine liver mRNA, and the factor was provisionally termed IFN-γ-inducing factor because it was first identified as an IFN-γ inducer in mice. Consequently, human interleukin-18 (hIL-18) was cloned as normal human liver mRNA (2). IL-18 is a non-N-linked, glycosylated, 18.3-kDa cytokine in its mature form and exhibits biologic activities in the monomeric form. IL-18 has been found to have a variety of biologic actions, including the stimulation of the proliferation of activated T cells, enhancement of the lytic activity of NK cells, induction of interferon-γ (IFN-γ), and granulocyte-macrophage colony-stimulating factor production by activated T cells and promotion of Th1-type helper (Th1) clone responses (1–4). It has also been reported that IL-18 inhibits osteoclast-like multinucleated cell formation in co-cultures of osteoblasts and hemopoietic cells of spleen or bone marrow origin (5). Thus, it is very obvious that IL-18 plays an important role in the immune system.

IL-18 shares some of its biologic activities with IL-12, although the primary structures of the two cytokines show no homology (2). In addition, in the experiments using murine Th1 clones and enriched human T cells, IL-18 and IL-12 acted on the T cells synergistically to induce IFN-γ production (1, 4). Interestingly, the amino acid sequence of IL-18 includes the IL-1 signature-like sequence (2) and has been shown to have 15% homology at the amino acid level with the IL-1β protein, but does not bear significant functional resemblance to the IL-1 family (3).

The identification of the receptor for IL-18 is important for investigation of the physiological role of IL-18 in nature. In this report, we describe the purification and identification of hIL-18R from a Hodgkin's disease-derived cell line, L428, and present some characterization of this molecule.

**EXPERIMENTAL PROCEDURES**

### Cell Lines and Reagents

C57M, CCRF-HSB-2, HPB-ALL, JM, MOLT-3, MOLT-4, MOLT-16, PEER, SKW-3 (human T cell leukemia), ARH-77, BALL-1 (human B cell leukemia), KG-1, HL-60, U-937 (human myelomonocytic cell leukemia), NALM-16, HEL (human non-T, non-B cell leukemia), and L-428 and HDLM (human Hodgkin's disease) cell lines were maintained in culture at 37 °C, in a 5% CO2 air mixture in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum (BioWhittaker Inc.). Recombinant human IL-1β (R&D Systems) and 125I-IL-1β (Amersham) were obtained commercially.

### Recombinant IL-18

Recombinant human IL-18 (rhIL-18) was produced by culture of Escherichia coli transformed with an expression vector containing hIL-18 cDNA. Purification of rhIL-18 to homogeneity from culture broths of transformed E. coli was described elsewhere (2). The purified rhIL-18 had a specific activity of 1 × 10^6 units/mg of protein (2). One unit of IL-18 is that amount of protein which induces 160 IU/ml IFN-γ production from 0.75 × 10^6 peripheral blood mononuclear cells in the presence of ConA.

### Labeling of Recombinant IL-18

Radiiodination of rhIL-18 using Bolton-Hunter reagent (ICN) was performed according to the manufacturer's instructions (6). Specific radioactivity of the preparations was in the range of 5–7 × 10^4 cpm/ng of protein.

### Binding of 125I-IL-18 to Human Cell Lines

All human cell lines were suspended in RPMI 1640 medium containing 0.1% bovine serum albumin, 0.1% NaN3, and 100 mM HEPES...
(binding medium). The binding reactions were performed on 2 × 10^6 cells for 1 h at 4°C in 150 μl of binding medium containing various concentrations of 125I-IL-18 or 125I-IL-1β with or without 500 nM unlabeled rhIL-18 or IL-1β. After incubation, the mixture was layered over 200 μl of phthalate oil (dibutylphthalate-diethylphthalate = 1:1), centrifuged at 150,000 × g for 5 min at 4°C, and cell-bound antigen or 125I-IL-18 of the cell pellet was determined using a γ-counter. Receptor binding data of cell lines with high specific binding were analyzed by the Scatchard coordinate system.

**Chemical Cross-linking**

L428 cells (5 × 10^6) were incubated for 1 h at 4°C in binding medium containing 10 μg of 125I-IL-18 or 125I-IL-1β with or without unlabeled rhIL-18 or IL-1β. The cells were suspended in 1 ml of PBS containing 1 mM bis(sulfoconiimidyl) substrates (BS3, Pierce) and incubated for 1 h on ice. After the addition of 50 μl of 1 M Tris-HCl (pH 7.4), the cells were lysed by the addition of 100 μl of PBS containing 12 mM CHAPS. After incubation at 4°C for 3 h, the lysates were centrifuged at 10,000 × g for 30 min at 4°C and the supernatants were electrophoresed on a 2–15% SDS-PAGE (Daichi Chemicals Co.) under reducing conditions. After electrophoresis, the gel was dried and exposed to x-ray film for 4 days.

**125I-IL-18 Receptor Binding Assays on Cell Extracts**

The assay used to semi-quantitate the extracted hIL-18R was modified from the solubilized granulocyte colony-stimulating factor receptor binding assay developed by Fukunaga et al. (7). The CHAPS-extracted receptor (50 μl) or partially purified hIL-18R preparations were mixed with 1 μg of 125I-IL-18 or 125I-IL-1β and 0.1% bovine serum albumin in the presence or absence of 500 nM unlabeled rhIL-18. The binding reaction was performed for 1 h at 4°C. After incubation, 50 μl of PBS containing 200 μg of bovine γ-globulin (Sigma) was added and the receptor-ligand complex was precipitated from the solution by the addition of 250 μl of PBS containing 20 mM polyethylene glycol. Precipitates were collected by centrifugation at 10,000 × g for 5 min at 4°C. The radioactivity of the pellet was quantitated as described above.

**Preparation of Anti-IL-18 Receptor mAb**

BALB/c mice were immunized through the intraperitoneal route with L428 cells at 2 × 10^6 cells/mouse. Mice received 11–12 booster injections of 2 × 10^6 cells over a 6-month period. For preparation of activated spleen cells, mice were injected intravenously with a partially purified hIL-18R preparation, on 2 consecutive days, starting 3 days prior to spleen cell harvest. Spleen cells were fused with SP2/0 cells according to the methods of Uchiyama et al. (8). Hybrioma supernatant reactivities were assayed by the system of inhibition of 125I-IL-18 binding to L428 cells. Antibodies were purified from murine ascites fluids by hydroxyapatite gel chromatography, since the mAb of interest, 117-10C, was of the IgM subclass. mAb 117-10C was conjugated to bovine serum albumin in the presence or absence of 500 nM unlabeled rhIL-18.

**Purification of IL-18 Receptor**

**Preparation of L428 Cell Extracts**

To obtain a large number of L428 cells as a source of hIL-18R, we used the “in vivo cell proliferation method (hamster method).” Briefly, this is a method to propagate human cells in the bodies of immunosuppressed hamsters instead of conventional cell culture methods (9).

A membrane fraction extract was prepared as described (13) with several modifications. Briefly, to a frozen pellet of L428 cells (10 × 10^9 cells, approximately 100 g) was added 500 ml of 10 mM Tris-HCl (pH 7.2) and 1 mM MgCl₂, and cells were homogenized on ice for 30 s five times. A 2-fold volume of 10 mM Tris-HCl (pH 7.2) containing 0.3 M sucrose and 1 mM MgCl₂ homogenate was added, and the homogenate was centrifuged at 600 × g for 10 min. A membrane-enriched fraction was obtained by centrifugation of the supernatant at 150,000 × g for 60 min. The pellet was suspended in 600 ml of PBS containing 12 mM CHAPS, 1 mM p-aminophenylmethanesulfonyl fluoride hydrochloride, and 10 mM EDTA and stirred at 4°C for 18 h. The suspension was centrifuged at 150,000 × g for 60 min, and the supernatant was used as the starting material for the experimental procedure. Purification steps described above were used to produce one lot of extract. The CHAPS-extracted samples from 45 lots, derived from a total of 4.5 × 10₁² cells, were pooled and used for further purification.

**Wheat Germ Lectin (WGL)-Sepharose, mAb 117-10C-Sepharose Chromatography—**Two hundred milliliters of WGL-Sepharose (Pharmacia) were applied to 600 ml of the extracted crude membrane fraction. After washing, the protein was eluted with PBS containing 12 mM CHAPS and 0.5 mM N-acetylglucosamine. The hIL-18R preparations recovered from WGL-Sepharose were dialyzed against PBS containing 2 mM CHAPS, further purified by mAb 117-10C-Sepharose chromatography, and eluted with 35 mM ethylamine (pH 10.8) containing 2 mM CHAPS. The eluates were immediately neutralized with 1 M HEPES (pH 7.2) and concentrated with an ultrafiltration membrane module (Asahikasei).

**N-terminal Amino Acid Sequencing and Peptide Mapping**—The eluates from mAb 117-10C-Sepharose chromatography were pooled and concentrated. The concentrated sample was electrophoresed on a 7.5% SDS-polyacrylamide gel, transferred onto a polyvinylidene difluoride membrane, and stained with CBB according to the methods of Matsudaira et al. (10). Polyvinylidene difluoride-blotted proteins were excised and subjected to N-terminal sequencing (10). For tryptic peptide mapping by the in-gel digestion procedure, the concentrates were electrophoresed on a 7.5% SDS gel in the presence of a reducing agent. After staining the gel with CBB, bands corresponding to the receptor were excised and digested with trypsin (Promega) according to the methods of Hellman et al. (11). The peptide fragments extracted from the gel slices were injected into a RPC C2/C18 SC2110 column in a SMART system (Pharmacia). Fractions were subjected to amino acid sequencing using a Perkin-Elmer protein sequencer model 473A.

**IL-1Rrp Expression Plasmid and Transfection of COS-1 Cells**

From the results of internal amino acid sequencing analysis and comparison with published sequences, the internal peptide fragments of the purified hIL-18R were found to be identical to hIL-1Rbp. We next intended to construct a mammalian expression vector for hIL-1Rbp. A peptide encoding amino acids 1–541 of hIL-1Rbp was designed into pCRScript and then into the expression vector, pCDNA/Amp (18). COS-1 cells were transfected by electroporation with expression constructs and harvested after 72 h of incubation as described previously (12). Using the transfected COS-1 cells as targets, binding assays and NF-κB gel shift assays were performed (13).

**RESULTS**

**Selection of L428 Cells as Strong Expressors of IL-18R**—To facilitate the purification and cDNA cloning of the hIL-18R, the strategy chosen was to select cells that exhibited high expression of hIL-18R on the cell surface, constitutively. Eighteen human leukemic cell lines of different phenotypes, including T, B, and myelomonocytic cell lines, were examined for hIL-18R expression by binding assay using 125I-IL-18. As shown in Table I, specific binding of rhIL-18 was strongest to L428 cells and next, to HDLM1 cells, with detectable levels found in C5/MJ, MOLT-16, PEER, and U937 cells, and slightly detectable levels also found in CCRF-HSB-2, MOLT-3, MOLT-4, SKW-3, and ARH-77 cells, respectively. From these results, we selected L428 cells as the primary material for hIL-18R purification.

Fig. 1 shows typical results of binding of 125I-IL-18 to L428 cells. A plot of the data in the Scatchard coordinate system yielded a straight line, suggesting that a single class of a binding site for IL-18 exists on L428 cells. The apparent dissociation constant (Kₐ) calculated from the data was about 18.5 nM with 18,000 binding sites/cell.

**Comparison of IL-18 and IL-1β Binding to L428 Cells**—To ascertain at this stage that IL-18 and IL-1β do not share the same receptors, competition assays were performed on L428 cells using 125I-IL-18 and 125I-IL-1β. As shown in Fig. 2, on the surface of L428 cells, specific binding of both 125I-IL-18 and 125I-IL-1β was observed. The binding of 125I-IL-18 to L428 cells was not inhibited by the addition of unlabeled IL-1β, and the specific binding of 125I-IL-18 to L428 cells was not inhibited by the addition of unlabeled rhIL-18, respectively. Affinity cross-linking for IL-18 on L428 cells showed the formation of a cross-linked complex which was observed at only one range, and which was estimated to have a molecular mass of 120–80 kDa (Fig. 3). Appearance of the band was inhibited by the addition of unlabeled rhIL-18 during binding of 125I-IL-18 to
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TABLE I
Expression of hIL-18R on human leukemic cell lines

| Cell line     | Origin (phenotype) | Specific count |
|---------------|-------------------|----------------|
| C5/MJ         | NT (T cell)       | 537            |
| CCRF-HSB-2    | ALL (T cell)      | 147            |
| HPB-ALL       | ALL (T cell)      | —              |
| JM            | ALL (T cell)      | —              |
| MOLT-3        | ALL (T cell)      | 107            |
| MOLT-4        | ALL (T cell)      | 104            |
| MOLT-16       | ALL (T cell)      | 501            |
| PEER          | ALL (T cell)      | 512            |
| SKW-3         | CLL (T cell)      | 118            |
| ARH-77        | MM (B cell)       | 167            |
| BALL-1        | ALL (B cell)      | —              |
| KG-1          | AML (myelomonocytoid cell) | 397 |
| HL-60         | APL (myelomonocytoid cell) | 101 |
| U-937         | LY (myelomonocytoid cell) | 505 |
| NALM-16       | ALL (non-T, non-B cell) | — |
| HEL           | EL (non-L, non-M cell) | — |
| L-428         | HD (non-L, non-M cell) | 5111 |
| HDLM          | HD (non-L, non-M cell) | 2511 |

a Abbreviations used are as follows: NT, normal virus-transformed T-cells; ALL, acute lymphoblastic leukemia; CLL, chronic lymphocytic leukemia; MM, multiple myeloma; APL, acute promyelocytic leukemia; LY, lymphoma; EL, erythroleukemia; HD, Hodgkin’s disease.

L428 cells. Since the mass of rhIL-18 is about 18 kDa, the size of the cross-linked complex suggested that hIL-18R exists as a single polypeptide of mass 100–60 kDa. In correlation with the results of binding assays, the formation of the cross-linked complex was not inhibited by the addition of unlabeled IL-1β. The mass of the cross-linked complex was observed at a range corresponding to a mass of 100–60 kDa, which range was different from that of the 125I-IL-18 and hIL-18R complex, even though both cytokines have almost the same mass (18.3 kDa and 17.5 kDa for IL-18 and IL-1β, respectively). These results suggested that the cross-linking of hIL-18R is distinct from the IL-1R molecule.

Screening for a mAb against hIL-18R—After immunization of BALB/c mice with L428 cells, spleen cells were fused to SP2/O cells and hybridoma supernatants were assayed for anti-IL-18R reactivity by inhibition of 125I-IL-18 binding to L428 cells. After initial screening, several positive clones were obtained. After a second screening, one positive clone of the IgM subclass (mAb 117-10C) was obtained, which efficiently inhibited 125I-IL-18 binding to L428 cells. In addition, the mAb was useful for flow cytometric analysis and inhibited the biological activity of rhIL-18; however, the mAb was not useful for Western blot analysis in the presence of reducing agents (data not shown).

Purification of hIL-18R from L428 Cell Membrane Extracts—Purification of the hIL-18R from extracted L428 membranes was monitored by an assay procedure that is based on the selective precipitation of the ligand–receptor complex by polyethylene glycol 6000. The quantity of extracted hIL-18R in the sample was estimated from the radioactive counts of the precipitate. As a starting material for purification of the hIL-18R, membrane-rich fractions of L428 cells were extracted with 12 mM CHAPS and the solubilized fractions were applied to WGL-Sepharose. As shown in Table II, about 30% of the binding activity of the solubilized membrane fraction was recovered in the eluted fraction of WGL-Sepharose, resulting in about a 16-fold purification. Next, after dialysis against 2 mM CHAPS in PBS to remove 0.5 M N-acetylglucosamine, the sample was applied to mAb 117-10C-Sepharose and eluted with alkaline buffers as described under “Experimental Procedures.” About 25% of the binding activity from WGL-Sepharose chromatography was recovered in the fraction eluted from mAb 117-10C-Sepharose, resulting in about a 8300-fold purification.

When the concentrated preparation was analyzed by SDS-PAGE in the presence of reducing agents, CBB staining revealed a broad band with a size in the range of 64–100 kDa (Fig. 4). As for the wide band for the purified hIL-18R, the reason is unclear. Possibly, hIL-18R protein is highly glycosylated and has a heterogeneity of sugar chains. The purification process is summarized in Table II, which indicates that the overall purification of hIL-18R was about 10,000-fold of the CHAPS-extracted fraction with a yield of 7.7%. From 4.5 × 1012 L428 cells, an estimated 23 μg of hIL-18R protein was obtained.

Partial Amino Acid Sequencing—Five micrograms from the 23 μg of purified hIL-18R were used for N-terminal amino acid sequence analysis. The results of N-terminal amino acid sequencing at positions 1 and 3 in Fig. 4 yielded the following residues: Glu-X-X-Pro/Gly-Ary-Pro-His-Ile-Thr-Glu/Val-Val-Glu. However, the N-terminal amino acid sequence at position 2 was unreadable, possibly because the N-terminal sequences of position 2 might be masked. To further clarify the data, the rest of the purified hIL-18R preparation was used for tryptic mapping and internal tryptic peptide fragments were sequenced. From the results of a homology search in the GenBank, PIR, and EMBL data bases, it was evident that the amino acid sequences of eight different hIL-18R peptide fragments all matched those of the hIL-1Rrp sequence (Fig. 5).

Expression of hIL-1Rrp cDNA in COS-1 Cells—We next intended to clone the hIL-1Rrp cDNA and construct a mammalian expression vector for hIL-1Rrp to determine if hIL-1Rrp is a functional hIL-18R. hIL-1Rrp cDNA was amplified by RT-PCR technique and inserted into a mammalian transient expression vector (pCDNA/Amp), and hIL-1Rrp was transiently expressed in COS-1 cells (Fig. 6). Specific binding of 125I-IL-18 to the hIL-1Rrp-expressing COS-1 cells was observed. The expressed hIL-1Rrp exhibited an apparent Kd value of 46 nM and 4.9 × 10⁴ specific binding sites per cell. This Kd value was
similar to the previously mentioned $K_d$ value for IL-18 binding to L428 cells.

To examine whether the expressed hIL-1Rrp could drive signal transduction after adding IL-18 protein to the transfected COS-1 cells, we performed experiments to determine whether the transcription factor NF-$\kappa$B was activated or not, because it has been reported that the cytoplasmic domain of IL-1Rrp was capable of activating NF-$\kappa$B DNA binding (14). As shown in Fig. 7, the hIL-18R (IL-1Rrp) is capable of inducing NF-$\kappa$B DNA binding ability in response to stimulation with rhIL-18. Since IL-1$\beta$ cross-reacted with mock-transfected COS-1 cells, the induction of NF-$\kappa$B to DNA binding abilities of the mock-transfected and transfected COS-1 cells in response to IL-1$\beta$ was also examined. However, there were no differences in the extent of NF-$\kappa$B activation between the mock-transfected and IL-1Rrp-transfected COS-1 cells in response to IL-1$\beta$.

**DISCUSSION**

Generally, cytokine receptors are present at low levels on the cell surface, possibly having a sugar chain and exhibiting a high molecular weight like most other membrane proteins. These characteristics make it technically difficult to purify receptor proteins. To solve this problem, we screened for hIL-18R-expressing cell lines by binding assay and attempted to establish an anti-hIL-18R mAb by immunization of mice with the selected hIL-18R-expressing cells.

![Table II](image)

| Purification of human IL-18R | Volume | Protein | IL-18R | Yield | Purification |
|-------------------------------|--------|---------|--------|-------|--------------|
| CHAPS extracts*              | 25,000 | 50,000  | 300    | 100   | 1            |
| WGL-Sepharose                | 4516   | 903     | 90     | 30    | 30           |
| mAb 117–10C-Sepharose        | 11     | 0.46    | 23     | 7.7   | 8333         |

* Extracts were taken from $4.5 \times 10^{12}$ L428 cells.

![Fig. 2](image)

**Fig. 2.** Inhibition of $^{125}$I-IL-18 and $^{125}$I-IL-1$\beta$ binding to L428 cells by adding unlabeled IL-18 and IL-1$\beta$. L428 cells were incubated for 1 h at 4 °C with 1.0 nM $^{125}$I-IL-18 or $^{125}$I-IL-1$\beta$, in the presence of a 500-fold molar excess of unlabeled rhIL-18, IL-1$\beta$, or none, as described under “Experimental Procedures.”

![Fig. 3](image)

**Fig. 3.** Analysis of hIL-18R and hIL-1R expression on L428 cells by cross-linking with $^{125}$I-IL-18 or $^{125}$I-IL-1$\beta$ in the presence of unlabeled rhIL-18 or IL-1$\beta$. L428 cells were incubated with 1.0 nM $^{125}$I-IL-18 or $^{125}$I-IL-1$\beta$ in the presence or absence of a 500-fold molar excess of unlabeled rhIL-18 or IL-1$\beta$. Incubations and cross-linking were performed as described under “Experimental Procedures.” Subsequently, samples were analyzed by electrophoresis on 2–15% gradient SDS-PAGE gels and autoradiography of the dried gels. The arrows show the positions of the IL-18 plus IL-18R complex and the IL-1$\beta$ plus IL-1R complex, respectively.

The selected Hodgkin’s disease cell line, L428 cells expressed hIL-18R at about 18,000 sites/cell. The resultant 10–20-fold difference in expression levels when compared with other cell lines screened facilitated the preparation of sufficient quanti-
ties of purified protein for amino acid sequencing.

The results of screening of hIL-18R-expressing cells from among 18 human leukemic cell lines of T, B, and myelomonocytic cell origin showed that expression of the hIL-18R was widespread but was expressed at higher levels by Hodgkin's disease cell lines. These results suggest that hIL-18R is expressed in a number of organs as is the IL-1R, which is in good agreement with the results of Northern blot analysis for IL-1Rrp mRNA expression (14). Further studies along these lines are being planned to clarify the distribution of hIL-18R in normal tissues.

Although there are no resemblances between IL-18 and IL-1 concerning their target cells and biological actions, the amino acid sequence of IL-18 includes an IL-1 signature-like sequence (2). A comparison of the IL-18 and IL-1 chains showed a parsimonious alignment over the length of the respective sequences that comprise the 12 b-strands of the distinctive b-trefoil fold (15). Therefore, it was likely that there was little possibility that IL-18 and IL-1 share the same receptor. Indeed,

**FIG. 4.** Analysis of the purified human IL-18R by SDS-polyacrylamide gel electrophoresis. The human IL-18R purified by mAb 117-10C-Sepharose elution (lane 1), leak eluate (lane 3), and a molecular size marker (lane 2) were mixed with SDS sample buffer containing a reducing agent and electrophoresed on a 7.5% SDS-PAGE gel. Subsequently, the gel was stained with CBB. The positions 1 and 3 (numbers in circles) show the purified hIL-18R positions, and position 2 shows an unidentified molecule (N-terminal amino acid sequence was blocked).

**FIG. 5.** Human IL-1Rrp amino acid sequences and the results of partial amino acid sequencing of IL-18R. The eluates from mAb 117-10C-Sepharose were concentrated, and the samples were electrophoresed on a 7.5% SDS gel in the presence of a reducing agent. Subsequently, N-terminal amino acid sequencing and internal amino acid sequencing were performed as described under "Experimental Procedures." The vertical arrow shows the putative N-terminal amino acid sequence of hIL-1Rrp. The amino acid sequences underlined show the actual determined amino acid sequences, I is the N terminus, and 2–9 are the results of tryptic mapping using the Perkin-Elmer protein sequencer model 473A.

**FIG. 6.** Scatchard analysis of specific binding of IL-18 to COS-1 cells transfected with hIL-1Rrp cDNA. Human IL-1Rrp cDNA was amplified by RT-PCR using L428 cell mRNA as a template. The cDNA was cloned into the expression vector, pcDNA/Amp. COS-1 cells were transfected by electroporation with expression constructs and harvested after 72 h of incubation. Two × 10⁶ COS-1 cells transfected with IL-1Rrp cDNA were incubated for 1 h at 4 °C with various concentrations of [125I]-IL-18 as described under "Experimental Procedures." Non-specific binding was measured by the addition of 500 nM unlabeled rhIL-18. The specific binding was determined as the difference between total and nonspecific binding and plotted according to Scatchard coordinates. □, total binding; ●, specific binding; ○, nonspecific binding.

**FIG. 7.** NF-κB gel shift assay of COS-1 cells transfected with hIL-1Rrp cDNA. COS-1 cells were transfected with expression constructs and incubated at 37 for 2 days. The culture media were then replaced with serum-free RPMI 1640 and the cells were incubated for 18 h and subsequently stimulated (30 min, 10 ng/ml) with rhIL-18 or IL-1β as indicated. Nuclear extracts were prepared and incubated with 32P-labeled NF-κB oligonucleotides (5'-ACCAAGAGGGATTTCACCTAAATC-3') and electrophoresed. The arrow points to the position of the induced NF-κB complex with DNA.

**hIL-1Rrp in normal tissues.**

Although there are no resemblances between IL-18 and IL-1 concerning their target cells and biological actions, the amino acid sequence of IL-18 includes an IL-1 signature-like sequence (2). A comparison of the IL-18 and IL-1 chains showed a parsimonious alignment over the length of the respective sequences that comprise the 12 b-strands of the distinctive b-trefoil fold (15). Therefore, it was likely that there was little possibility that IL-18 and IL-1 share the same receptor. Indeed,
the results of binding assays and affinity cross-linking showed that IL-18 and IL-1β did not share the same receptor expressed by L428 cells (Figs. 2 and 3). In addition, using human Burkitt lymphoma Raji cells, which express IL-1RII (16), we also confirmed that IL-18 does not compete for the IL-1R expressed on Raji cells by binding assay (data not shown).

From the results of hIL-18R detection on L428 cells by affinity cross-linking, the molecular mass of the hIL-18R was estimated to be in the range of 60–100 kDa, in good agreement with the molecular size of purified hIL-18R determined after CBB staining as shown in Fig. 4. On the cell surface of L428 cells, there appears to be a single polypeptide of 60–100 kDa, which binds IL-18, and IL-18R remains in the monomer form after binding IL-18.

Recently, some cell surface molecules that showed homology to the amino acid sequence of IL-1RI have been cloned, namely the murine IL-1 receptor accessory protein (IL-1RaAc, 23% amino acid homology to IL-1RI), murine T1/ST2 (26% amino acid homology to IL-1RI), and IL-1Rrp (27% amino acid homology to IL-1RI) (14, 17, 18). Concerning murine T1/ST2, a putative ligand for T1/ST2 has been cloned (19, 20), and as regards IL-1RaAc, it has been reported that IL-1RaAc forms a complex with IL-1RI or IL-1RII to bind IL-1α or IL-1β, but not IL-1R antagonist (IL-1Ra) (17). As regards IL-1Rrp, IL-1Rrp cDNA has been cloned by RT-PCR methods using degenerated primers for conserved amino acid sequences in the IL-1RI sequence (14). Nevertheless, IL-1α does not bind IL-1Rrp, for which the ligand remained unknown to date. It has been reported that a chimeric receptor, in which the IL-1Rrp cytoplasmic domain was fused to the extracellular and transmembrane region of the IL-1 receptor, responded to IL-1 following transfection into COS-1 cells by activation of NF-κB and induction of IL-8 transcriptional promoter function (14). In this report, we ascertained that COS-1 cells transfected with expression vector containing the IL-1Rrp cDNA conferred not only IL-18 binding affinity but also the capacity of NF-κB activation. In addition, in experiments using the anti-hIL-18R mAb, this mAb inhibited the biological activities of IL-18 on normal stimulated human peripheral blood mononuclear cells, and the transfected COS cells were IL-18R-positive by FACS analysis.2 From these results, we conclude that a functional IL-18 receptor component is IL-1Rrp.

It has been reported that murine Th1 clones responded to IL-18 resulting in the augmentation of IFN-γ production and proliferation, whereas little or no proliferation in response to mL18 was observed in murine Th2 clones in which IL-1R was expressed (4, 16). Since amino acid sequences of the cytoplasmic region of IL-18R show especially high homology to those of IL-1R, the pattern of signal transduction by IL-18 would be expected to be similar to that induced by IL-1. Therefore, we think that the distribution of different receptor-bearing cells causes the differences observed between the biological effects of IL-18 and IL-1. In fact, in the case of murine Th clones, mRNA expressions of the IL-18R were observed in only the Th1 clones and not in Th2 clones as determined by RT-PCR methods (data not shown). These results indicate that IL-18R probably exists in Th1 type cells and not in Th2 type cells. In addition, from the results of binding assay on L428 cells using labeled IL-18 or labeled IL-1, IL-18R seems to be structurally related to IL-1R; however, IL-18R does not seem to be associated with IL-1R.

In short, we have demonstrated that 1) hIL-18R molecule has been purified from CHAPS extraction of L428 cells using a series of chromatographic methods; 2) internal amino acid sequences of the purified sample matched those of IL-1Rrp; and 3) when expressed in COS-1 cells, the cDNA of IL-1Rrp conferred IL-18 binding properties and the capacity of signal transduction. From these results, we conclude that IL-1Rrp is a functional receptor component of IL-18.

Acknowledgments—We thank Dr. M. Micallef for helpful discussions and suggestions.

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