



Expression and responsiveness of human interleukin-18 receptor (IL-18R) on hematopoietic cell lines

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Interleukin-18 (IL-18) is a new inflammatory cytokine sharing biological functions with IL-12. The human IL-18 receptor (IL-18R) was recently identified and was found to be expressed on normal peripheral blood lymphocytes. To further characterize IL-18R, we analyzed IL-18R expression using a series of human hematopoietic cell lines selected from various cell lineages. We found the IL-18R expression on cells of T and B lineages as expected from analysis on normal cells. The IL-18R expression, however, was found not to be restricted to any specific maturation stages of T and B cells. In addition, we detected IL-18R expression in myeloid, monocytoid, erythroid and megakaryocytic cell lines, indicating that normal counterparts of these cell lineages could express IL-18R and participate in *in vivo* reactions caused by IL-18. Biochemical studies showed that IL-18R proteins exist as heterogeneous molecules ranging from 60 to 110 kDa. Deglycosylation experiments indicated that the heterogeneity could not be explained only by a difference in glycosylation. We also found that tumor necrosis factor- α (TNF- α) modulated the IL-18R expression, which implies an important *in vivo* effect of TNF- α on IL-18-induced reaction. Analyzing the responsiveness of IL-18R, we found that only KG-1 responded to IL-18 stimulation. This suggests that certain inhibitory mechanisms of IL-18 responsive genes are involved in the all IL-18R-positive cell lines except KG-1. *Leukemia* (2000) 14, 1052–1059.

Keywords: IL-18 receptor; expression; deglycosylation; KG-1; TNF- α

Introduction

Interleukin-18 (IL-18) was originally identified as an interferon- γ (IFN- γ) inducing factor in mice suffering from endotoxin shock. The mouse and human cDNAs were then cloned from livers as novel cytokines.^{1,2} Most IL-18 functions, such as IFN- γ induction, and the activation of NK, NKT and Th1 cells, are shared by IL-12.^{2–5} Physiological functions of IL-18 were also observed in an IL-18 gene knockout mouse which showed impaired NK and Th1 cell activities.⁶ Involvement of IL-18 in pathological processes was also reported in autoimmune disease (diabetes⁷ and rheumatoid arthritis⁸) and infections.^{9–11} Elevated IL-18 was observed in some patients with leukemia, especially those with acute lymphoblastic leukemia (ALL) and chronic myelocytic leukemia (CML).¹²

The receptor for human IL-18 was recently identified as an already-reported orphaned receptor, IL-1Rrp (IL-1 receptor-related protein) by Torigoe *et al.*¹³ They purified the IL-18R protein from a Hodgkin's disease cell line, L-428, and determined its amino acid sequence after lectin- and antibody-based affinity chromatography. They clearly showed by cold target inhibition assay and transfectant experiments that IL-1Rrp specifically binds IL-18 but not IL-1 β . COS-1 cells transfected with the human IL-1Rrp gene were shown to transmit an IL-18 signal from the receptor to the nucleus via NF- κ B activation,

indicating that IL-1Rrp is a functional receptor. Using IL-1Rrp gene knockout mice, Hoshino *et al.*¹⁴ confirmed that mouse IL-1Rrp was also an IL-18-binding receptor and was essential for IL-18-mediated signaling events. They demonstrated that IL-18R^{-/-} mice had an *in vivo* defect in Th1 cell development as well as an impairment in NK cell activity, as is the case for IL-18-deficient mice. Kunikata *et al.*¹⁵ analyzed the IL-18R expression of human peripheral blood cells by two-color flow cytometry and found that most CD19⁺ B cells and a percentage of CD8⁺ T cells constitutively expressed IL-18R. They induced IL-18R expression on CD56⁺ NK cells and CD4⁺ T cells with IL-12 and/or PHA stimulation. In contrast to the human system, resting murine T cells did not express IL-18R.¹⁶ Only after stimulation with anti-CD3 and anti-CD28 mAb in the presence of IL-12, was IL-18R induced on CD4⁺T cells at marginal levels and on CD8⁺ cells at higher levels. It was also demonstrated that IL-18R was selectively and persistently expressed on Th1 cells but not Th2 cells using both long-term cultured clones and newly polarized cells.¹⁷ Thus, IL-18R serves as a cell surface marker for distinguishing Th1 from Th2 cells in the mouse system. Selective expression on Th1 cells is consistent with the selective biological effect of IL-18 on Th1 cells. In this study, we analyzed IL-18R expression by flow cytometry using a variety of human hematopoietic cell lines to see if there are any specific expression patterns in terms of cell lineage or maturation stage. Also, we examined the molecular nature of IL-18R protein and the responsiveness of IL-18R positive cell lines to IL-18 stimulation.

When evaluating IL-18 function, many investigators have paid special attention to the combination effect of IL-18 and IL-12 because of their remarkable synergy.^{18,19} This synergy, observed in a number of experimental systems, results in a strong induction of IFN- γ which eventually leads to an enhanced antitumoral^{20,21} or antimicrobial effect,²² prevention of allergic asthma,²³ and also severe toxicity. The mechanism of the synergy was mainly explained by induction or up-regulation of IL-18R with IL-12.²⁴ The ability of IL-12 to up-regulate IL-18R was observed in both T cells and B cells.²⁵ The effect of other cytokines on IL-18R expression has not been reported yet. In this study, we also examined the modulating effect of IL-12 and tumor necrosis factor- α (TNF- α) on IL-18R expression.

Materials and methods

Monoclonal anti-human IL-18R antibody

Clone #117-10C was used as an antibody for the detection of human IL-18R. This mAb was produced by Torigoe *et al.*¹³ and characterized by Kunikata *et al.*¹⁵ The isotype of this clone is IgM, κ . Biotinylated antibody was used for flow cytometric detection.

Human hematopoietic cell lines

Established cell lines derived from leukemia/lymphoma patients and *in vitro* transformed (with human T lymphotropic virus type I (HTLV-I) or Epstein–Barr virus (EBV) cell lines were maintained in our laboratory and used for IL-18R analyses. KARPAS-299 was kindly provided by Dr Hans G Drexler (DSMZ-German Collection of Microorganisms and Cell Cultures).²⁶ ED-S⁻ and ATL-16T^o were kindly provided by Dr Michiyuki Maeda (Kyoto University).²⁷ IZ-86 was kindly provided by Dr Eiji Tatsumi (Kobe University). SALT-3 was kindly provided by Dr Kimitaka Sagawa (Kurume University).²⁸ MAT was kindly provided by Dr Jun Okamura (Kyushu Cancer Center). KMS-18 was kindly provided by Dr Takemi Otsuki (Kawasaki Medical School).²⁹ All cell lines were grouped, by immunophenotype, into seven categories: T cell, B cell, myeloid cell, monocytoid cell, erythroid cell, megakaryocytic cell, and miscellaneous. RPMI-1640 (Nissui, Tokyo, Japan) supplemented with 5% heat-inactivated fetal calf serum (FCS, Gibco, Grand Island, NY, USA) was used for maintaining the cells.

Cytokines and enzyme-linked immunosorbent assay (ELISA)

Recombinant human IL-18 and human TNF- α were produced and purified by Hayashibara Biochemical Laboratories (Okayama, Japan). Recombinant human IL-12 was purchased from Genzyme Corporation (Cambridge, MA, USA). Cytokine levels in the culture supernatants were measured using ELISA kits for IFN- γ and IL-6 (Genzyme Corporation), and for granulocyte–macrophage colony-stimulating factor (GM-CSF, Endogen, Woburn, MA, USA) following the protocols recommended by the manufacturers.

Flow cytometry

The cells were incubated first with 5 μ g/ml of biotinylated anti-human IL-18R for 30 min, and then stained with phycoerythrin-conjugated streptavidin for another 30 min. All procedures were carried out at room temperature in the presence of 0.1% sodium azide. Fluorescent cells were analyzed by flow cytometry using an EPICS Profile II analyzer. Biotinylated mouse IgM antibody (PharMingen, San Diego, CA, USA) was used as an isotype-matched control antibody in some of the cases to rule out the possibility of non-specific staining.

Immunoblotting

Biochemical analysis of IL-18R was performed by an immunoblotting method modified from one described previously.³⁰ Cells growing in logarithmic phase were harvested and washed three times with phosphate-buffered saline, and then lysed for 15 min in an extraction buffer containing 0.1% NP-40, 50 mM Tris, pH 7.2, 0.15 M NaCl, 2 mM EDTA, 10 mM iodoacetamide, 0.02% NaN₃, and 1 mM phenylmethylsulfonyl fluoride. The lysates were centrifuged at 20000 g for 20 min at 4°C, and the supernatants were used for immunoblotting detection. The proteins were separated in 12% acrylamide gels under non-reducing conditions and transferred on to a nitrocellulose membrane in transfer buffer containing 25 mM Tris, 192 mM glycine and 20% methanol. After trans-

fer, the membrane was treated with Block Ace (Dainippon Pharmaceutical Company, Osaka, Japan) for 30 min to block non-specific binding and was then incubated with purified mAb #117-10C for 1 h at room temperature. After washing the membrane with a washing buffer containing 0.1% Tween 20 in 10% Block Ace solution, it was incubated for another hour with horseradish peroxidase-conjugated sheep anti-mouse immunoglobulin (Amersham, Piscataway, NJ, USA). Antigen–antibody complex on the membrane was visualized by enhanced chemiluminescence method using detection reagents (Amersham).

Deglycosylation by endoglycosidase F/N-glycosidase F

N-linked oligosaccharides were removed by a combination of enzymes of the endoglycosidase F/N-glycosidase F (Boehringer Mannheim, Indianapolis, IN, USA) according to the manufacturer's instructions. Briefly, cell lysates containing the IL-18R protein were first denatured by boiling for 5 min in the presence of SDS at a final concentration of 0.5% to increase the deglycosylation rate and were then treated with the glycosidases. Before adding the enzymes, an excess amount of NP-40 was added to the denatured samples to avoid inactivation of the enzyme by SDS. The deglycosylation reaction was performed overnight at 37°C by adding 0.1 units of the endoglycosidase F/N-glycosidase F to cell extracts corresponding to approximately 2×10^6 cells. The deglycosylated proteins of IL-18R were analyzed by immunoblotting as described above. The absence of contaminating protease activities in the endoglycosidase F/N-glycosidase F was guaranteed by the manufacturer in their specifications.

In vitro cytokine treatment

To analyze the modulation of IL-18R expression by cytokines, one million cells were incubated with 10 ng/ml of recombinant human IL-12 or with 100 Japan Reference Units per milliliter of recombinant human TNF- α in RPMI-1640 medium supplemented with 10% FCS and penicillin/streptomycin in 5% CO₂ at 37°C. After 24 h incubation, the cells were harvested and used for flow cytometric detection of IL-18R expression. Ligand responsiveness was monitored by treating the cells with recombinant human IL-18. One million cells suspended in 2 ml of RPMI-1640 medium containing 10% FCS were incubated with 100 ng/ml of IL-18 in a 24-well microtiter plate for 48 h at 37°C. The amount of IFN- γ , GM-CSF, and IL-6 in the supernatants was measured by ELISA.

Results

Flow cytometric detection of IL-18R

Hematopoietic cells and cell lines were selected from T, B, myeloid, monocytoid, erythroid, and megakaryocytic cell lineages to see if there were any specific patterns for IL-18R expression. Cell lines derived from Hodgkin's lymphoma were also included because IL-18R was originally characterized using this type of cell. Results of flow cytometry are listed in Table 1 showing the expression intensity in a semi-quantitative manner. Of 39 cell lines analyzed, 24 cell lines were positive for IL-18R expression and IL-18R-positive cell lines were found in all six lineages. In the case of T and B cells, both

Table 1 IL-18R expression in human hematopoietic cell lines

Cell lines	Intensity ^a	Origin
<i>T cells</i>		
ATL-16T ^o	+++	HTLV-I-T
SALT-3	+++	ATL
PEER	++	ALL
MOLT-13	++	ALL
MAT	++	LY
HUT-102	++	MF
MOLT-16	+	ALL
MT-1	+	ATL
KARPAS-299	+	LY
RPMI-8402	-	ALL
DND-41	-	ALL
MOLT-3	-	ALL
ALL-SIL	-	ALL
JURKAT	-	LY
ED-S ⁻	-	ATL
IZ-86	-	ATL
<i>B cells</i>		
Hair-M	++	HCL
JC-1	++	HCL
B278TO	++	EBV-T
B302	++	EBV-T
ARH-77	+	MM
Ri-1	+	LY
NALM-1	+	CML
NALM-6	+	ALL
RAMOS	-	BL
AL-1	-	BL
U-698-M	-	LY
BALM-13	-	ALL
BALM-14	-	ALL
KMS-18	-	MM
<i>Myeloid cells</i>		
KG-1	++	AML
MOLM-6	+	CML
<i>Monocytoid cells</i>		
U-937	+	LY
THP-1	-	AMOL
MOLM-14	-	AMOL
<i>Erythroid cells</i>		
HEL	+	EL
<i>Megakaryocytic cells</i>		
MOLM-1	+	CML
<i>Others</i>		
L-428	+++	HD
HDLM-2	++	HD

HTLV-I-T, human T lymphotropic virus type I-transformed; ATL, adult T cell leukemia; ALL, acute lymphoblastic leukemia; LY, lymphoma; MF, mycosis fungoides; HCL, hairy cell leukemia; EBV-T, Epstein-Barr virus-transformed; MM, multiple myeloma; BL, Burkitt's lymphoma; AML, acute myeloblastic leukemia; CML, chronic myeloid leukemia; AMOL, acute myelomonoblastic leukemia; EL, erythroleukemia; HD, Hodgkin's disease.

^aIntensity of IL-18R expression was graded as from negative (-) to very strong (+++).

mature cells (ATL-16T^o, SALT-3, Hair-M and JC-1) and less mature cells (MOLT-13, PEER, MOLT-16, NALM-1 and NALM-6) expressed IL-18R. Relatively high expression of IL-18R was detected in certain types of cell lines, such as HTLV-I-associated cells (ATL-16T^o and SALT-3), hairy cell leukemia-derived cells (Hair-M and JC-1), EBV-transformed cells

(B270TO and B320), and Hodgkin's lymphoma-derived cells (L-428 and HDLM-2). Histograms of flow cytometry are shown as representative positive and negative cell lines (Figure 1).

Detection of human IL-18R protein by immunoblotting

Human IL-18R expression was further analyzed by immunoblotting method (Figure 2). Human IL-18R proteins were detected as heterogeneous bands at a molecular size of 60–110 kDa. These bands were specific for IL-18R because the secondary antibody alone did not reveal them, as shown in L-428. There are two major bands around 70 kDa and 90 kDa in most of the cell lines, and some minor bands were also evident in L-428 and ATL-16T^o. As an exception, MOLT-16 had bands of a higher molecular weight than all other cell lines. Their molecular weights were between 100 and 110 kDa. SALT-3 contained the highest amount of IL-18R protein when the same cell numbers were loaded, while MOLT-16 showed a relatively low amount. Under reducing conditions, these bands became less detectable by immunoblotting, indicating that the epitope detected by mAb #117-10C was sensitive to disulfide cleavage.

Deglycosylation of human IL-18R protein

We further analyzed the molecular nature of IL-18R by deglycosylation treatment to address questions about heterogeneity indicated after the immunoblotting. We selected L-428, SALT-3 and MOLT-16 as representative cell lines, and treated their extracts with the deglycosylation enzymes, endoglycosidase F and N-glycosidase F. These enzymes can cleave N-linked oligosaccharide residues from glycoproteins. As shown in Figure 3, protein bands of L-428 and SALT-3 between 70 and 90 kDa as well as those of MOLT-16 located at between 100 and 110 kDa were shifted to lower molecular weight bands after the deglycosylation. The bands at position I corresponding to about 60 kDa have the same molecular size estimated from the amino acid sequence of the mature and non-glycosylated form of IL-18R protein. The other lower bands, designated II, III and IV, are at approximately 53, 40 and 35 kDa, respectively. Therefore, even after deglycosylation IL-18R proteins remained heterogeneous.

Up-regulation of human IL-18R with cytokine treatment

We next investigated the modulating effects of cytokines on human IL-18R expression. IL-12 has been shown to up-regulate the IL-18R expression of murine Th1 cells as well as B cells. TNF- α was also used for this analysis because preliminary IFN- γ induction experiments suggested the involvement of TNF- α in IL-18R modulation. The results are shown in Figure 4. IL-12 treatment did not change the IL-18R expression of any cell lines tested either upward or downward. In contrast to this, TNF- α treatment revealed modulating effects on some cell lines. The IL-18R expression of MOLT-16, PEER and KG-1 was up-regulated by TNF- α , while that of U-937 was down-regulated. Modulated expressions in MOLT-16, PEER, KG-1 and U-937 were obvious and reproducible. TNF- α had almost no effect on the IL-18R expression of L-428, HDLM-2, Hair-

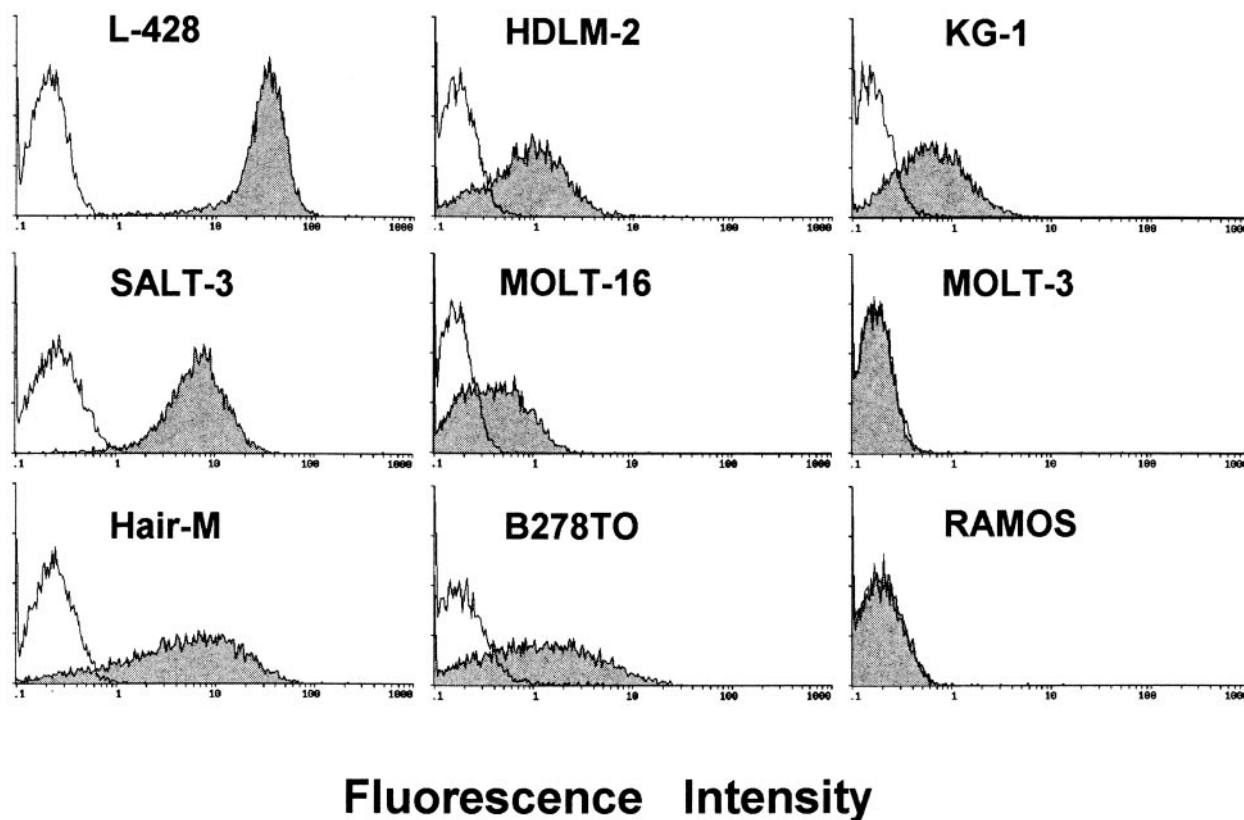


Figure 1 Expression of human IL-18R on hematopoietic cell lines detected by flow cytometry. Cells were stained first with biotin-conjugated anti-human IL-18R antibody (clone #117-10C, IgM) and then with phycoerythrin-conjugated streptavidin. Shaded histograms and open histograms represent staining with or without an anti-human IL-18R antibody, respectively. Note that the cell lines showed a certain degree of human IL-18R expression varying from very strong (eg L-428), to moderate (eg MOLT-16), to negative (eg MOLT-3 and RAMOS). The lineages and origins of the cell lines are described in Table 1.

M and a receptor negative cell line, MOLT-3. In these treatments, TNF- α had growth inhibitory effects of MOLT-16, KG-1 and U-937, but not on PEER or Hair-M. IL-12 had no growth effects on any cell line used in this experiment.

Responsiveness of human IL-18R-positive cell lines to IL-18

To determine whether human IL-18R is functionally reactive, we treated human IL-18R-positive cells with IL-18 and measured the cytokine inducing ability. Among the various lineages, only KG-1 responded to IL-18 stimulation (Figure 5). In the absence of IL-18, KG-1 did not produce any IFN- γ , GM-CSF or IL-6. With 100 ng/ml of IL-18 stimulation, KG-1 produced 873 IU/ml of IFN- γ , 241 pg/ml of GM-CSF and 147 pg/ml of IL-6 after 48 h culture. None of the other cell lines tested (a total of 23 cell lines) responded at all to IL-18 treatment. Even cell lines with higher IL-18R expression such as L-428 and SALT-3 did not produce IFN- γ and did not show enhanced production of GM-CSF or IL-6.

Discussion

The characterization of IL-18R is a key to understanding the physiological and pathological functions of IL-18. Identification of IL-18R-positive cells is useful in analyzing IL-18 responsiveness in certain cell populations *in vivo*, and mol-

ecular detection of IL-18R is necessary for the study of signal transduction mechanisms. In the present study, we investigated the expression and biochemical nature of IL-18R as well as the responsiveness of IL-18R-positive cells to IL-18 stimulation using a large panel of hematopoietic cell lines.

Using flow cytometric analyses, we found 24 cell lines were positive for IL-18R out of 39 cell lines tested. The distribution pattern of IL-18R expression showed that IL-18R was expressed in a non-lineage-specific manner and independently of the stage of maturation at all cell line levels. Certain types of cell lines, such as HTLV-I-associated, hairy cell leukemia-derived, EBV-transformed and Hodgkin's disease-derived cell lines, were found to express high levels of IL-18R. The limited number of cell lines tested does not allow for generalization, but it is tempting to speculate on their association with the high level of IL-18R expression.

As for a comparison with normal hematopoietic cells, there is a report on analyses of expression by human peripheral blood lymphocytes by Kunikata *et al*.¹⁵ They showed that without stimulation, a large proportion (82%) of CD19⁺ B cells, the majority (68%) of CD8⁺ T cells, and 40% of CD56⁺ NK cells expressed IL-18R while only a small proportion (15%) of CD4⁺ T cells did so. The results of our study seem to support their data as regards T cell and B cell IL-18R expression. In particular, mature B cell expression confirmed by our analyses on cell lines would provide a useful tool to evaluate the role of B cells in IFN- γ production induced by IL-18/IL-12 treatment.¹⁸ In the mouse system, preferential responsiveness to IL-18 stimulation and selective expression

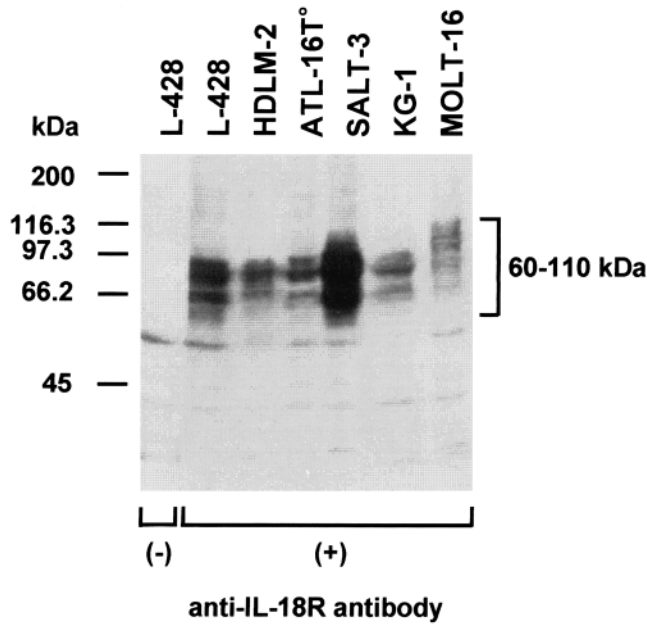


Figure 2 Immunoblotting detection of human IL-18R. Lysates were prepared from each cell line using NP-40 extraction buffer as described in Materials and methods. The proteins corresponding to 1×10^6 cells were loaded on to respective lanes and separated by SDS-PAGE under non-reducing conditions. Human IL-18R proteins blotted on to a nitrocellulose membrane were incubated with anti-human IL-18R antibody. Antibodies bound to the membrane were visualized by enhanced chemiluminescent methods using horseradish peroxidase-conjugated anti-mouse immunoglobulin. The far left lane was incubated without anti-human IL-18R antibody and showed non-specific reaction, whereas all other lanes had the antibody. Note that specific bands were detected between 60 and 110 kDa. The positions of standard molecular weight markers are shown on the left side.

of IL-18R has been reported for Th1 cells compared with Th2 cells.¹⁷ We could not address this preferential expression in human cell lines, since definite classification of Th1/Th2 cell types cannot be applied to human leukemia T cell lines. IL-18R expression by non-lymphoid cells has not been thoroughly investigated in normal cells. There is only one report showing the possibility that normal macrophages express IL-18R.¹⁹ Therefore, our findings of IL-18R expression in myeloid, monocytoid, erythroid and megakaryocytic cell lines are interesting because it indicates that normal counterparts of these lineages may also express IL-18R and participate in *in vivo* reactions caused by IL-18.

Our results of IL-18R expression by myeloid and monocytoid cell lines suggest another interesting possibility. Since myeloid and monocytoid cells are potential sources of the IL-18 ligand, the expression of both receptor and ligand in the same cells may result in autocrine proliferation or activation. In the case of leukemia patients, some of them show elevated level of IL-18 as reported by us.¹² Therefore, it would be interesting to compare the relationship between IL-18R expression in patients with leukemia, especially myeloid leukemia patients, with serum IL-18 levels to determine the possibility of autocrine stimulation.

We demonstrated that the IL-18R protein exists as a set of quite heterogeneous molecules ranging from 60 to 110 kDa. Most of the cell lines tested exhibited two major bands of approximately 70 and 90 kDa, while MOLT-16 produced higher molecular weight bands at 100–110 kDa. One possibility for the heterogeneity was the heavy and varying extent

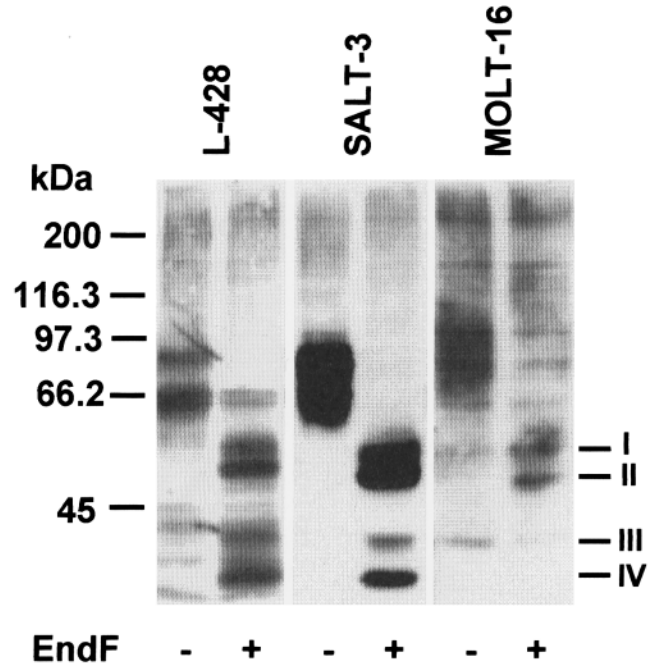


Figure 3 Deglycosylation of human IL-18R proteins. Cell extracts containing IL-18R proteins were treated with the endoglycosidase F/N-glycosidase F after denaturing the samples as described in Materials and methods. The deglycosylated IL-18R proteins corresponding to 2×10^6 cells prepared from L-428, SALT-3 and MOLT-16 were separated under non-reducing conditions by SDS-PAGE and detected by immunoblotting using the chemiluminescence method. Exposure time of chemiluminescence detection varies among the different cell lines. Protein samples treated with the enzymes or left untreated are shown in parallel. The positions of standard molecular weight markers are shown on the left side. The bands detected in the deglycosylated samples are shown by arrowheads and designated as bands I to IV.

of glycosilation of the protein. Therefore, we tested this possibility by removing sugar chains from the IL-18R of three cell lines including MOLT-16. Even after deglycosilation treatment the IL-18R proteins were detected as a set of heterogeneous molecules, indicating that the heterogeneity cannot be explained by glycosilation alone. In that experiment, we identified some bands with molecular weights much lower than expected. The polypeptide mass of mature IL-18R deduced from the cDNA is 60 kDa,¹³ but the molecules detected in L-428, SALT-3 and MOLT-16 are at 53, 40 and 35 kDa. It is unlikely that the smaller bands detected after deglycosilation are artifacts produced by peptidases in the original cell preparation, because no such bands were detected in the control samples untreated with enzyme. Possible contamination of peptidases in the endoglycosidases preparation can be ruled out when one considers the manufacturer's specifications. Also, cross-reactivity of the anti-IL-18R antibody does not seem to be the reason for the heterogeneity because the IL-18R proteins prepared from COS-1 transfected cells showed similar heterogeneity (data not shown). Therefore, we performed RT-PCR with a various combination of primers to investigate the possible existence of alternate forms of IL-18R at the RNA level. As far as we could detect, there were no PCR bands implying the existence of an alternative form of IL-18R even in MOLT-16 (data not shown). Further analysis at the protein level is required to address the heterogeneity issues.

Another interesting observation after immunoblotting is that

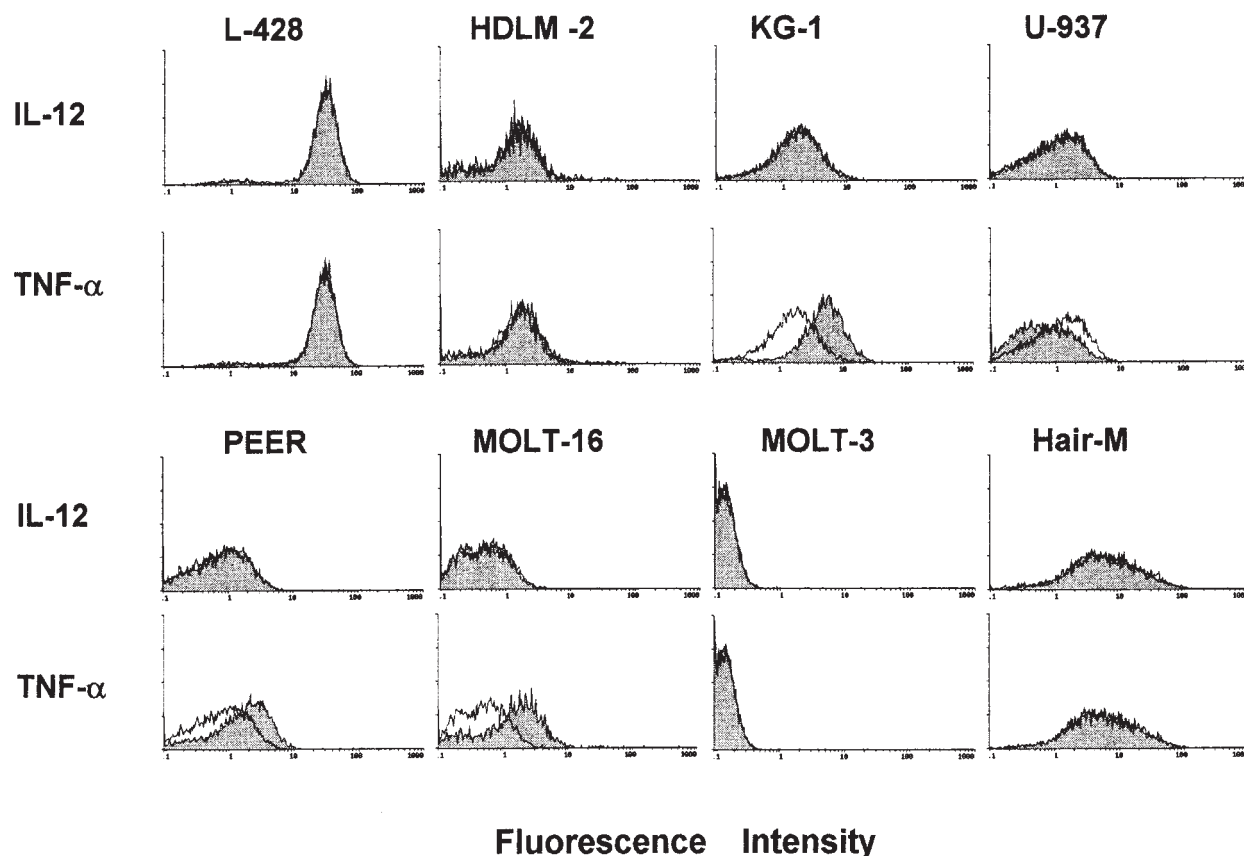


Figure 4 Effects of cytokine treatment on human IL-18R expression. Cell lines were cultured in the presence of IL-12 (10 ng/ml) or TNF- α (100 Japan reference unit per milliliter) for 24 h. IL-18R expression was detected with anti-IL-18R antibody. The IL-18R expression of cytokine-treated cells (shaded histograms) was compared with that of untreated cells (open histograms).

SALT-3 apparently contains far more IL-18R protein than the other cell lines with high expression, L-428 and ATL-16T $^{\circ}$. When the total protein amount was adjusted to the same level for each lane, SALT-3 still revealed much more IL-18R protein than the other cell lines (data not shown). One possible explanation is that SALT-3 may contain more IL-18R proteins intracellularly than the other cell lines.

IL-12 has been documented as a strong synergistic factor for many IL-18 functions, and the mechanism of synergy was partly explained by up-regulation of IL-18R expression with IL-12.²⁴ The up-regulation of IL-18R by IL-12 was clearly demonstrated in murine Th1 cell lines²⁴ and human peripheral T and NK cells.¹⁵ However, in our experiment human hematopoietic cell lines did not respond to IL-12 treatment at all for IL-18R modulation. Non-responsiveness to IL-12 was observed among almost all cell types. This is probably explained by the lack of or lower level of IL-12 receptor expression among the human hematopoietic cell lines tested. In contrast to IL-12, TNF- α was effective in modulating IL-18R expression. TNF- α up-regulated IL-18R expression in three cell lines and down-regulated it in one cell line. Modulation of the effects of TNF- α was accompanied by growth inhibition in three cell lines. This finding suggests that TNF- α may control IL-18 responsiveness *in vivo* in inflammatory reactions, positively or negatively.

Among several IL-18R-positive cell lines, only KG-1 showed the ability to produce IFN- γ , GM-CSF and IL-6 upon IL-18 stimulation. None of the other IL-18R-positive cell lines was responsive to IL-18 for cytokine production. The peculiarity

of KG-1 as an IL-18 responsive cell line was utilized in bioassays of human and mouse IL-18.³¹ At the same time, these results imply that IL-18R cannot transmit a signal from receptor to nucleus in non-responder cell lines. Recently, a novel member of the IL-1 receptor family, designated AcPL, was cloned by Born *et al*³² after searching the expression sequence tag database. They showed that both AcPL and IL-18R (IL-1R β) are required for induction of NF- κ B activation in response to IL-18, and that the dominant negative version of AcPL specifically inhibited IL-18 signaling, implicating AcPL as an essential component of functional IL-18R complex. Since we speculated that the non-responsiveness of IL-18-stimulated cytokine production is due to a lack of AcPL expression, we analyzed the mRNA expression of AcPL by the RT-PCR method. However, we detected AcPL messages in both IL-18 responder (KG-1) and non-responder (such as L-428, SALT-3 and KARPAS-299) cell lines (data not shown). Moreover, it was reported that transcription factor NF- κ B activation was induced by IL-18 stimulation in L-428.¹³ Therefore, non-responsiveness to IL-18 stimulation could be explained by impairment at the promoter activation/suppression level in L-428 and probably in some other non-responder cell lines.

In conclusion, we demonstrated a wide distribution of IL-18R among a variety of hematopoietic cell lineages, and a molecular heterogeneity of the IL-18R proteins. We also presented evidence that TNF- α had modulating effects on IL-18R expression and that none of the IL-18R-positive cell lines except KG-1 responded to IL-18 by producing cytokines. These findings help clarify some of the characteristics of IL-

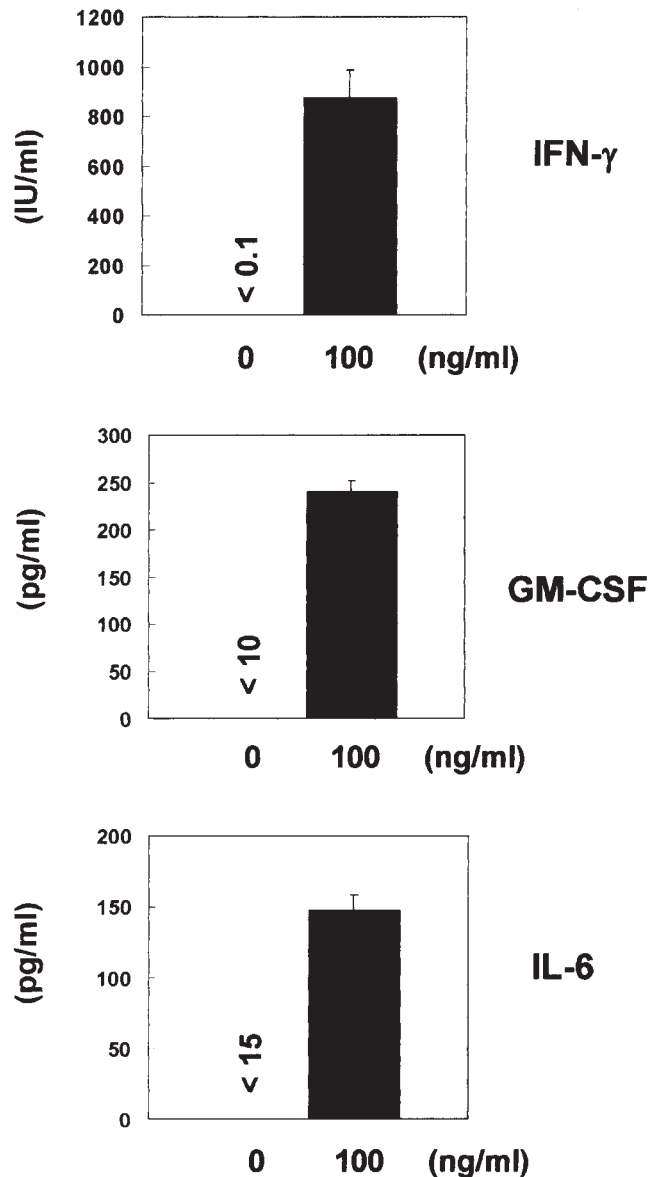


Figure 5 Responsiveness of KG-1 to the IL-18 treatment. All the IL-18R-positive cell lines (total 24 cell lines) were analyzed for their cytokine production abilities in response to the treatment of IL-18. The cell lines were incubated with 100 ng/ml of IL-18 in a 24-well microtiter plate for 48 h at 37°C. The amount of IFN- γ , GM-CSF and IL-6 in the supernatants was measured by ELISA. Since only KG-1 responded to IL-18 treatment to produce these cytokines and none of the other cell lines (23 cell lines) showed any responsiveness, only the results of KG-1 are shown here. Without treatment, KG-1 did not spontaneously produce any of the cytokines tested. Detection limits for IFN- γ , GM-CSF and IL-6 are <math>< 0.1</math> units/ml, <math>< 10</math> pg/ml, and <math>< 15</math> pg/ml, respectively. Data are expressed as means \pm s.d.

18R as well as the functions of IL-18 in physiological and pathological processes.

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