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Preferential Production of Interleukin-1β over Interleukin-1 Receptor Antagonist Contributes to Proliferation and Suppression of Apoptosis in Leukemic Cells

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Normal human monocytes were isolated in a nascent state by centrifugal elutriation and used for the study of interleukin-1 (IL-1) and interleukin-1 receptor antagonist (IL-1ra) expression. Neither IL-1β nor IL-1ra mRNA was present in monocytes just after the isolation, but they were induced simultaneously in response to various stimulants. In contrast, only IL-1β mRNA was expressed in monocytic leukemia cell line JOSK-1, while little or no IL-1ra mRNA was detected even after stimulation. Dominant expression of IL-1β over IL-1ra was also observed in fresh leukemia cells including monocytic leukemias, i.e., IL-1β mRNA was constitutively expressed in 26 out of 36 cases (72.2%), whereas IL-1ra mRNA was present only in 8 cases (22.2%). The signal intensity of IL-1β mRNA was stronger than that of IL-1ra even in IL-1ra-positive cases. Apoptotic cell death of monocytes was significantly inhibited by IL-1β, and it was enhanced by IL-1ra. In fresh leukemia cells, ^{3}H-thymidine uptake was generally higher in IL-1-producing cases than in IL-1ra-producing cases, and was increased by the addition of IL-1β in all cases tested. Cell proliferation was inhibited by either IL-1ra or anti-IL-1β antibody in IL-1-producing cases, while it was enhanced by anti-IL-1ra antibody in IL-1ra-producing cases. These results suggest that the balance between IL-1 and IL-1ra is necessary for homeostasis of the mononuclear phagocytosis system. The imbalance between these two counter-acting cytokines might contribute to the altered growth and accumulation of leukemic cells.

Key words: Interleukin-1β — Interleukin-1 receptor antagonist — Monocyte — Leukemia — Apoptosis

Monocytes play a pivotal role in host defense against infection and tumors, and are a major source of inflammatory cytokines and growth factors.[1,2] Among monocyte-derived cytokines, interleukin-1 (IL-1) plays the most important role and is believed to mediate various monocyte-specific biological activities.[3] IL-1 mediates fever, neutrophilia, acute-phase response and production of cytokines by T-lymphocytes.[4] Recently, a specific inhibitor of IL-1 has been identified in adherent immunoglobulin-stimulated monocytes and its cDNA has been cloned.[5–7] This inhibitor is structurally related to both IL-1α and IL-1β, competitively blocks the binding of IL-1 to its receptors and, thus, has been named IL-1 receptor antagonist (IL-1ra).[8] Administration of IL-1ra has been shown to reduce the severity of IL-1-induced pathologic conditions such as sepsis, arthritis, colitis, type I diabetes and graft versus host disease.[9,10]

Recent evidence suggests that the balance between the amount and secretion of IL-1 and IL-1ra may represent a homeostatic mechanism that limits the severity of these conditions. For example, IL-1ra showed a dramatic increase following the induction of IL-1β during experimental endotoxemia[11] or immune complex colitis.[12] This is consistent with the idea that IL-1ra acts as an endogenous mediator of the negative feedback mechanism that down-regulates the proinflammatory activity of IL-1 by restoring the balance between these counter-acting monokines. In this context, it is reasonable to speculate that loss of balance between IL-1 and IL-1ra might result in pathologic states. Recently, an imbalance of IL-1 and IL-1ra synthesis has been postulated to contribute to the pathogenesis of many disorders, including rheumatoid arthritis,[13] Lyme disease,[14] Hodgkin's disease[15] and psoriasis.[16]

With this background in mind, we examined the production of IL-1β and IL-1ra in normal monocytes and leukemic cells, and studied the effects of these factors on growth and survival of monocytes and leukemic cells in order to investigate the roles of IL-1 and IL-1ra in the biology of monocytes and the pathogenesis of leukemia.

MATERIALS AND METHODS

Cell preparation and culture Normal human monocytes were purified from the peripheral blood of healthy volunteers by centrifugal elutriation in a Hitachi SRR6Y rotor (Hitachi Koki Co., Ltd., Tokyo).[17] The purity of the monocyte specimen was approximately 90% on the basis

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of morphological examination. Fresh leukemia cells were isolated from patients with acute myeloblastic leukemia (AML) and chronic myelomonocytic leukemia (CMML) as previously described.[18] Only samples containing more than 90% blasts were used in this study. All samples were obtained after informed consent had been obtained, and under institutional review board-approved protocols.

Human monocytic leukemia cell line JOSK-I was established in our laboratory and maintained as previously described.[19]

Monocytes were cultured at $2-5\times10^6$ cells/ml for given culture periods in Dulbecco's modified Eagle's medium (Gibco, Grand Island, NY). Autologous serum (0.2%) was added for long-term culture (up to 72 h). To avoid activation by plastic adherence, monocytes were cultured in polypropylene tubes (Falcon2059) unless otherwise indicated.

Fresh leukemic cells were cultured at 5×10^5 cells/ml for 72 h in RPMI-1640 medium (Flow Laboratories, McLean, VA) containing 10% heat-inactivated fetal calf serum (FCS) (Commonwealth Serum Laboratories, Melbourne, Australia) with factors or antibodies as indicated. DNA synthesis was monitored in terms of ^{3}H-thymidine uptake with a pulse-labeling time of 16 h.[20]

Factors and reagents Recombinant human IL-1β, recombinant human IL-1ra and goat anti-human IL-1ra antibody were purchased from R&D Systems (Minneapolis, MN). Anti-human IL-1β monoclonal antibody was obtained from Genzyme (Cambridge, MA). Lipopolysaccharide (LPS), immunoglobulin G (IgG) and 12-O-tetradecanoylphorbol-13-acetate (TPA) were purchased from Sigma (St. Louis, MO).

DNA clones The following cDNA clones were used in this study: a 1.0 kb *Pst*I fragment of human IL-1β cDNA (provided by Dr. Hiroyo Matsui, Ajinomoto Bioscience Institute, Yokohama) and a 1.8 kb *Hin*dIII fragment of human β-actin cDNA (provided by Dr. Kiyoshi Nose, Institute of Medical Science, University of Tokyo, Tokyo). A 423 bp fragment of IL-1ra cDNA corresponding to nucleotides 102 to 524 was generated by reverse transcription-polymerase chain reaction based on the published sequence.[5–7]

Northern blotting Total cellular RNA was isolated by cesium chloride centrifugation after lysing the cells in 4 *M* guanidium isothiocyanate solution. Ten-microgram samples were electrophoresed in a 1.0% formaldehyde-containing agarose gel and blotted onto a synthetic nylon membrane as described.[20] The membranes were hybridized with each cDNA probe, which had been labeled with ^{32}P-dCTP (Du Pont-New England Nuclear) by the oligonucleotide random priming method. To monitor the equal loading of RNA, the membranes were rehybridized with β-actin probe.

Western blotting Cell lysate was prepared as previously described.[20] The samples were electrophoresed in a 12% SDS-polyacrylamide gel and transferred onto a nitrocellulose membrane (TEF Co., Nagano). The membranes were incubated overnight in either 10 μg/ml of anti-human IL-1β monoclonal antibody or 1 μg/ml of goat anti-human IL-1ra antibody after blocking with 4% bovine serum albumin (fraction V, Sigma) in tris-buffered saline for 1 h.[21] The membranes were developed with nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate after 2 h incubation with 1:7500 dilution of anti-rabbit IgG conjugated with alkaline phosphatase (Promega, Madison, WI) or 1:2500 dilution of anti-goat IgG conjugated with alkaline phosphatase (Chemicon International, Temecula, CA).

Enzyme-linked immunosorbent assay IL-1β and IL-1ra concentrations in the conditioned medium were determined by using enzyme-linked immunosorbent assay kits (R&D Systems) according to the manufacturer's instruction.

Analysis of DNA fragmentation in agarose gels After incubation of $1-5\times10^6$ cells at 50°C for 1 h in 100 μl of DNA isolation buffer (10 m*M* EDTA, 50 m*M* Tris-HCl, pH 8, 0.5% sarcosyl) containing 0.5 mg/ml proteinase K (Sigma), RNase was added to a final concentration of 0.15 mg/ml. DNA was extracted with 1 volume of phenol, and then twice with 1 volume of chloroform: isoamyl alcohol. The aqueous phase was adjusted to 0.5 *M* NaCl and precipitated with two volumes of ethanol at −20°C overnight. The pellet was dried and resuspended in TE buffer. Horizontal electrophoresis of DNA was performed for 2 h at 50 V in a 1.0% agarose gel in TBE buffer (90 m*M* Tris, 90 m*M* boric acid, 2 m*M* EDTA, pH 8.0) using a mini-electrophoresis system, MUPID-2 (Cosmo Bio Co., Tokyo). DNA samples (1–2 μg) were loaded in wells. A 1 kb-DNA ladder (Gibco) was used as a molecular weight marker. DNA was visualized by ethidium bromide staining after the electrophoresis.

Statistical analysis The significance of differences was determined by using Student's *t* test and the chi-square test.

RESULTS

Effects of various reagents on IL-1β and IL-1ra mRNA expression in normal monocytes Regulation of IL-1β and IL-1ra mRNA expression was investigated in nascent human monocytes isolated by centrifugal elutriation, since monocytes are a major source of these two counter-acting cytokines.[21, 22] As shown in Fig. 1, monocytes expressed neither IL-1β nor IL-1ra mRNA just after isolation (T-0). Monocytes did not express either of the messages when the cells were cultured without FCS in polypropylene tubes (non-adherent condition) for 6 h. FCS (10%), LPS (10 μg/ml), non-adherent IgG (25

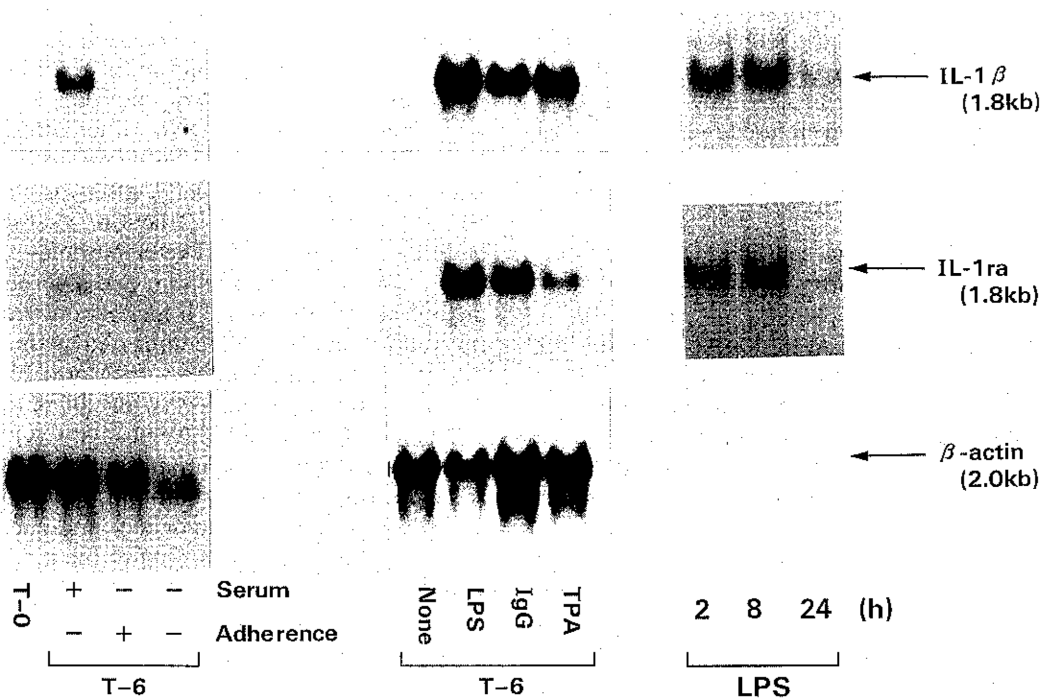


Fig. 1. Effects of various reagents on IL-1β and IL-1ra mRNA expression in monocytes. Monocytes were cultured for 2–24 h in the absence or presence of either FCS (10%), LPS (10 μg/ml), IgG (25 μg/ml) or TPA (4 n*M*) in polypropylene tubes. For plastic adherence (Adherence +), the cells were cultured in tissue-culture dishes. Monocytes were subjected to RNA isolation without culture for the T-0 specimen. IL-1β and IL-1ra mRNA expression was examined by Northern blotting.

μg/ml) and TPA (4 n*M*) could induce both IL-1β and IL-1ra mRNA without plastic adherence after 6 h culture. The time-course of the induction of IL-1β and IL-1ra mRNA was investigated in LPS-stimulated monocytes. Both messages were apparently induced after 2 h of stimulation, reached the maximal level at 6–8 h and were down-regulated after 24 h. These results indicate that production of IL-1β and IL-1ra is balanced in normal human monocytes, although the ratio of the intensities of induced messages is variable according to the stimulant.

Production of IL-1β and IL-1ra proteins by normal monocytes Production of IL-1β and IL-1ra by monocytes was confirmed at the level of gene products by Western blotting. Monocytes were cultured in the absence or presence of various stimulants in polypropylene tubes and harvested after 24 h. Intracellular IL-1β and IL-1ra proteins were detected with monospecific antibodies as previously described.[21] As shown in Fig. 2, a 34 kDa band corresponding to proIL-1β and a 22/21 kDa doublet of IL-1ra protein were readily detected in monocytes cultured for 24 h without any stimulant, although little or none of the corresponding mRNAs was detected by Northern blotting. This discrepancy has already been reported by others.[22] The amount of proIL-1β was increased by the additions of LPS, IgG and TPA, and that of IL-1ra was also increased by LPS and IgG (Fig. 2). Extracellular IL-1β and IL-1ra were simultaneously measured by ELISA. As shown in Table I, the amounts of both cytokines were significantly increased in the conditioned medium of monocytes treated with either LPS, IgG or TPA.

Effects of IL-1β and IL-1ra on survival and apoptosis of cultured monocytes Monocytes were cultured with 0.2% autologous serum in polypropylene tubes and harvested after 24, 48 and 72 h for isolation of DNA. Viability of the cells declined to approximately 80% after 72 h. Typical DNA fragmentation was observed after 24 h (Fig. 3A, Control), indicating that monocytes undergo apoptosis in the absence of appropriate stimuli. Apoptotic cell death was significantly inhibited when IL-1β was added to the culture at the concentration of 1 n*M* (Fig. 3A, IL-1β), and the subsequent experiments showed that as little as 0.1 n*M* could inhibit apoptosis (data not shown). Specificity of the effect was confirmed by abrogation of the effect with IL-1ra or anti-IL-1β neutralizing antibody (data not shown). In contrast, IL-1ra enhanced DNA fragmentation of cultured monocytes (Fig. 3B). This acceleration of apoptosis was also shown to be

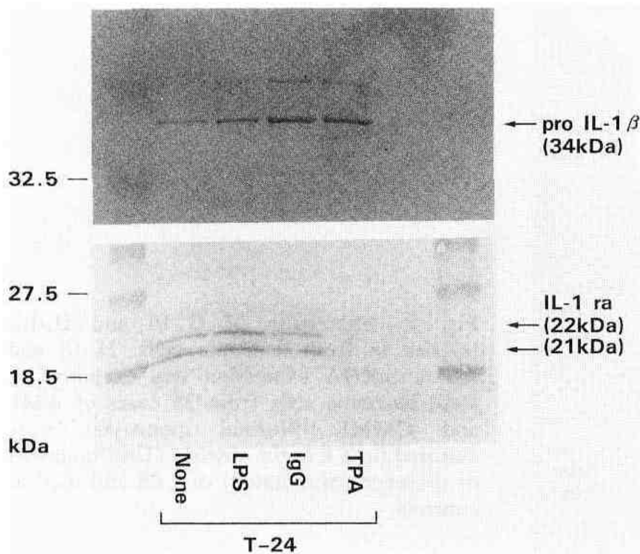


Fig. 2. Production of IL-1β and IL-1ra proteins by monocytes. Monocytes were cultured for 24 h in the absence (None) or presence of LPS (10 μg/ml), IgG (25 μg/ml) or TPA (4 n*M*) in polypropylene tubes. Intracellular IL-1β and IL-1ra were detected by Western blotting using specific antibodies. The positions of the molecular size markers are indicated on the left side.

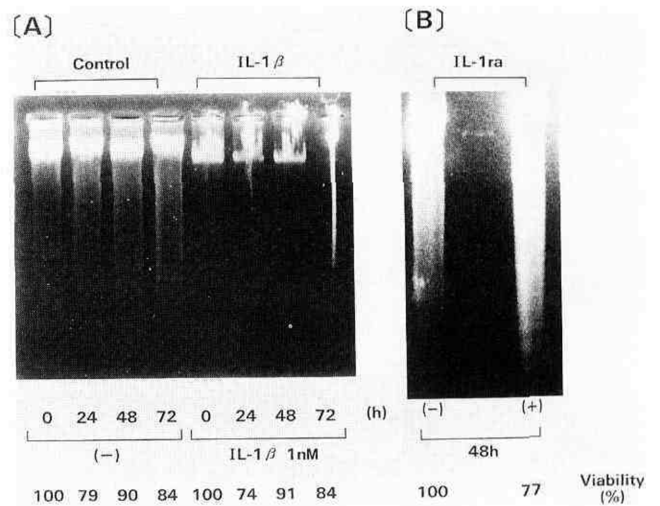


Fig. 3. Effects of IL-1β and IL-1ra on apoptosis of cultured monocytes. Monocytes were cultured in the absence (Control) or presence of either (A) IL-1β (10 ng/ml) or (B) IL-1ra (500 ng/ml) with 0.2% autologous serum in polypropylene tubes. Cells were harvested at given time points for DNA isolation. DNA was visualized by ethidium bromide staining after 1.0% agarose gel electrophoresis. Cell viability was determined by the erythrosin B dye exclusion test.

Table I. IL-1β and IL-1ra Concentrations in Monocytes Conditioned Medium

Stimulant	IL-1β (ng/ml)[a]	IL-1ra (ng/ml)[a]
None	0.9±0.1	0.3±0.1
LPS	8.8±1.2	7.5±1.1
IgG	3.2±0.8	13.4±2.1
TPA	4.7±0.9	4.2±1.2

a) Monocytes were cultured in the absence (None) or presence of LPS (10 μg/ml), IgG (25 μg/ml) or TPA (4 n*M*), and the supernatants were collected after 24 h. IL-1β and IL-1ra concentrations were determined by enzyme-linked immunosorbent assay.

specific for IL-1ra by abrogation of the effect with IL-1β or anti-IL-1ra neutralizing antibody (data not shown). These results indicate that survival of monocytes is regulated by these cytokines in an autocrine or paracrine fashion.

Expression of IL-1β and IL-1ra mRNA in monocytic leukemia cell line JOSK-I Human monocytic leukemia cell line JOSK-I was established in our laboratory.[19] Subsequent studies revealed that this cell line could produce IL-1 constitutively and proliferate in response to IL-1.[23, 24] Expression of IL-1β and IL-1ra mRNA was examined in JOSK-I cells by Northern blotting. As

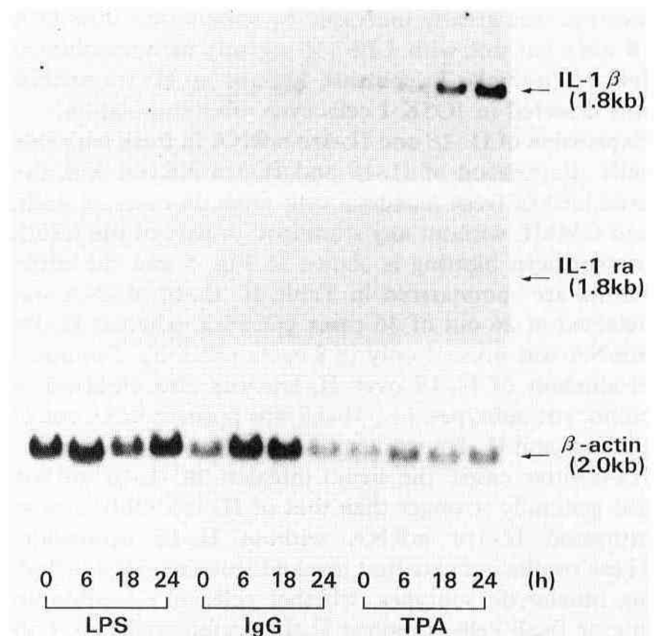


Fig. 4. Expression of IL-1β and IL-1ra mRNA in monocytic leukemia cell line JOSK-I. JOSK-I cells were cultured in the presence of LPS, IgG or TPA up to 24 h. RNA was isolated at given time points and subjected to Northern blot analysis to evaluate IL-1β and IL-1ra mRNA expression.

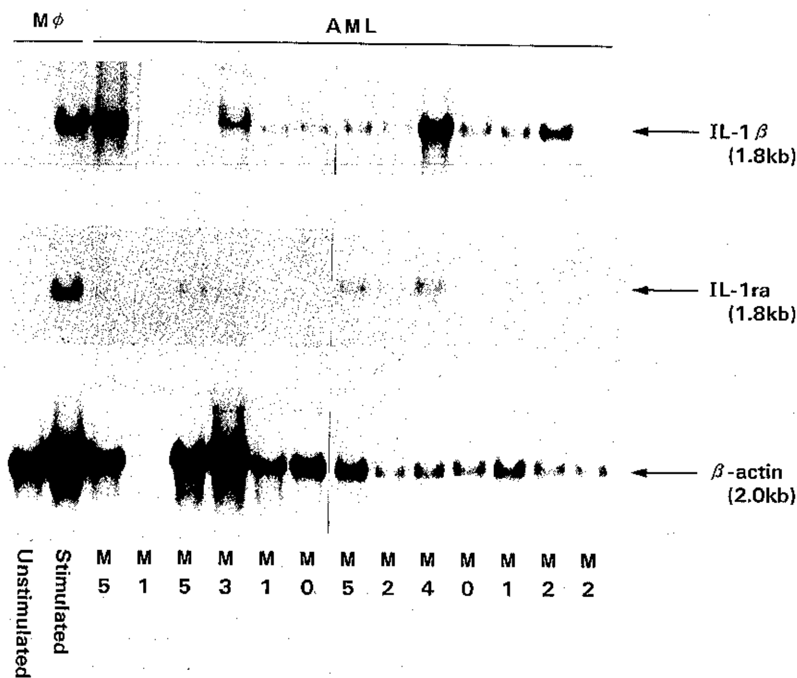


Fig. 5. Expression of IL-1β and IL-1ra mRNA in fresh leukemia cells. IL-1β and IL-1ra mRNA expression was examined in fresh leukemia cells from 36 cases of AML and CMML. Normal monocytes were cultured for 6 h in the absence (Unstimulated) or presence (Stimulated) of LPS and used as controls.

shown in Fig. 4, a small amount of IL-1β mRNA was present in unstimulated JOSK-I cells. The level of the message was greatly increased by stimulation with TPA (4 n*M*) but not with LPS (10 μg/ml) or non-adherent IgG (25 μg/ml). In contrast, little or no IL-1ra mRNA was detected in JOSK-I cells even after stimulation.

Expression of IL-1β and IL-1ra mRNA in fresh leukemia cells Expression of IL-1β and IL-1ra mRNA was also examined in fresh leukemia cells from 36 cases of AML and CMML without any stimulant. A part of the results of Northern blotting is shown in Fig. 5 and the entire results are summarized in Table II. IL-1β mRNA was detected in 26 out of 36 cases (72.2%), whereas IL-1ra mRNA was present only in 8 cases (22.2%). Dominant production of IL-1β over IL-1ra was also observed in monocytic subtypes, i.e., IL-1β was positive in 11 out of 12 cases and IL-1ra was expressed in 6 cases. Even in IL-1ra-positive cases, the signal intensity of IL-1β mRNA was generally stronger than that of IL-1ra. Only 3 cases expressed IL-1ra mRNA without IL-1β expression. These results indicate that myeloid leukemia cells including monocytic subtypes, whether cells of a continuous line or fresh cells, produce IL-1β preferentially over its counter-acting partner, IL-1ra.

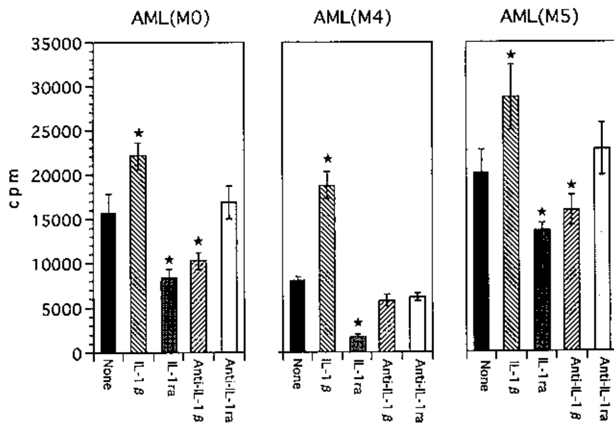
Effects of IL-1β and IL-1ra on proliferation of fresh leukemia cells The effects of IL-1β, IL-1ra, anti-IL-1β neutralizing antibody and anti-IL-1ra neutralizing antibody on proliferation of fresh leukemia cells were investigated in suspension culture. Cell proliferation was monitored in terms of ^{3}H-thymidine uptake after 72 h culture. Sufficient numbers of the cells were available from 3 patients with IL-1β-producing, IL-1ra non-producing [IL-1(+)/IL-1ra(−)] cells and 3 patients with IL-1β non-producing, IL-1ra-producing [IL-1(−)/IL-1ra(+)] cells. Spontaneous incorporation of ^{3}H-thymidine was greater in leukemic cells from IL-1(+)/IL-1ra(−) cases than in those from IL-1(−)/IL-1ra(+) cases ($P<0.01$; Fig. 6, None). IL-1β could significantly enhance ^{3}H-thymidine uptake in all cases ex-

Table II. Expressions of IL-1β and IL-1ra mRNA in Fresh Leukemia Cells

	Case No.	IL-1β(+)[a]	IL-1ra(+)[a]
AML			
M0	4	2	0
M1	6	4	0
M2	11	7	1
M3	3	2	1
M4	4	4	2
M5	6	5	2
CMML	2	2	2
Total	36	26 (72.2%)	8 (22.2%)

a) IL-1β(+) denotes IL-1β mRNA-positive cases. IL-1ra(+) denotes IL-1ra mRNA-positive cases.

amined ($P<0.05$). As shown in Fig. 6A, cell proliferation was inhibited by either IL-1ra or anti-IL-1β antibody in all 3 cases with IL-1β production ($P<0.05$), whereas anti-IL-1ra did not have any effect. In contrast, anti-IL-1ra could significantly enhance cell proliferation in IL-1ra-producing cases ($P<0.05$), while IL-1ra and anti-IL-1β did not show any effect (Fig. 6B). These results indicate that growth of leukemic cells is, at least in part, positively regulated by IL-1β derived from leukemic cells, and is also negatively regulated by IL-1ra from leukemic cells in autocrine or paracrine manners.



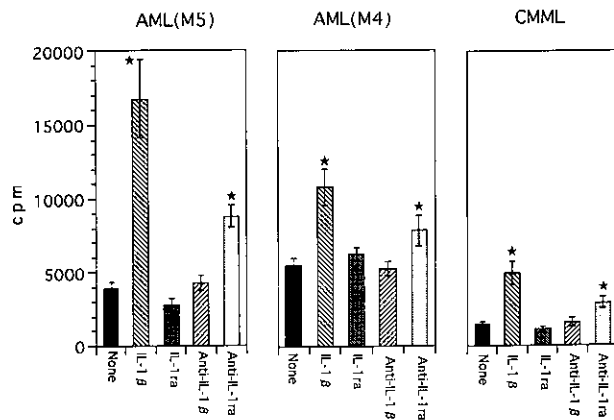


Fig. 6. Effects of IL-1β and IL-1ra on proliferation of fresh leukemia cells. (A) Fresh leukemia cells with IL-1β production were cultured for 72 h in the absence (None) or presence of IL-1β (10 ng/ml), IL-1ra (500 ng/ml), anti-IL-1β (10 μg/ml) or anti-IL-1ra (10 μg/ml). Cell proliferation was monitored in terms of ^{3}H-thymidine incorporation. Columns represent mean$\pm$SD (bar) of the results from 3 independent experiments. *; $P<0.05$ by Student's t test. (B) The same experiments were performed with cells from 3 IL-1ra-producing cases.

Effects of IL-1β and IL-1ra on survival and apoptosis of fresh leukemic cells DNA was isolated from AML cells with IL-1β production (M0) and from AML cells with IL-1ra production (M5) at day 3 of the experiments described in Fig. 6. DNA fragmentation was not observed in AML(M0) cells, but was induced after treatment with IL-1ra and anti-IL-1β neutralizing antibody in accordance with the inhibition of cell proliferation (Fig. 7). A small amount of DNA ladder was present in AML(M5) cells after the culture (Fig. 7, None) and it was increased in the presence of IL-1ra. Both IL-1β and anti-IL-1ra could suppress DNA fragmentation of AML(M5) cells, in accordance with the increase in ^{3}H-thymidine uptake.

DISCUSSION

In order to investigate the role of IL-1 and IL-1ra in the biology of normal monocytes and in the pathogenesis of leukemic cells, we examined the production of IL-1β and IL-1ra in normal monocytes and leukemic cells, and studied the effects of these factors on growth and survival of monocytes and leukemic cells. Monocytes were isolated in a nascent state by centrifugal elutriation and used for the study. Both IL-1β and IL-1ra mRNAs were always induced simultaneously in normal monocytes by various stimulants, including FCS, LPS, non-adherent IgG and TPA, although the ratio of IL-1β and IL-1ra varied depending on the stimulant. This indicates that

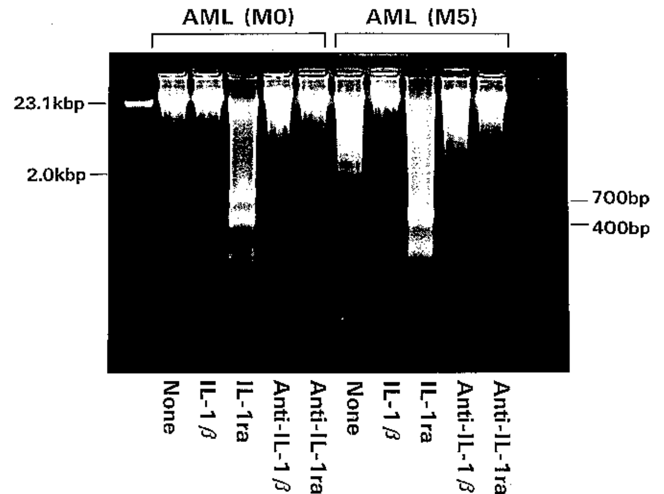


Fig. 7. Effects of IL-1β and IL-1ra on apoptosis of fresh leukemia cells. DNA was isolated from AML cells after 72 h culture in the absence (None) or presence of IL-1β (10 ng/ml), IL-1ra (500 ng/ml), anti-IL-1β (10 μg/ml) or anti-IL-1ra (10 μg/ml). DNA was visualized by ethidium bromide staining after 2.0% agarose gel electrophoresis. A 1 kb DNA ladder and *Hin*dIII-digested λ DNA were used as molecular size markers.

monocytes are essentially committed to produce both cytokines together, although the regulatory mechanisms of production of these factors are possibly different and some stimulants such as adherent IgG[25,26] and transforming growth factor-β[27] were reported to preferentially induce IL-1ra in monocytes. However, very little information is available regarding the mechanisms of possible differential regulation of IL-1 and IL-1ra. The promoter region of the IL-1ra gene has been isolated and sequence analysis did not reveal a specific region involved in preferential transcription of IL-1ra.[28] Further investigation is required to clarify the precise mechanims, if it exists, of differential regulation of IL-1 and IL-1ra production. In the present study, both IL-1β and IL-1ra were induced simultaneously in normal monocytes by all stimulants tested, indicating that both cytokines tended to be produced coordinately under physiological conditions, although certain stimulants could generate them separately. In contrast, the monocytic leukemic cell line JOSK-I produced IL-1β preferentially over IL-1ra. This is also the case with fresh leukemia cells, i.e., the majority of fresh AML cells including monocytic leukemias constitutively expressed IL-1β mRNA, but IL-1ra mRNA was present only in 20% of the cases.

Monocytes are derived from a small population of primitive hematopoietic stem cells in bone marrow.[1] They differentiate in the bone marrow microenvironment and are finally released into the peripheral blood as specific effector cells of the self-defense mechanisms.[2,3] After spending hours to days in a functional state, terminally differentiated monocytes should be eliminated from the body through a particular mechanism, which is now known as apoptosis. Although the precise mechanisms involved in monocyte survival and death are poorly understood, recent evidence suggests that exogenous stimuli may regulate the viability of monocytes. Pro-inflammatory cytokines such as IL-1β or tumor necrosis factor-α and LPS have been reported to prolong survival of monocytes by preventing apoptotic cell death,[29,30] whereas IL-4 enhances apoptosis in stimulated human monocytes.[31] The role of IL-1 in maintenance of monocyte viability was confirmed here, and IL-1ra was shown to inhibit the effect of IL-1β and to induce apoptosis as previously reported for IL-4.[31] This suggests that coordinate production of IL-1β and IL-1ra in monocytes might play an important role in maintaining the numbers of activated monocytes by prevention of accumulation of monocytes released from apoptotic cell death by IL-1β. This may represent an endogenous homeostatic mechanism to prevent the accumulation of activated monocytes, since abnormal accumulation of mononuclear phagocytes may cause lethal tissue damage, as reported in transgenic mice carrying the granulocyte-macrophage colony stimulating factor (GM-CSF) gene.[32]

AML originates from primitive hematopoietic cells, including monocyte precursors, and is characterized by a loss of differentiation capacity and an abnormal accumulation possibly due to escape from apoptotic cell death.[33] Since hematopoietic growth factors are known to maintain the viability of hematopoietic cells through the prevention of apoptosis,[34] it is reasonable to speculate that these factors also play an important role in abnormal accumulation of AML cells. We and others have reported that autocrine production of hematopoietic growth factors such as IL-1β[35-37] or GM-CSF[38] supports the growth and survival of AML cells *in vitro*. In contrast, IL-1ra could inhibit the growth of leukemic cells including AML,[39,40] chronic myelocytic leukemia (CML)[41] and juvenile CML[42] *in vitro*. Constitutive production of IL-1β in AML cells was also confirmed in this study. In contrast, IL-1ra mRNA was not present in the majority of the fresh cases, including monocytic sub-

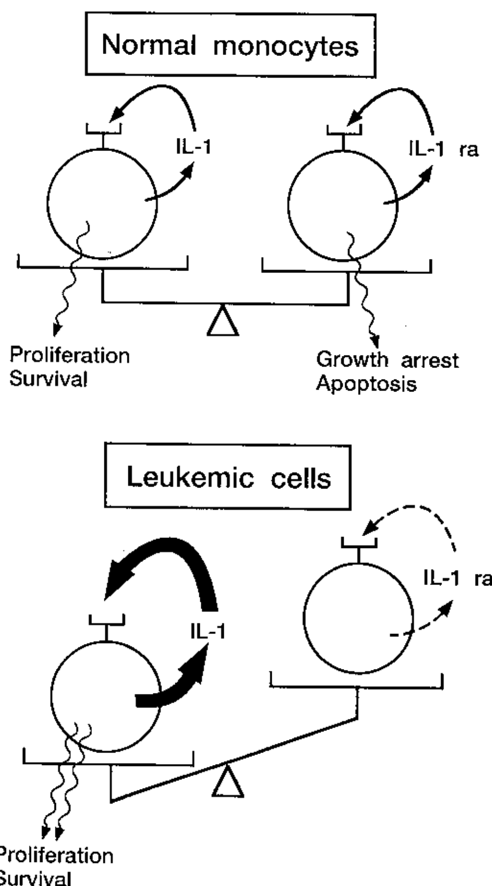


Fig. 8. The roles of IL-1β and IL-1ra in homeostasis of the mononuclear phagocytosis system. The balance between IL-1β and IL-1ra production is necessary for homeostasis of monocytes. Preferential production of IL-1β over IL-1ra contributes to the pathogenesis of leukemic cells.

types, or in monocytic leukemia cell line. Even in IL-1ra-positive cases, the signal intensity of IL-1β mRNA was stronger than that of IL-1ra. Cell proliferation was significantly inhibited and apoptosis was induced by either IL-1ra or anti-IL-1β antibody in IL-1β-producing AML cells. In IL-1ra-producing cases, the capacity of spontaneous growth was lower than that of IL-1β-producing cases and cell proliferation was induced by preventing apoptosis in the presence of either IL-1β or anti-IL-1ra antibody. The mechanisms of the proliferation of AML cells which produce only IL-1ra might be as follows. (1) Other growth factors such as GM-CSF may be involved in cell proliferation of these cases. (2) Abnormal activation of the molecules involved in the signal transduction such as MAP kinase may be present in these cells. (3) IL-1ra produced by these cells may be functionally defective. Taken together, our results indicate that the imbalance between IL-1β and IL-1ra might contribute to the altered growth and abnormal accumulation of leukemic cells.

In summary, the balance between IL-1β and IL-1ra is considered to be necessary for homeostasis of the mononuclear phagocytosis system. Dominant production of IL-1β over IL-1ra thereby contributes to the pathogenesis of certain subsets of leukemia cases, as illustrated in Fig. 8. The usefulness of IL-1ra for the treatment of some cases of leukemia may be explained by this mechanism.

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