Role for Interleukin-1 (IL-1) in Benzene-Induced Hematotoxicity: Inhibition of Conversion of Pre-IL-1α to Mature Cytokine in Murine Macrophages by Hydroquinone and Prevention of Benzene-Induced Hematotoxicity in Mice by IL-1α

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BENZENE (BZ), A WIDELY used industrial chemical and ubiquitous environmental pollutant, is a hematotoxin that causes aplastic anemia and acute myelogenous leukemia in humans and rodent models.1,2 The stromal macrophage, a target of BZ toxicity,3,4 is involved in hematopoietic regulation5 through synthesis of several cytokines, including interleukin-1 (IL-1), which synergizes with IL-3 to promote development of the pluripotent stem cell to myeloid and lymphoid stem cells.6,7 IL-1 is also involved in lymphocyte development and in induction of cytokine production in stromal fibroblasts and endothelial cells.8,9,10 Inhibition of IL-1 production by stromal macrophages could result in development of aplastic anemia. Evidence for monocyte dysfunction and deficient IL-1 production in aplastic anemia was provided by two studies in which markedly depressed IL-1 production was observed in adherence-separated and LPS-stimulated monocytes from 75% to 80% of patients with severe aplastic anemia as compared with normal control subjects.11,12 Hydroquinone (HQ), a hepatic metabolite of BZ, concentrates within the BM,13 where it can undergo peroxidase-mediated activation in stromal macrophages to 1,4-benzoquinone. P388D1 macrophages (unpublished observations) as well as peritoneal stromal macrophages14 convert HQ to 1,4-benzoquinone, which can form adducts with DNA and the sulfhydryl groups of cysteine residues in cellular proteins. BZ-induced aplastic anemia may result from an interference of benzoquinone with IL-1 production and/or secretion in stromal macrophages. We report that HQ, at concentrations that are noncytotoxic and have no effect on DNA or protein synthesis, prevents conversion of the 34-Kd pre-IL-1α to the mature 17-Kd cytokine in lysates of murine macrophage-derived P388D1 cells and purified BM stromal macrophages and that stromal macrophages from BZ-treated mice produce the 34-Kd pre-IL-1α when stimulated in culture with LPS but cannot convert the precursor to the mature cytokine; this suggests that HQ, derived from metabolism of BZ, also prevents conversion of the precursor in vivo, thus causing BM depression. This suggestion was supported by the finding that injection of mice with recombinant IL-1α before BZ administration bypassed the need for the stromal macrophages to produce IL-1 and prevented BM depression. Our results are in accordance with two reports indicating that LPS-induced secretion of IL-1 by murine BM-derived macrophages15 and P388D1 cells16 is decreased in vitro after exposure to HQ, as measured in conditioned medium (CM) by a nonspecific thymocyte proliferation assay.

MATERIALS AND METHODS

Animals. C57Bl/6 inbred male mice were obtained from Jackson Laboratories, Bar Harbor, ME at age 6 weeks ( ± 18 to 20 g). Animals were housed at an AAALAC-approved central facility (73° ± 3°F; humidity 53% ± 7%; 12-hour light cycle) in polycarbonate cages with hardwood bedding (four mice per cage) and fed Purina Rodent Chow and water ad libitum. Animals were accl-

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were incubated without HQ. On completion of the incubation mature fragment consisting of amino acids 115 through 270; activity for F, and C₃ expression of high effector activity in an antibody-dependent cell-mediated cytotoxic system, and production of IL-1 in response to LPS or PMA. We independently confirmed that our P388D₃ cells phagocytize sheep RBCs, possess nonspecific esterase activity, and reduce nitroblue tetrazolium.

Isolation of resident stromal macrophages from murine BM. Stromal macrophages were obtained by flushing the BM from the femurs into sterile culture dishes with RPMI 1640 containing 10 mmol/L HEPES, 10 U/mL heparin, penicillin (100 U/mL), and streptomyccin (50 μg/mL). A narrow adherent layer consisting primarily of macrophages, fibroblasts, and neutrophils was established by incubating the cultures at 37°C in 5% CO₂ for 2 hours. Nonadherent cells were removed by washing with PBS-A; stromal macrophages were selectively grown in the adherent layer by culturing them for 7 days in DMEM containing 10% FCS, penicillin/streptomycin, and 750 U/mL rhM-CSF. This protocol produced a confluent, uniform cell population that morphologically resembled macrophages. More than 95% of the cells were positive for nonspecific esterase activity.

Exposure to HQ. Before treatment, P388D₃ cells (1 x 10⁶/mL) were plated in 24-well culture plates and incubated (37°C, 5% CO₂) 12 hours to permit adherence. P388D₃ cells, or purified stromal macrophages prepared as above, were washed twice with 1.0 mL PBS-A and incubated with 1.0 mL HQ (0.5–10 μmol/L) in RPMI 1640 supplemented with 10 mmol/L HEPES or PBS-A and 2% wt/vol α-D-glucose for 6 hours at 37°C in 5% CO₂. Control cells were incubated without HQ. On completion of the incubation period, the medium was removed and the cells were washed twice in PBS-A before exposure to LPS (40 μg/mL) in RPMI supplemented with 10% FCS for 24 hours (37°C, 5% CO₂). Cells serving as the positive control received LPS; the negative control group was incubated without LPS. Cells were lysed by freezing and thawing three times in 200 μL of a solution containing 10 mmol/L Tris-HCl, pH 8.0, 22°C 1 mmol/L EDTA, 0.01% Triton X-100 and the following protease inhibitors: 2 mmol/L PMSF, 100 μmol/L leupeptin, and 100 μmol/L pepstatin. A. Lysates were stored at −20°C.

DNA synthesis after exposure to HQ. DNA synthesis after exposure to HQ was assayed by monitoring [³H]-thymidine incorporation into trichloroacetic acid (TCA)-precipitable material using a filter method. The TCA-soluble fractions were also analyzed by counting by 5 mL Aquasol 2 to determine whether differences occurred in [³H]-thymidine uptake between control and treated cell populations. After HQ exposure, cells were incubated in RPMI with 10% FCS, LPS (40 μg/mL), and 1 μCi/mL [methyl-³H]-thymidine for periods of up to 24 hours. Negative controls did not receive LPS. After incubation, the media were removed and the cells were washed twice with 1 mL PBS A and lysed as described above.

[³H]-thymidine incorporation into DNA was measured as TCA-precipitable material by spotting 100 μL cell lysate onto Whatman grade-3, 2.5-cm filter paper discs that were washed with ice-cold 10% TCA, twice with 5% TCA, once with 95% ethanol, and then dried. The discs were placed in vials, and the DNA was solubilized by exposure to Protosol (0.5 mL) for 30 minutes. Formula 949 scintillator was added, and the samples were counted with an Intertechnique SL-30 liquid scintillation spectrometer.

Protein synthesis after exposure to HQ. After HQ exposure, ³C-amino acid uptake and incorporation into protein was determined by incubating the cells in RPMI + 10% FCS containing 25 μCi uniformly labeled ³C-amino acids for the indicated times. The cells were washed and lysed, and the TCA-precipitable fraction was obtained by spotting 50 μL lysate onto paper discs. The discs were washed and counted as described for [³H]-thymidine incorporation into DNA.

Determination of intracellular and membrane-bound IL-1α. Total protein concentration was determined on cell lysates, and separation of lysate protein was performed using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (15% acrylamide, 0.8-mm thick, 14 x 14 cm, 150 V). Equal amounts of total cellular protein were added to each well. The proteins were electrophoretically transferred to a nitrocellulose membrane (Schleicher & Schuell, BAS3 0.2 μm) by a Genie Vertical Plate Electrophoretic Blotting System (Idea Scientific Co, Cornwallis, OR) (40 minutes at 12 V). The blot was blocked in a 1% BSA in PBS-A solution containing sodium azide with gentle rocking for 12 hours at 37°C. The blot was then washed five times in PBS-A + 0.05% Tween 20 before exposure to polyclonal rabbit anti-murine–IL-1α (1/500 dilution) in PBS-A/Tween 20/1% BSA (1 hour, 22°C, gentle rocking). The polyclonal neat rabbit serum was elicited using more than 99% pure rmU-IIα and is specific for IL-1α (Genzyme). The blot was again washed extensively in PBS-A/Tween 20/azide before exposure for 1 hour to ³¹I-labeled anti-rabbit IgG (2 x 10⁶ counts) in PBS-A/Tween 20/1% BSA. On completion, the blot was washed in PBS-A/Tween 20 and air-dried overnight. Autoradiography was performed by exposure to Kodak Ortho G film at −70°C for 72 hours; film was developed with a Kodak X-O-Mat M20 Processor. In some cases, densitometric analysis of the immunoblots was performed on an LKB Bromma (Uppsala, Sweden) Ultrascan XL Laser Densitometer.

Handling of BZ. The laboratory practices recommended by the NIH Guidelines for laboratory use of chemical carcinogens were followed. As long as the animals were expiring BZ, they were
housed in a carcinogen-rated hood under conditions approved by the Institutional Animal Research Committee.

Treatment of animals with BZ. BZ in corn oil (12 mL/kg body weight) was administered intraperitoneally (IP) twice daily for 2 days at doses indicated in Figs 1 through 8. Injections were separated by 6 hours. The animals were killed by cervical dislocation 18 hours after the last treatment, and one femur per animal was used.

Administration of indomethacin and rMuIL-1α. A fresh stock solution of indomethacin in ethanol was diluted in PBS-A to 0.17 mg/mL (4.3%). Indomethacin was coadministered IP with BZ. Control animals received corn oil and 4.2% ethanol in PBS-A. Recombinant IL-1α in RPMI 1640 containing 0.2% BSA was administered (2,000 U per animal; 4 × 10⁻⁶ U/kg body weight) 18 hours before the first daily BZ treatment. Controls received RPMI 1640 plus 0.2% BSA. Heat-denatured rMuIL-1α was prepared by exposure to 100°C for 20 minutes.

Preparation of an adherent stromal layer from BZ-treated animals. Male C57Bl/6 mice were exposed to BZ (600 or 800 mg/kg) twice daily for 2 days as described above. Indomethacin 2 mg/kg was concomitantly administered with BZ to one experimental group according to the above protocol. Controls received corn oil. The animals were killed 18 hours after the final BZ treatment, and the femoral BM plug was extruded using RPMI 1640 with 10 mmol/L HEPES, pH 7.4, containing 10 U/mL heparin. The BM cells were then incubated at 37°C in 5% CO₂ for 2 hours to yield an adherent layer consisting primarily of macrophages, fibroblasts, and neutrophils. The adherent layer was washed, and IL-1α production was induced in the macrophages by incubation in RPMI 1640 supplemented with 10% FCS and 40 μg/mL LPS for 18 hours. The cells were then washed, lysed, and analyzed for the presence of IL-1α by Western immunoblotting.

Determination of BM cellularity. The epiphysial plate on each end of the femur was removed, and 3 mL of a solution of cold RPMI 1640 medium supplemented with 10 U/mL heparin and 10 mmol/L HEPES, pH 7.4, was forced through the femur with a syringe with a 25-gauge needle. The marrow was collected in a tissue culture tube, and the RBCs were lysed by suspending the BM cells in 1.0 mL of a cold solution consisting of 155 mmol/L NH₄Cl, 0.1 mmol/L EDTA, and 10 mmol/L KHCO₃. The nucleated cells were collected by centrifugation, the pellet was dispersed into a single-cell suspension in 5 mL RPMI 1640-HEPES, and the number of cells was counted with a hemocytometer and tested for viability by trypan blue exclusion.

Effect of IL-1 on BZ-induced BM depression. Male C57Bl/6 mice (23 to 28 g) received two doses of BZ (800 mg/kg) in corn oil every 6 hours for 2 days before being killed. rMuIL-1α (2,000 U/animal) was administered in RPMI 1640 containing 0.2% BSA 17 hours before each daily BZ exposure. Controls received corn oil and RPMI 1640 containing 0.2% BSA. Eighteen hours after the final BZ injection, the animals were killed and their BM cells were obtained as described for the determination of BM cellularity.

RESULTS

Inhibition of conversion of pre-IL-1α to mature cytokine by HQ. Exposure of adherent P388D cells to LPS for 24 hours results in production of 34-Kd pre-IL-1α as well as its conversion to the 17-Kd mature cytokine as analyzed by Western immunoblots of cell lysate protein with polyclonal rabbit anti-murine IL-1α antibody which does not cross-react with murine IL-1β (Fig 1, lanes B and C). This experiment was representative of five experiments that produced identical results. Exposure of the cells to HQ at concentrations greater than 0.5 μmol/L for 6 hours before LPS stimulation caused virtually complete inhibition of the conversion of the 34-Kd precursor to the 17-Kd mature cytokine (Fig 1, lanes D and E) but had no inhibitory effect on the levels of pre-IL-1α, which also indicates that HQ was not inhibiting LPS stimulation of the cells. Some 34-Kd precursor is produced in these cells in the absence of LPS induction (Fig 1, lane A). HQ at concentrations between 0.5 and 5.0 μmol/L had no significant effect on cell viability, as measured by trypan blue exclusion. As shown in Fig 2, exposure of adherent P388D cells to 1 μmol/L HQ for time periods ranging from 0.5 to 4.0 hours before LPS stimulation demonstrated that more than 30-minute exposure was required for HQ to inhibit conversion of pre-IL-1α to the mature cytokine.

Lack of effect of HQ on protein and DNA synthesis. To ensure that HQ was not causing a general cytotoxicity to the P388D cells, we investigated the effect of HQ on protein and DNA synthesis. Protein synthesis was evaluated by allowing adherent P388D cells to incorporate a complete mixture of ³H amino acids for time periods of up to 24 hours after exposure to 1 μmol/L HQ for 6 hours (Fig 3). Protein synthesis was unaffected by HQ in a 24-hour period.

Exposure to concentrations of 1 to 50 μmol/L HQ for 6 hours followed by incubation of the cells with [³H]-thymidine for 24 hours had no effect on thymidine uptake
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Fig 2. The effect of time of exposure to HQ on conversion of pre-IL-1α. Adherent P388D, cells were incubated with 1.0 μmol/L HQ for 0.5, 1.0, 2.0, and 4.0 hours (lanes A through D) under conditions described in the legend to Fig 1. Controls (lanes E and F) were incubated for 4 hours without HQ. The cells were washed, IL-1α production was stimulated with LPS (lane F did not receive LPS), and cell lysates were prepared as described in the legend to Fig 1. Lysate protein (20.2 μg per lane) was separated by SDS-PAGE, and IL-1α was identified by Western blotting followed by autoradiography as described in the Materials and Methods section.

Fig 3. Effect of HQ on protein synthesis in P388D, cells. Adherent P388D, cells were incubated with 1.0 μmol/L HQ for 6 hours, washed, and incubated in RPMI 1640 supplemented with 10% FCS and a complete mixture of [14C]amino acids (25 μCi) for time periods indicated. After 24 hours, cells were lysed and [14C]-amino acid incorporation into TCA-precipitable material was determined as described in the Materials and Methods section.

Fig 4. Effect of HQ on DNA synthesis in P388D, cells. Adherent P388D, cells were incubated with 1.0 μmol/L HQ for 6 hours as previously described, washed, and incubated in RPMI 1640 supplemented with 10% FCS and [3H]-thymidine (10 μCi) for the time periods indicated. After 24 hours, cells were lysed and [3H]-thymidine incorporation into TCA-precipitable material was determined as described in the Materials and Methods section.

Fig 5. Inhibition of conversion of pre-IL-1 to mature cytokine by HQ in mouse stromal macrophages. Inhibition of conversion of pre-IL-1α to the mature cytokine in P388D, macrophagelike cells necessitated demonstration that a similar situation occurs in BM stromal macrophages. Stromal macrophages were obtained by culturing femoral BM cells with rhM-CSF for 7 days. A population of more than 95% stromal macrophages was confirmed by morphologic and biochemical analysis (data not shown). Stromal macrophages grown to confluence were incubated with concentrations of HQ ranging from 0.5 to 10.0 μmol/L for 6 hours, followed by LPS stimulation for 24 hours. Immunoblot analysis of cell lysates indicated that stromal macrophages do not express detectable levels of either pre-IL-1α or the 17-Kd mature cytokine in the absence of LPS stimulation (Fig 5, lane A), whereas in the presence of LPS both forms of the cytokine are present (Fig 5, lane B). Exposure to HQ concentrations of 0.5 to 10.0 μmol/L results in complete inhibition of conversion of the precursor to the cytokine (Fig 5, lanes C through F). This experiment was performed three times with identical results.

Inhibition of conversion of pre-IL-1α to the mature cytokine in stromal macrophages from BZ-treated animals. Inhibition of conversion also had to be demonstrated in stromal macrophages from mice treated with BZ under conditions into the cells (data not shown) or on [3H]-thymidine incorporation into DNA in a 24-hour period (Fig 4).
known to cause myelotoxicity measured as a depression of BM cellularity, a commonly used parameter to measure BZ-induced myelotoxicity. Male C57Bl/6 mice were treated with BZ (800 mg/kg) twice daily for 2 days as described in the Materials and Methods section. Indomethacin (2 mg/kg), a prostaglandin H synthase (PHS) inhibitor, was concomitantly administered with BZ to one experimental group according to a protocol in which indomethacin has been shown to prevent BZ-induced BM cell depression and genotoxicity. Indomethacin at this dosage level has no effect on hepatic metabolism of BZ, but does prevent oxidation of HQ to reactive species by PHS-peroxidase in macrophages. Eighteen hours after the final BZ treatment, the femoral BM was obtained and the number of nucleated BM cells was determined (data not shown). In this protocol, administration of BZ twice daily for 2 days produces a 45% to 55% reduction in BM cellularity, measured as a decrease in nucleated cells (data not shown). This dose of BZ causes no significant change in body weight, and the cells flushed from the femur are more than 95% viable. Coadministration of indomethacin with BZ prevents BM depression; indomethacin alone has no effect on body weight or cell viability.

The ability of stromal macrophages from the BZ-treated animals to convert pre-IL-1α to the 17-Kd cytokine was assessed by placing BM cells from each of the animals of three experimental groups into culture to establish a BM adherent layer consisting primarily of macrophages and fibroblasts. IL-1α production in stromal macrophages of the adherent layer was stimulated by incubation in medium supplemented with FCS and LPS for 18 hours. The cells were lysed, and the presence of the 34-Kd pre-IL-1α and the 17-Kd cytokine was determined by Western immunoblotting. Stromal macrophages from control animals stimulated with LPS produced both 34-Kd precursor and 17-Kd cytokine (Fig 6, lane A). Stromal macrophages from animals treated with 800 mg/kg BZ showed the presence of the 34-Kd precursor when stimulated with LPS but did not convert the precursor to the mature cytokine (Fig 6, lane C). Indomethacin, which inhibits PHS-peroxidase and thus oxidation of HQ to p-benzoquione, only prevented BZ-induced inhibition of precursor processing when it was coadministered with BZ but increased the amount of mature cytokine produced (Fig 6, lane B). This experiment was performed four times with identical results.

**IL-1α prevents BZ-induced myelotoxicity.** The observations that HQ can prevent conversion of pre-IL-1α to IL-1α in stromal macrophages in vitro and that stromal macrophages obtained from BZ-treated animals also are incapable of effecting the conversion suggests that the depression of BM cellularity by BZ results from a lack of IL-1. If this is indeed true, BZ-induced myelotoxicity should be prevented by bypassing the requirement for stromal macrophages to provide the cytokine. Experiments were performed to determine whether administration of IL-1α to mice before BZ administration could prevent BZ-induced myelotoxicity. Male C57Bl/6 mice were pretreated with mature, recombinant biologically active rMuL-1α (2,000 U per animal) 18 hours before the first daily BZ injection. Figure 7 shows data from a typical experiment indicating that native IL-1α completely protects against the BM depressive effects of BZ, whereas heat-inactivated IL-1α showed no protective effect. Pretreatment with rMuIL-1α alone had no effect on BM cellularity (data not shown). Similar
results were obtained in experiments in which a BZ dose of 600 mg/kg body weight was used.

DISCUSSION

HQ, a toxic metabolite of BZ that accumulates in BM, prevents processing of the 34-Kd pre-IL-1α to the 17-Kd mature cytokine, as measured in lysates of P388D1 macrophagelike cells by Western immunoblots with specific polyclonal rabbit anti-murine IL-1α antibody (Fig 1). HQ in a concentration range greater than 10-fold had no effect on the amount of LPS-induced pre-IL-1α (Figs 1 and 2). Because cell lysates were used, only cytoplasmic and membrane-bound IL-1α was measured. HQ was not causing a general cytotoxic effect on P388D1 cells because cell viability, as well as DNA and protein synthesis, was unaffected.

HQ was also shown to prevent conversion of pre-IL-1α to the cytokine in stromal macrophages grown from femoral BM cells cultured with rhM-CSF for 7 days. In contrast with P388D1 cells, stromal macrophages did not express detectable levels of pre-IL-1α or the mature 17-Kd cytokine in the absence of LPS (Fig 5) and showed a greater sensitivity to HQ in inhibition of conversion of the precursor to the cytokine. In stromal macrophages, HQ at 0.5 μmol/L completely prevented conversion of the precursor to the cytokine (Fig 5) but had no effect on production of pre-IL-1α.

Murine macrophages produce mainly pre-IL-1α, which appears to be selectively phosphorylated; this phosphorylation has been postulated to be a prerequisite for binding of the precursor to the cell membrane. Mature IL-1α and IL-1β are produced from their high-molecular-weight precursors by proteolytic cleavage that generates the N-terminus of the 17-Kd cytokines; however, the precise mechanism and site at which processing occurs have not been completely elucidated. Our results obtained with lysates of well-washed cells suggest that in the mouse macrophage pre-IL-1α processing does not occur extracellularly but instead occurs in the cytosol or on precursor bound to the cell membrane.

Kobayashi et al recently demonstrated that processing of pre-IL-1α to the mature 17-Kd cytokine in human adherent monocytes is catalyzed by a sulfhydryl-dependent calcium-activated neutral protease (CANP) that has been identified as calpain. Membrane-associated pre-IL-1α may be cleaved by membrane-bound calpain and benzoquinone produced from HQ in the cytosol of the stromal macrophages may inactivate calpain by forming a covalent adduct with the SH group of the cysteine residue at the active site, thus preventing cleavage of pre-IL-1α to the mature cytokine. We previously demonstrated that murine peritoneal and P388D1 macrophages (unpublished observations) effect oxidation of 14C-HQ to radiolabeled benzoquinone, which covalently binds proteins. In the presence of cysteine, protein binding is inhibited and the decrease in protein binding can be accounted for by formation of the monocysteine-hydroquinone conjugate. Benzoquinone has been shown to inactivate other sulfhydryl-dependent enzymes. Experiments are currently being performed to determine whether benzoquinone binds the cysteine at the active site of calpain.

Reasor et al recently reported, in preliminary form, that 1.0 μmol/L HQ caused a 30% to 40% decrease in chymotrypsinlike activity in extracts of P388D1 cells concomitant with a decrease in release of IL-1 into the conditioned medium; they suggested that HQ may decrease IL-1 release by inhibiting proteolytic conversion of the molecule from its membrane-bound precursor form.

The presence of membrane-associated IL-1α in the stromal macrophages allows it to participate in autocrine stimulation as well as paracrine stimulation of stromal fibroblasts, possibly by direct transfer of IL-1 to the fibroblast, as has been demonstrated for other hematopoietic growth factors, and subsequent induction in the fibroblast of CSF production required for survival and differentiation of progenitor cells.

Mice treated with BZ (600 or 800 mg/kg body weight) for 3 days show significant BM depression, as measured by a decrease in nucleated BM cells; BM cell depression is considered an indication of BZ-induced myelotoxicity. Under these conditions, stromal macrophages from BZ-treated mice produce pre-IL-1α in response to LPS in culture but are incapable of converting the 34-Kd precursor to the 17-Kd mature cytokine (Fig 6). These in vivo results confirm those obtained with mouse stromal macrophages treated with HQ in vitro which were incapable of processing pre-IL-1α to the 17-Kd cytokine. Indomethacin, which prevents the myelotoxic and genotoxic effects of BZ when coadministered with BZ to mice and which prevents the oxidation of HQ to benzoquinone by PHS-peroxidase in the macrophages, not only prevented the inhibition of precursor processing observed in stromal macrophages from BZ-treated animals when the macrophages were exposed to LPS in culture but also showed an increase in the amount of 17-Kd cytokine produced. This may occur for the following reasons. BZ is known to increase prostaglandin E2 levels in BM. Indomethacin prevents production of prostaglandin E2, which blocks the ability of myeloid progenitor cells to respond to CSF and which negatively regulates production of IL-1 in the macrophages. Together, these results suggest that the depression of BM cellularity observed in these experiments may result from an inability of the stromal macrophages to process pre-IL-1α to mature IL-1α, which is responsible for inducing stromal fibroblasts to produce CSF required for differentiation of stem cells. The demonstration that BZ-induced myelotoxicity is completely prevented by the absence of the stromal macrophages to process pre-IL-1α to mature IL-1α, which is responsible for inducing stromal fibroblasts to produce CSF required for differentiation of stem cells. The demonstration that BZ-induced myelotoxicity is completely prevented by bypassing the need for the stromal macrophages to provide IL-1 supports this hypothesis. Administration of native but not heat-inactivated rMuIL-1α to mice before administration of a dose of BZ that significantly reduced BM cellularity completely prevented the BM depressive effects of BZ (Fig 7). BZ-Induced BM depression leading to aplastic anemia appears to occur because of a lack of IL-1α. The lack of IL-1α production by stromal macrophages may be caused by bioactivation of the toxic metabolite, HQ, by PHS-peroxidase to benzoquinone which could inhibit calpain, the sulfhydryl-dependent protease responsible for conversion of the precursor to the mature cytokine.
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Role for interleukin-1 (IL-1) in benzene-induced hematotoxicity: inhibition of conversion of pre-IL-1 alpha to mature cytokine in murine macrophages by hydroquinone and prevention of benzene-induced hematotoxicity in mice by IL-1 alpha

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