Effect of Interleukin-1 (IL-1) Blockade on Cytokine Synthesis: I. IL-1 Receptor Antagonist Inhibits IL-1–Induced Cytokine Synthesis and Blocks the Binding of IL-1 to Its Type II Receptor on Human Monocytes

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Interleukin-1 (IL-1) induces IL-1, tumor necrosis factor α (TNFα), and IL-6 gene expression and synthesis in a variety of cells. In this study, we investigated the ability of human recombinant IL-1 receptor antagonist (IL-1ra) to inhibit IL-1–induced cytokine production in human peripheral blood mononuclear cells (PBMC) and isolated monocytes. IL-1ra alone at concentrations as high as 1 μg/mL did not induce IL-1α, IL-1β, TNFα, or IL-6 synthesis. Suppression of IL-1–induced IL-1, TNFα, or IL-6 synthesis was dose-dependent (P ≤ .0001). At a twofold molar excess, IL-1ra inhibited IL-1-induced IL-1 or TNFα synthesis by 50% (P < .01); an equimolar concentration of IL-1ra inhibited synthesis of these two cytokines by over 20% (P < .05). A 10-fold molar excess of IL-1ra over IL-1β reduced IL-1β–induced IL-1α by 95% (P = .01) and IL-1α–induced IL-1β by 73% (P < .01). IL-1ra added to PBMC 8 hours after stimulation with IL-1β was still able to inhibit IL-1α, TNFα, and IL-6 synthesis (P ≤ .01). A similar reduction in IL-1β–induced IL-1α was observed when IL-1β was removed from the cultures after 8 hours of stimulation (P < .05), suggesting a prolonged presence of IL-1 or restimulation of IL-1 receptors on monocytes is required for the induction of cytokines. In elutriated monocytes, a 10-fold molar excess of IL-1ra reduced IL-1β–induced IL-1α by 82% (P < .05), TNFα by 64% (P = .05), and IL-6 by 47% (P < .05). IL-1ra was bound to purified monocytes, cross-linked, and immunoprecipitated. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis showed a band at 85 Kd corresponding to the 68-Kd IL-1 receptor type II (IL-1RttII). Excess unlabeled IL-1β or IL-1ra blocked the binding of 125I-IL-1β to the IL-1RtII. We conclude that IL-1ra inhibits IL-1–induced cytokine synthesis and competes with IL-1 for the IL-1RtII on human monocytes. © 1992 by The American Society of Hematology.
during the previous 2 weeks. Blood was drawn into syringes containing heparin (20 U/mL final concentration; LyphoMed Inc, Rosemont, IL). PBMC were isolated by centrifugation through Ficoll (Sigma) and Hypaque (90%; Winthrop Laboratories, New York, NY). Preparations of Ficoll-Hypaque used ultrafiltered water containing less than 100 pg/mL of endotoxin. PBMC were washed twice in sterile 0.9% sodium chloride (Abbott Laboratories, North Chicago, IL) before being resuspended at a concentration of 5 × 10⁶ cells/mL in RPMI. The cell suspension was supplemented with 2% vol/vol heat-inactivated AB serum and 1 µg/mL indomethacin. PBMC (500 µL) were then aliquoted into 5-mL polypropylene tubes and the tubes placed in a 37°C water bath. Fifteen minutes later, 250 µL of either RPMI or IL-1ra in RPMI was added (except as indicated). Cells were incubated an additional 30 minutes in the 37°C water bath. At that time 250 µL of either RPMI or IL-1 in RPMI was added. All cells were then incubated for 24 hours (unless stated otherwise) at 37°C in a humidified atmosphere containing 5% CO₂. After incubation, PBMC cultures were immediately frozen at −70°C (unless separated).

Where indicated, IL-1β was removed from the cultures at specific times. Cell suspensions were centrifuged at 50g for 5 minutes; supernatants were removed and frozen at −70°C. Cell counts performed on these supernatants showed less than 4% of the original number of PBMC. Supernatants were replaced by an equivalent volume of RPMI with 1% AB serum that had been maintained at 37°C under identical conditions. In these same experiments, PBMC were again separated from the cultures before freezing. Cell suspensions were centrifuged at 300g for 5 minutes; supernatants were removed and frozen at −70°C. Cell pellets were washed once with RPMI, resuspended in 1 mL RPMI with 1% AB serum, and then frozen at −70°C.

PBMC cultures or PBMC pellets were subjected to three freeze-thaw cycles to yield maximal recovery of cell-associated cytokines. Samples were assayed in duplicate for IL-1α, IL-1β, TNFα, or IL-6 by radioimmunoassay (RIA). Previous studies have shown no cross-reactivity between these RIA. The limit of detection for each RIA was 40 to 80 pg/mL IL-1α, 80 to 160 pg/mL IL-1β, 80 to 160 pg/mL TNFα, and 80 to 160 pg/mL IL-6. In addition, IL-1ra (1 µg/mL) was added to each tube of IL-1α RIA standards (20 pg/mL to 10 ng/mL). Inhibition of IL-1α cell binding by its RIA was unaffected by the presence of IL-1ra. A similar experiment showed the binding of IL-1β was not affected by the presence of IL-1ra (data not shown).

Magnetic microspheres. PBMC were incubated in PBS (Sigma) with mouse antihuman Leu M3 monoclonal antibody (MoAb; Becton Dickinson, Mountain View, CA) for 30 minutes at 4°C, washed in PBS, and then incubated with magnetic microspheres at 10 rpm for 2 hours at 4°C. Magnetic microspheres (Dynabeads M-280 sheep antimouse IgG; Dynal Inc, Great Neck, NY) were precoated with affinity-purified, sheep polyclonal IgG against all mouse IgG subclasses. Leu M3⁺ cells attached to the microspheres were then separated from Leu M3⁻ cells by a magnet. Leu M3⁺ cells were resuspended in PBS and rotated for an additional hour at 4°C. The Leu M3⁺ cells were once again pelleted with a magnet before removing the supernatant. PBMC (treated in the same manner as Leu M3⁺ cells except for the addition of Leu M3 MoAb and microspheres), Leu M3⁻ cells, and Leu M3⁺ cells were resuspended separately in RPMI with 2% AB serum and 1 µg/mL indomethacin. Experiments as described above for stimulating human PBMC were then performed. Fluorescein-conjugated cell sorter (FACS) analysis (Epic 4; Coulter Electronics, Hialeah, FL) of a representative of two experiments showed that the unfractionated PBMC contained 10% Leu M3⁺ cells.

Elutriation. Monocytes were separated from total PBMC by countercurrent centrifugal elutriation (J-6B centrifuge, elutriation rotor, 20-mL elutriation chamber; Beckman Instruments Inc, Fullerton, CA) as described by Stevenson. PBMC were gently agitated to achieve a single cell suspension before being resuspended at a concentration of 1 × 10⁶ cells/mL in RPMI with 0.25% AB serum at 4°C. PBMC were then loaded at a flow rate of 5.25 mL/min into an elutriator maintained at 4°C and 560g. While the flow rate was kept constant, centrifugation speed was decreased to 490g. Subsequently, flow rate was increased by 500 µL/min every 10 minutes. Cell fractions were collected in 50-µL aliquots and stored at 4°C until quantitation. As determined by staining with α-naphthylacetate esterase (Sigma) and Diff-Quik (Baxter Scientific Products, McGaw Park, IL), the populations designated ‘monocytes’ contained 93% to 97% monocytes. Viability of monocytes was greater than 99%, as determined by trypan blue exclusion and phagocytosis of latex beads. The populations labeled ‘lymphocytes’ contained 8% to 7% lymphocytes, as determined by Diff-Quik staining. Viability of lymphocytes was greater than 99%, as determined by trypan blue exclusion. In some experiments, monocytes and lymphocytes were washed twice before being resuspended in RPMI with 2% AB serum and 1 µg/mL indomethacin. Cells were stimulated as described for human PBMC.

Cell culture. Raji cells were obtained from American Type Culture Collection (Rockville, MD). EL4-6.1 cells were a kind gift of Dr Robert Newton (E.I. du Pont de Nemours & Co, Glenolden, PA). Both cell lines were cultured in RPMI containing 5% FCS.

Cross-linking of 125I-IL-1B to 125I-IL-1R. Using the methods of Spriggs et al, elutriated monocytes were suspended at 5 × 10⁶ cells/mL in RPMI with 1% AB serum and 10⁻⁷ mol/L dexamethasone. The monocytes were then incubated for 12 hours at 37°C. Subsequently, the monocytes were centrifuged at 300g for 10 minutes. The cell pellet was then resuspended for 1 minute at 4°C in 300 µL of 150 mmol/L sodium chloride, 100 mmol/L glycine HCl, pH 3.2 before being diluted to 50 mL in PBS (4°C). Monocytes, Raji cells, and EL4-6.1 cells were washed twice in cold RPMI before being resuspended in binding buffer. Either 25 × 10⁶ monocytes, 8 × 10⁶ Raji cells, or 20 × 10⁶ EL4-6.1 cells were then rotated at 10 rpm at 4°C overnight with 1 nmol/L 125I-IL-1B in the presence of either 1 µmol/L IL-1β or 100 µmol/L IL-1α. The final volume was 1 mL. Cells were then washed with cold PBS and cross-linked in the presence of 2.7 mL/mg L-sulfodisuccinimidyl-suberate (Pierce, Rockford, IL) for 2 hours at 4°C. After a wash with cold PBS, the cells were resuspended in 13,000g for 15 minutes at 4°C.

Immunoprecipitation. Using the methods of Rangnekar et al and Clark et al, rabbit antihuman IL-1B antibody was used to precipitate the solubilized IL-1B/IL-1R complexes. Briefly, 100 µL of packed IgGSorb (The Enzyme Center, Malden, MA) was incubated with 50 µg of affinity-purified goat antirabbit IgG (Southern Biotechnology Associates, Birmingham, AL) at 10 rpm for 6 hours at 4°C. After washing twice in CLB, the conjugated IgGSorb was resuspended in 1 mL cold PBS. Aliquots of 216 µL conjugated IgGSorb were then mixed with 24 µL rabbit antihuman IL-1B and incubated at 10 rpm overnight at 4°C. The antihuman IL-1B-conjugated IgGSorb was washed twice with CLB before being resuspended in 160 µL CLB. Before immunoprecipitation, cell lysates were incubated for 30 minutes on ice with 10 µL of IgGSorb conjugated with goat antirabbit IgG to remove nonspecific binding proteins. Monocytes, Raji cells, and EL4-6.1 cell lysates were combined with 14 µL of antihuman IL-1B–conjugated IgGSorb and then incubated at 10 rpm overnight at 4°C. Immunoprecipitates were pelleted by centrifugation at 13,000g for 15 minutes at 4°C.
minutes at 4°C followed by washing in CLB. Supernatants were frozen at −70°C.

**Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).** The immunoprecipitated IL-1β/IL-1R complexes were resolved by SDS-PAGE with a 10% polyacrylamide gel. Each immunoprecipitate was solubilized in 20 μL of loading buffer (62 mmol/L Tris-HCl, pH 6.8, 10% vol/vol glycerol, 1% SDS, and 0.0005% bromphenol blue) and heated at 100°C for 2 minutes. Prestained protein molecular weight standards (GIBCO-BRL, Gaithersburg, MD) were applied to the gel. The gel was dried with heat under vacuum. Autoradiography was performed by exposure of XAR-5 film (Eastman Kodak Co, Rochester, NY) for 2 days at −70°C using an intensifying screen (Cronex Lightning Plus; Dupont, Wilmington, DE).

**Statistics.** Statistical analysis was performed using the Student’s t-test for paired samples and analysis of variance using Fischer’s least significant difference. Data are expressed as mean ± standard error of the mean (SEM).

**RESULTS**

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**IL-1ra does not induce IL-1α, IL-1β, TNFα, or IL-6 production by PBMC.** In PBMC from nine donors, there was less than 100 pg/mL of IL-1α, IL-1β, or IL-6 in either the untreated or IL-1ra-treated (10 ng/mL to 1 μg/mL) samples. The concentration of TNFα in untreated PBMC cultures (414 ± 102 pg/mL) did not significantly differ (P = .53) from the TNFα produced by PBMC treated with 1 μg/mL IL-1ra (458 ± 13 pg/mL).

**IL-1ra inhibits IL-1-induced IL-1 production in PBMC.** In the present study we investigated whether IL-1ra could interfere with IL-1-induced IL-1 production in PBMC. PBMC from four donors were precultured with or without IL-1ra. The cells were then stimulated with IL-1β (Fig 1). A 10-fold molar excess of IL-1ra over IL-1β resulted in a 95% reduction in IL-1α synthesis (P = .01). Pretreatment with IL-1ra (Fig 1) also resulted in a 73% reduction in IL-1α-induced IL-1β synthesis (P < .01). Because IL-1ra blocks IL-1-induced PGE2 synthesis,27 we added indomethacin to these cultures. The presence of indomethacin (1 μg/mL) did not modify the amount of inhibition by IL-1ra (data not shown).

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**Fig 1.** Effect of IL-1ra on IL-1-induced IL-1 production in PBMC. PBMC were incubated in the absence or presence of IL-1ra (1,000 ng/mL). Thirty minutes later, the cells were stimulated with either IL-1β (100 ng/mL) or IL-1α (100 ng/mL).RIA were performed to determine the total concentration of either IL-1α or IL-1β. Total IL-1α and IL-1β are depicted as the mean ± SEM of four donors. *P ≤ .01 when comparing PBMC pretreated with IL-1ra (E) with cells not pretreated with IL-1ra (□).

**Fig 2.** Effect of increasing concentrations of IL-1ra on IL-1β-induced cytokines in PBMC. PBMC were cultured with different concentrations of IL-1ra. Thirty minutes later, all cells were stimulated with IL-1β (100 ng/mL). (A) IL-1α, (B) TNFα, and (C) IL-6 are depicted as the mean ± SEM of four donors. *P < .05 when comparing PBMC treated with IL-1ra with PBMC untreated with IL-1ra. P ≤ .0001 using analysis of variance.

**Inhibition of IL-1β-induced IL-1α and TNFα by IL-1ra is dose-dependent.** The preceding data showed that IL-1β-induced cytokines can be inhibited by a 10-fold molar excess of IL-1ra. PBMC from four donors were incubated in the presence of increasing concentrations of IL-1ra. All samples were then stimulated with IL-1β (Fig 2A). IL-1ra suppressed IL-1β-induced IL-1α by over 20% (P < .05) at an equimolar ratio of IL-1ra to IL-1β. A twofold molar excess of IL-1ra over IL-1β inhibited IL-1α synthesis by 50% (P < .01). Similar concentrations of IL-1ra had the same effect of IL-1β-induced TNFα (P < .01) (Fig 2B). Inhibition of IL-1β-induced IL-6 by IL-1ra was also dose-
IL-1ra inhibits IL-1β-induced cytokines

**Fig 3.** Effect of time of addition of IL-1ra on IL-1β-induced IL-1α in PBMC. PBMC were treated with IL-1ra (1,000 ng/mL) either before, at the same time as, or 4, 8, or 24 hours after stimulation with IL-1β (100 ng/mL). IL-1ra was not added at 48 hours. Forty-eight hours after stimulation with IL-1β, the cell suspensions were frozen at -70°C. This figure depicts the mean ± SEM of four donors. *P ≤ .01 when comparing PBMC treated with IL-1ra with PBMC untreated with IL-1ra.

**Fig 4.** Effect of removal of IL-1β on IL-1α synthesis in PBMC. PBMC were stimulated with IL-1β (100 ng/mL). At the times specified, the cultures were centrifuged and the media replaced with RPMI. Media was not replaced at 48 hours. Forty-eight hours after stimulation with IL-1β, cell-associated IL-1α was determined by RIA. *P ≤ .05 when comparing PBMC in which IL-1β was removed from the cultures with PBMC exposed to IL-1β for 48 hours.

IL-1ra added to PBMC either before or at the same time as stimulation with IL-1β resulted in greater than 75% inhibition of TNFα synthesis (P = .001). Treating PBMC with IL-1ra 8 hours after stimulation with IL-1β also resulted in 46% (P = .001) inhibition of TNFα synthesis (data not shown). Similar data was obtained for inhibition of IL-1β-induced IL-6 by IL-1ra (data not shown).

Removal of IL-1β from the culture media reduces synthesis of cell-associated IL-1α. The results of the previous experiments show that IL-1 at a concentration several orders of magnitude greater than that sufficient to occupy all IL-1R on PBMC could be displaced after 8 hours and still reduce IL-1β-induced cytokines. These observations suggested that IL-1R on PBMC require prolonged occupancy by IL-1 to transmit a signal that stimulates the synthesis of IL-1, TNFα, and IL-6. Therefore, we removed IL-1β at different times from the PBMC cultures and assessed cytokine synthesis. Five minutes, 1, 4, 8, or 24 hours after stimulation with IL-1β, the culture media containing IL-1β was replaced with RPMI containing 1% AB serum. After 48 hours of culture, PBMC were isolated and cell-associated IL-1α was determined. As shown in Fig 4, removal of IL-1β 5 minutes or 1 hour after stimulation resulted in 70% less IL-1α (P < .05). Removal of IL-1β after 4, 8, or 24 hours reduced IL-1α by 64% (P < .05), 62% (P < .05), and 31% (P = .05), respectively. The concentrations of IL-1α in the media removed at time points of 5 minutes, 1, 4, 8, and 24 hours were all less than 50 pg/mL. To control for any contribution of centrifugation to these results, parallel cultures were centrifuged at the specified times. PBMC were then resuspended in the same IL-1β containing media. Centrifugation did not significantly affect the synthesis of IL-1α (data not shown).

IL-1ra inhibits IL-1β-induced IL-1α in Leu M3+ cells. Leu M3+ cells in the PBMC preparation are the primary source of IL-1. It is possible that IL-1ra inhibits IL-1 by blocking the production of a T-cell product that induces monocytes to make IL-1. We used magnetic microspheres to separate Leu M3+ cells from the other cells in PBMC. PBMC, Leu M3+ cells, and Leu M3− cells were then cultured with or without IL-1ra before being stimulated with IL-1β. As illustrated in Fig 5, 2.5 x 10⁶ PBMC stimulated with IL-1β produced 4.2 ng/mL IL-1α, whereas 2.5 x 10⁶ Leu M3+ cells stimulated with IL-1β produced 18 ng/mL IL-1α. Leu M3− cells produced 950 pg/mL IL-1α. Pretreatment of PBMC and Leu M3+ cells with IL-1ra reduced IL-1α production to 150 pg/mL and 800 pg/mL IL-1α, respectively.

IL-1ra inhibits IL-1β-induced IL-1α, TNFα, and IL-6 in elutriated monocytes. Monocytes and lymphocytes obtained by elutriation of PBMC from four donors were incubated in the presence or absence of IL-1ra before being stimulated with IL-1β. Elutriated monocytes pretreated with IL-1ra produced 82% less IL-1α than untreated monocytes (P < .05) (Fig 6A). Monocytes exposed to IL-1ra exhibited a 64% reduction in IL-1β-induced TNFα (P = .05) (Fig 6B). Monocytes pretreated with IL-1ra before stimulation with IL-1β also produced 47% less IL-6 than untreated monocytes (P < .05) (Fig 6C). Lymphocytes at a concentration of 2.5 x 10⁶ cells/mL produced less...
Fig 5. Effect of IL-1ra on IL-1β-induced IL-1α production in Leu M3+ cells. Leu M3+ cells were separated from total PBMC using magnetic microspheres. PBMC, Leu M3+ cells, and Leu M3- cells were then cultured in the absence or presence of IL-1ra (1,000 ng/mL). Thirty minutes later, all cells were stimulated with IL-1β (100 ng/mL). The concentration of IL-1α in untreated cells (■) and in cells treated with IL-1ra (□) was determined by RIA. Data are expressed as picograms of cytokine per milliliter per 2.5 x 10⁶ cells. Results from one of two donors is shown. The broken line represents the limit of detection of the RIA.

Fig 6. Effect of IL-1ra on IL-1β-induced cytokine production in monocytes and lymphocytes. Monocytes and lymphocytes were separated from total PBMC by elutriation. All cells were then cultured in the absence or presence of IL-1α (1,000 ng/mL). Thirty minutes later, all cells were stimulated with IL-1α (100 ng/mL). The concentration of cytokines produced by untreated cells (■) and by cells treated with IL-1α (□) was determined by RIA. Data are expressed as picograms of cytokine per milliliter per 2.5 x 10⁶ cells. (A) IL-1α, (B) TNFα, and (C) IL-6 are depicted as the mean ± SEM of four donors. *P ≤ .05. The broken line represents the limit of detection of the RIA.

IL-1ra did not itself induce IL-1, TNFα, or IL-6 synthesis in PBMC, supporting the concept that IL-1ra does not possess agonist activity. However, IL-1β-induced IL-1α was inhibited 95% by a 10-fold molar excess of IL-1ra over IL-1β; IL-1α-induced IL-1β was also inhibited (73%) by a 10-fold excess of IL-1ra). These differences may be attributable to the fact that IL-1ra is a more potent inhibitor of IL-1β binding to the IL-1RtII.

In PBMC, a twofold molar excess of IL-1ra inhibited IL-1β-induced IL-1α and TNFα by 50%. An equimolar concentration of IL-1ra inhibited synthesis of these cyto-
kines by over 20%. In contrast, Arend et al27 found that a fivefold molar concentration of IL-1ra was required to produce a 50% inhibition of IL-1β−induced murine thymocyte proliferation. Fifty percent inhibition of IL-1β−induced PGE2 production in human synovial cells and rabbit chondrocytes required 14-fold and 78-fold molar excesses of IL-1ra, respectively. In bovine nasal cartilage explants, 50% inhibition of IL-1α−induced PGE2 synthesis required a 20-fold molar excess of IL-1ra.28 Seckinger et al29 needed concentration ratios of IL-1ra to IL-1 of 10 to block bone resorption in fetal rat long bones by 50%. Carter et al30 reported that a ninefold molar excess of IL-1ra was necessary for a 50% inhibition of IL-1α−augmented endothelial cell adhesiveness for neutrophils.

The above studies using IL-1RtI bearing cells are to be contrasted with the present studies using IL-1RtI−bearing cells. IL-1ra may be a more potent inhibitor in monocytes than in macrophages, Chizzonite et al33 were unable to find mRNA encoding the IL-1RtI. In murine macrophages, Chizzonite et al35 were unable to find mRNA encoding the IL-1RtI.

Cross-linking studies confirmed that IL-1ra blocks 125I−IL-1β binding to the human monocyte IL-1RtI. This finding was not unexpected; we have previously shown that IL-1ra can inhibit the binding of IL-1 to the IL-1RtI on human PMN and B-lymphoma cells.13 In addition, Spriggs et al40 showed that the IL-1RtI was present on adherent human monocytes. While Spriggs et al did find messenger RNA (mRNA) encoding the IL-1RtI in human monocytes and THP-1 cells, neither nor our cross-linking studies showed a band corresponding to the IL-1RtI. In murine macrophages, Chizzonite et al35 were unable to find mRNA encoding the IL-1RtI.

The addition of IL-1ra before or at the same time as IL-1β resulted in similar inhibition of IL-1β−induced cytokine synthesis. However, IL-1ra also reduced IL-1β−induced IL-1α, TNFα, and IL-6 production even when the IL-1ra was added 8 hours after stimulation with IL-1β. This was an unexpected observation. In PBMC treated with IL-1β, a similar reduction in IL-1α synthesis was observed when IL-1β was removed from the cultures 8 hours after stimulation. Together, these observations suggest that the classical model of ligand-receptor signal triggering is different for IL-1 on these IL-1RtI−bearing cells. Exposing 2.5 × 107 PBMC to 100 ng/mL IL-1β results in 109 IL-1β molecules per cell. Because human monocytes4 and lymphocytes32 possess less than 103 IL-1R per cell, the conditions of our PBMC cultures represent over 106 molecules of IL-1β per IL-1R. Thus, prolonged exposure of IL-1R to saturating concentrations of IL-1 may be required to induce cytokine synthesis on these cells. Alternatively, constant restimulation of recycling IL-1R for 8 hours may be required for cytokine synthesis. In that case, in monocytes that have previously internalized the IL-1/IL-1RtI complex, IL-1ra may prevent IL-1β from binding to IL-1R that have recycled to the cell surface during the first 8 hours. This concept is supported by the recent finding that expression of the IL-1RtI on Raji cells is dependent on cell cycle with only 5% to 16% of cells expressing the receptor at any one time point.35

An alternative explanation is that, during the first 8 hours of incubation with IL-1, IL-1R are expressed on a subpopulation of monocytes that did not possess surface receptors at the time of stimulation. IL-1ra could then prevent binding of IL-1β to the newly expressed IL-1R. This explanation is supported by the fact that subpopulations of human monocytes respond differently to another cytokine, granulocyte-macrophage colony-stimulating factor.36 Although our data show that IL-1ra competitively inhibits the binding of IL-1β to the IL-1RtI on human monocytes, it does not exclude the possibility that human monocytes express both types of IL-1R. Human monocytes may be similar to human B-cell lines in expressing both IL-1R.37 Uhl et al38 have found that elutriated human monocytes have a single class of receptors for IL-1β with a binding constant of 600 pmol/L and a receptor density of approximately 100 binding sites per cell. Cross-linking studies showed this monocyte IL-1R has the same molecular weight (80 Kd) as the IL-1RtI. Thus, it is possible that in human monocytes both IL-1R are expressed and act cooperatively in signal transduction with small amounts of the IL-1RtI being required for the induction of cytokine synthesis. However, the requirement for prolonged exposure to IL-1 for the induction of cytokine synthesis, as shown in the present study, argues against a role for the IL-1RtI that induces protein synthesis after a short exposure to IL-1.

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