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Bacterial Lipopolysaccharide Causes Rapid Shedding, Followed by Inhibition of mRNA Expression, of the IL-1 Type II Receptor, with Concomitant Up-Regulation of the Type I Receptor and Induction of Incompletely Spliced Transcripts

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The IL-1 type I receptor (IL-1RI) is part of a signaling complex together with the IL-1R accessory protein, whereas available information is consistent with a “decoy” model of function for the IL-1 type II receptor (IL-1RII). The present study was designed to investigate the effect of bacterial LPS on IL-1RI in human monocytes. LPS causes rapid release of the IL-1RII, an effect blocked by a metalloprotease inhibitor. Subsequently, LPS-treated monocytes showed a drastic reduction of IL-1RII mRNA. In contrast, LPS induced IL-1RII and, to a lesser extent, IL-1AcP expression. LPS-induced augmented expression of the canonical 5-kb IL-1RI mRNA was accompanied by the appearance of 2.4-kb IL-1RII transcripts. The use of probes representative of different regions of the IL-1RII mRNA, as well as cDNA cloning, revealed that the 2.4-kb inducible band includes incompletely spliced, polyadenylated transcripts potentially encoding truncated versions of the receptor. The observation that the prototypic proinflammatory molecule LPS has divergent effects on IL-1Rs, with inhibition of IL-1RII and stimulation of IL-1RI and IL-1R accessory protein, is consistent with the view that these molecules subserve opposite functions in the pathophysiology of the IL-1 system. The rapid shedding of IL-1RII by monocytes early in recruitment may serve to buffer the systemic action of IL-1 leaking from sites of inflammation. This early event, followed by prolonged inhibition of IL-1RII expression and up-regulation of IL-1RI, may render monocytes more responsive to IL-1 at sites of inflammation. The Journal of Immunology, 1999, 162: 2931–2938.
The finding that antiinflammatory mediators augment expression of the decoy IL-1RII is consistent with IL-1RII being an important anti-IL-1 pathway. The present study was designed to investigate whether bacterial LPS, a prototypic proinflammatory molecule, affects IL-1Rs in human monocytes. LPS caused a rapid (10–20 min) release of the decoy IL-1RII via metalloprotease activation. Rapid stripping of the decoy IL-1RII was followed by profound inhibition of decoy IL-1RII mRNA expression. Concomitantly, expression of IL-1RI and IL-1RAcP mRNAs were up-regulated. Stimulation of IL-1RI expression by LPS was associated with the appearance of novel 2.4-kb mRNAs, which were characterized by PCR and cDNA cloning as heterogeneous, incompletely spliced, polyadenylated transcripts.

Materials and Methods

Cells

Human monocytes were separated from peripheral blood of human healthy donors by Percoll gradient centrifugation (11). Briefly, whole blood was fractionated on Ficoll gradient centrifugation (Seromed-Biochem KG, Berlin, Germany), and mononuclear cells were collected from the layer, layered on 46% Percoll (Pharmacia, Uppsala, Sweden), and centrifuged at 2000 rpm for 30 min at room temperature. Monocytes (>98% pure as assessed by morphology) were resuspended at 5 x 10^6 cells/ml in RPMI 1640 (Seromed-Biochem) and 2 mM HEPES (Merck, Darmstadt, Germany). All reagents contained <0.125 EU/ml of endotoxin as checked by Limulus amoeboocyte lysate assay (Microbiological Associates, Rockville, MD).

Stimulation conditions and reagents

Monocytes were incubated in endotoxin-free RPMI 1640 at 5 x 10^6 cells/ml with or without stimuli for the indicated times at 37°C in the presence of 5% CO₂, LPS (Escherichia coli 005:B5; Difco, Detroit, MI) was used at 500 ng/ml. Actinomycin D (AcD; Sigma Chemical Co., St. Louis, MO) was used at 1 µg/ml. Cycloheximide (CH; Sigma) was used at 10 µg/ml. Human IL-1β (Dompé, Aquila, Italy) was used at 10 ng/ml. TNF-α (BASE/Knoll, Ludwigshafen, Germany) was used at 500 U/ml. IL-1Ra (Cetus, Emeryville, CA) was used at 10 µg/ml. The mAb against TNF-α, B154.2, was a kind gift from Dr. G. Trinchieri (Wistar Institute, Philadelphia, PA). A 1:2000 dilution of ascertes neutralized 1000 U/ml of rTNF-α. The metalloprotease inhibitor BB-94 was from British Biotech (Oxford, U.K.); L,68083, a specific elastase inhibitor was from Pharmacia (Uppsala, Sweden), and mononuclear cells were collected from the ring, layered on top of 46% Percoll (Pharmacia, Uppsala, Sweden), and centrifugated at 2000 rpm for 30 min at room temperature. Monocytes (>98% pure as assessed by morphology) were resuspended at 5 x 10^6 cells/ml in RPMI 1640 (Seromed-Biochem) and 2 mM HEPES (Merck, Darmstadt, Germany). All reagents contained <0.125 EU/ml of endotoxin as checked by Limulus amoeboocyte lysate assay (Microbiological Associates, Rockville, MD).

IL-1 binding assay

The assay was conducted as described (29). Briefly, 10 x 10^6 cells were incubated with or without the indicated stimuli in serum-free medium at 37°C in 5% CO₂ for 20 min in 50-ml conical polypropylene tubes (Falcon-Becton Dickinson Labware, Lincoln Park, NJ). Cells were then washed with binding buffer (RPMI 1640, 0.2% BSA, pH 7.4), and 4 x 10^6 cells were incubated with 600 pM 125I-labeled IL-1β (sp. act. 180 µCi/mg; NEN, Bad Homburg, Germany) in the presence or absence of a 100 M excess of cold cytokine in 50 µl of binding buffer at 4°C for 4 h in poly-styrene 96 round-bottom well microplates (Falcon) on a shaking platform. Preliminary experiments showed that binding reached the plateau (4 h) under these conditions. To separate bound from free 125I-labeled IL-1β, cells were resuspended, transferred to Eppendorf tubes, washed in binding buffer, reususpended in 70 µl of binding buffer, and finally layered on the top of a 200-µl cushion of 20% sucrose (Merck) and 1% BSA in 400-µl polypropylene tubes (Beckman Instruments, Palo Alto, CA) and centrifuged at 10,000 rpm for 30 s at room temperature. The cellular pellets were counted in a gamma counter. To obtain a saturation curve, untreated or LPS-treated monocytes were incubated with increasing amounts of 125I-labeled IL-1β, in the presence or absence of a 100 excess of cold cytokine. Scatchard analysis was performed by the LIGAND program (Version 4.1; National Institutes of Health, Bethesda, MD) to determine the affinity and numbers of receptors for IL-1β.

Affinity cross-linking

Cells (3 x 10^6) were stimulated with 50 ng/ml LPS in 1 ml of RPMI 1640 at 37°C for 20 min. Medium was recovered and concentrated 10 times by membrane filtration (cutoff, 10,000; Amicon, Beverly, MA). One hundred microliters were added to 1 nM 125I-labeled IL-1β, with or without a 200 M excess of cold IL-1β or 10 µg/ml M1 (blocking mAb anti-IL-1RI) or M22 (blocking mAb anti-IL-1RII) (7, 34) kindly provided by Dr. J. E. Sims (Immunex, Seattle, WA), and incubated at 4°C for 4 h. After addition of 1 µM disuccinimidyl suberate (Pierce, Rockford, IL) at 4°C for 30 min, samples were analyzed by 8% SDS-PAGE under reducing conditions, and dried gels were exposed to autoradiography for 1–3 days.

RNA extraction and Northern blots

RNA was extracted and purified using the guanidine isothiocyanate (Merck) method as described previously (35, 36). Total cellular RNA (10 µg) was run in standard formaldehyde-agarose gel, blotted onto Gene Screen Plus membranes (New England Nuclear, Boston, MA), and fixed at 80°C for 2 h. Membranes were pretreated and hybridized in 50% formamide (Merck) with 10% dextran sulfate (Sigma) and washed twice with 2 x SSC (1 x SSC, 0.15 mol/L NaCl, and 0.015 mol/L sodium citrate) and 0.1% SDS (Merck) at 60°C for 1 h, and finally washed twice with 0.1 x SSC at room temperature for 5 min. Membranes were exposed for 4–24 h at −80°C with intensifying screens. RNA transfer to membranes was checked by UV irradiation, as shown in each figure.

Hybridizations for detection of IL-1RII transcript were performed, with HindIII-EcoRI cDNA probe corresponding to probe b in Fig. 7A. Hybridizations for detection of IL-1RII transcript were performed using the probe corresponding to the 750-bp fragment subcloned in the EcoRI site of the plasmid pHu175 gift by Dr. J. E. Sims (Immunex).

Hybridization for identification of IL-1RAcP transcripts was performed with an Asp18-NorI full-length hIL-1RAcP cDNA.

RT-PCR

To amplify different sequences of coding or noncoding regions of the IL-1RII gene, we performed RT-PCR (37). Briefly, 1 µg of total RNA from LPS-treated monocytes was reverse transcribed in reverse transcriptase buffer (5 mM MgCl₂, 50 mM KCl, and 10 mM Tris-HCl, pH 8.3) with 2.5 µM random hexamers, 1 mM each deoxynucleotide triphosphate, 1.0 µM RNase inhibitor, and 2.5 µM Moloney leukemia virus reverse transcriptase (Perkin-Elmer/Cetus, Norwalk, CT). Samples were incubated for 10 min at room temperature and then at 42°C for 15 min, 99°C for 5 min, and 5°C for 5 min. Then, each cDNA reaction was divided into two Eppendorf tubes, and a specific pair of primers designed to amplify cDNAs coding for IL-1RI, and as an internal control human β actin, was added to each. Amplification was performed in 2 mM MgCl₂, 50 mM KCl, 10 mM Tris-HCl, 0.2 mM each deoxynucleotide triphosphate, 2.5 µM 100 µM Taq DNA polymerase (Perkin-Elmer Cetus), and 4 µg/ml of each specific primer (see below). Amplification was performed in an automated thermal cycler (Perkin-Elmer Cetus) at 95°C for 1.5 min, 55°C for 1.5 min, and 72°C for 1.5 min. Amplification was stopped at 35 cycles. The specific primers were designed to amplify the following sequences: probe a, sequence 123–199 (exons 4–6, extracellular region); probe b, sequence 753–1192 (exons 7–10, extracellular and transmembrane regions); probe c, sequence 1051–1231 (exon 10, transmembrane region); probe d, sequence 1199–1399 (exon 11, intracellular region); probe e, sequence 1470–1970 (exon 12, intracellular region and a part of 3’ untranslated); probe f, sequence 2427–2966 (3’ untranslated); probe g, sequence 3201–3683 (3’ untranslated); and probe h, sequence 4003–4476 (3’ untranslated).

The nucleotides in these sequences can be found under GenBank accession number M27492. In this sequence, the translation termination codon is at nucleotide 1790, and the alternative consensus sequence for pol(A)’ addition is at nucleotide 2309. The PCR products were subcloned in pMOS Blue T-vector (pMOS Blue T-vector kit, RPN 1719; Amersham, Arlington Heights, IL) and sequenced by the T7 sequencing kit (Pharmacia).

cDNA cloning

Total RNA was isolated from monocytes stimulated for 4 h with 500 µg/ml LPS. Poly(A)’ RNA was further purified by affinity chromatography on oligo(dT) cellulose. A cDNA library was constructed in the λ-ZAP II vector (Stratagene, La Jolla, CA) as described (38). A total of 3 x 10^8 plaques were screened on nitrocellulose membranes in duplicate by standard procedures and screened as described (38). Eighty-four plaques were found to be positive for probes b and e, and only three plaques were found to hybridize with probe b and not with probe e.

Results

Induction of rapid shedding of the decoy IL-1RII by LPS

In a first series of experiments, we examined whether LPS affected rapid release of the decoy IL-1RII in human monocytes, a cell type
that expresses predominantly (>80% of the IL-1 binding capacity) the type II decoy R, IL-1RII (3, 11). As shown in Fig. 1A, exposure to LPS for 30 min caused a profound reduction of the monocyte capacity to bind IL-1, with an ED$_{50}$ of ~1 ng/ml and virtually complete inhibition at 10 ng/ml. The number of IL-1R/cell was 5016 ± 214 in the untreated population and 356 ± 11 in LPS-treated monocytes. No substantial change in receptor affinity was detected (4.12 ± 10$^{-9}$ M in untreated cells and 1.88 ± 10$^{-9}$ M in LPS-treated cells) (Fig. 1B). The effect of LPS was detected at 10 min and was maximal at 20 min (not shown).

Disappearance of IL-1 binding sites from the cell surface could be due to internalization or release. The internalization process of receptor-bound IL-1 was not affected by LPS, in that the percentage of internalized IL-1R was not different in untreated vs LPS-treated cells (data not shown). To examine the possibility that LPS induced the release of IL-1R from treated monocytes, cells were incubated with LPS, and the cell-free supernatants were recovered and examined by cross-linking with $^{125}$I-labeled IL-1$\beta$. As shown

FIGURE 1. Effect of LPS on IL-1 binding and cross-linking in human monocytes. A, Effect of LPS on IL-1 binding to human monocytes. Monocytes were exposed to different concentrations of LPS for 30 min and examined for IL-1 binding. Results, expressed as percentage of specific IL-1 binding with respect to monocytes treated with medium alone, are means ± SD of three independent experiments. B, Saturation curve and Scatchard analysis of $^{125}$I-labeled IL-1 binding to monocytes treated with LPS. Monocytes, treated or not with LPS (50 ng/ml) for 30 min at 37°C, were incubated with different doses of $^{125}$I-labeled IL-1 ranging from 0.25 to 2 nM, in the presence or absence of a 100 M excess of unlabeled IL-1$\beta$. Two experiments were performed; data are means of one representative experiment performed in triplicate (of two experiments performed). C, Affinity cross-linking of the soluble IL-1R released from LPS-treated monocytes. Monocytes were cultured without (lane 1) or with LPS (50 ng/ml) (lane 2) for 30 min in serum-free RPMI 1640. Supernatants were then recovered, concentrated, mixed with $^{125}$I-labeled IL-1, cross-linked with disuccinimidyl suberate, and analyzed by SDS-PAGE. Competitors added before cross-linking were a 100-fold excess of cold (unlabeled) cytokine (lane 3), 10 $\mu$g/ml anti-IL-1RI (mAb M1) (lane 4), and anti-IL-1RII (mAb M22) (lane 5), respectively.

FIGURE 2. Effect of anti-TNF-α mAb and protease inhibitors on decoy RII release on monocytes. A, Monocytes were incubated with LPS (50 ng/ml) or TNF-α (50 ng/ml) in the presence or absence of mAb B154.2 against TNF-α for 30 min at 37°C. All samples were then examined for binding of $^{125}$I-labeled IL-1$\beta$. Data are shown as percentage, with range, of specific binding of labeled IL-1 of a representative experiment performed in triplicate. B, Monocytes were incubated with BB-94 (1 $\mu$g/ml), E-64 (10 mM), and L680833 (2 mM) for 20 min at 37°C and then stimulated with LPS (50 ng/ml) for 20 min. All samples were then examined for binding of $^{125}$I-labeled IL-1$\beta$. Data are shown as percentage, with range, of specific binding of labeled IL-1 in two different experiments.
in Fig. 1C, LPS-treated monocytes released an IL-1 binding molecule of ~78 kDa; after subtraction of the IL-1 mass, the molecular mass of this protein was ~60 kDa, as expected for a soluble form of IL-1RII (11, 32, 33). The specific nature of the cross-linking was demonstrated by competition with an excess of unlabeled IL-1. The IL-1 soluble binding material was totally inhibited by the presence of a blocking mAb (M22) directed against the IL-1 decoy IL-1RII but not of an anti-IL-1RI mAb (M1). Thus, the loss of IL-1 binding induced by LPS is largely, if not exclusively, accounted for by the release of the soluble form of the IL-1 decoy IL-1RII from the monocyte surface.

TNF-α is a potent inducer of decoy IL-1RII release on monocytes and polymorphonuclear neutrophils (30), and LPS induces TNF-α production in monocytes. We tested whether LPS-induced decoy IL-1RII release was mediated by TNF-α produced by monocytes after incubation with LPS. We incubated monocytes with a blocking mAb specific for TNF-α, in the presence or absence of LPS. As a positive control, we also incubated monocytes with TNF-α and mAb. As shown in Fig. 2A, while the TNF-α action on decoy IL-1RII release was blocked by the specific Ab, the LPS effect was not significantly modified.

We recently showed that metalloprotease inhibitors block release of decoy IL-1RII in different systems (31). We tested whether the LPS-induced release of decoy IL-1RII could be inhibited by treatment of monocytes with metalloprotease inhibitors. As shown in Fig. 2B, when cells were treated with different protease inhibitors, BB-94, a specific metalloprotease inhibitor, significantly blocked the LPS-induced release of decoy IL-1RII.

**Effects on IL-1R expression**

Having identified a rapid action of LPS on surface levels of the IL-1 decoy IL-1RII, we investigated “late” effects on mRNA levels. As shown in Fig. 3A, resting monocytes express high levels of decoy IL-1RII mRNA, and these were markedly reduced by LPS in a dose-dependent way (at a dose of 500 ng/ml, 10-fold, mean of 20 experiments). This effect was maximal between 6 and 8 h after treatment with LPS (Fig. 3B).

Monocytes express barely detectable levels of IL-1RI mRNA (Fig. 3A). Upon exposure to LPS, monocytes showed a dramatic augmentation of steady-state IL-1RI transcripts (at a dose of 500 ng/ml, 30-fold, mean of 35 experiments). The effect of LPS on IL-1RI expression was maximal at 4 h and declined thereafter (Fig. 3B). As shown in Fig. 3C, expression of IL-1RACp was also augmented by LPS (8-fold, two experiments).

To obtain initial indications as to the mechanisms involved in LPS stimulation of IL-1RI expression, we used metabolic inhibitors and measured transcript half-life. As shown in Fig. 4, A and B, the transcriptional inhibitor ActD and the protein synthesis inhibitor CH completely abolished the divergent effects of LPS on expression of IL-1RI and of the decoy IL-1RII. In an effort to assess whether LPS affected mRNA stability, monocytes were exposed to LPS for 4 h, treated with ActD to inhibit transcription, and analyzed by Northern blot at different times to evaluate the mRNA half-lives (Fig. 4C). The estimated mRNA half-lives were 2 and 2.45 h for IL-1RI in control and LPS-treated cells, respectively, and 2.15 and 1.30 h for IL-1RII in control and LPS-treated monocytes, respectively.

The finding that LPS induction depended upon intact protein synthesis raised the possibility that LPS may be acting on monocytes via induction of the primary cytokines IL-1 and TNF. IL-1β and, to lesser extent, TNF-α augmented expression of IL-1RI and inhibited that of decoy IL-1RII in human monocytes (not shown). However, addition of excessive amounts of IL-1Ra and of a blocking anti-TNF-α mAb, alone or in combination, did not affect LPS with regard to expression of IL-1RI and decoy IL-1RII (Fig. 5).

**Identification, characterization, and cloning of novel, inducible IL-1RI transcripts**

In the course of these studies we observed that LPS induced the appearance of a 2.4-kb IL-1RI-related band, visible after more prolonged exposure of the blots (after ~24 h). Blots from resting and LPS-treated monocytes were therefore hybridized with the IL-1RI and IL-1RII probes separately. As shown in Fig. 6 (representative of 15 experiments), LPS induced expression of a novel 2.4-kb transcript, which hybridized with the IL-1RI probe and was detected after longer (~24 h) exposure of the blots. By densitometric analysis of results at an LPS concentration of 500 ng/ml, the intensity of the 2.4-kb band was 10–20-fold lower than that of the 5-kb one in this series of experiments. Induction of the 2.4-kb IL-1RI transcript closely paralleled that of the canonical 5-kb mRNA in terms of time course (not shown) and dose-response relationship.

In an effort to obtain indications as to the nature of the novel LPS-inducible IL-1RI-related transcripts, specific probes representative of different regions of the mature IL-1RI transcript were generated by RT-PCR. These probes (labeled a-h), representative of coding and noncoding regions (Fig. 7A) of IL-1RI mRNA, were...
used in Northern analysis of RNA from resting and LPS-stimulated cells.

All probes recognized the 5-kb IL-1RI transcript. In contrast, probes e, f, g, and h did not hybridize to the 2.4-kb IL-1R transcripts; therefore a part of the coding sequence (exon 12) and the 3' untranslated region were not present in the smaller 2.4-kb mRNA species (Fig. 7B). Since probe e comprises coding and noncoding sequences of the IL-1RI, we constructed two new probes, e1 and e2 (Fig. 7A), which correspond to the coding and noncoding regions, respectively. Neither the e1 nor the e2 probe hybridized to the small 2.4-kb transcript (Fig. 7C), which indicated that part of the 3' coding sequence may have been missing in this band.

To define the structure of the 2.4-kb IL-1RI-transcripts, we constructed a cDNA library from LPS-stimulated monocytes. The library was screened in duplicate using probes b and e; we selected for analysis clones negative with probe e and positive with probe b. We obtained 84 positive clones with probes b and e (IL-1RI wild-type) and 3 positive clones (clones 27, 21, and 25) that were positive with probe b and negative with probe e. These clones were sequenced; Fig. 8A shows a schematic representation of the structure of these clones. In all 3 clones, exon 12 and the 3' untranslated region are absent and polyadenylation is present. Intronic sequences (39) corresponding to introns 7 and 9 were present in clone 27, those of introns 5 and 6 in clone 21, and those of intron 11 in clone 25. In clone 21, the poly(A) tail was preceded by a novel sequence, X (GenBank accession number AF054830).

By PCR we amplified the intronic regions and used them as probes in Northern blot experiments using total and poly(A)^+ RNA from LPS-stimulated monocytes. As shown in Fig. 8B, a probe corresponding to intron 9 of IL-1RI recognized the 2.4 transcript in total (Fig. 8B, lanes 1 and 2) and poly(A)^+ RNA (Fig. 8B,
lane 3) from LPS-induced monocytes. The same 2.4-kb band was also recognized by a probe representative of sequence X (data not shown).

To confirm these data, we used primers corresponding to a fragment spanning exonic (exon 8) and intronic (intron 9) regions to amplify total RNA of LPS-stimulated monocytes. As expected, a band of ~970 bp was amplified (Fig. 8C), further supporting the existence of these transcripts in LPS-treated monocytes.

**Discussion**

The present study was designed to investigate the effects of the prototypic proinflammatory molecule LPS on IL-1Rs in human monocytes. LPS had different and divergent effects on IL-1RI and IL-1AcP vs IL-1RII, with inhibition on IL-1RII and induction of IL-1RI and IL-1AcP. The action of LPS on the decoy IL-1RII, the predominant (>80%) IL-1 binding molecule on the surface of mononuclear phagocytes (3, 11), was biphasic, with rapid shedding induced in minutes followed by later slow and persistent inhibition of mRNA expression, which took 2–4 h to plateau. The effect of LPS on IL-1RII transcripts was associated with an increased rate of mRNA degradation. In contrast, LPS augmented expression of IL-1RI mRNA, otherwise barely detectable in monocytes. Thus, LPS has divergent effects on IL-1Rs in human monocytes, with inhibition of decoy IL-1RII (the largely predominant form in myelomonocytic cells) and induction of IL-1RI.

LPS induced rapid (within minutes) loss of the IL-1 binding capacity in monocytes. The loss of IL-1 binding capacity in LPS-treated monocytes was due to the disappearance of the receptors from the cell surface and associated with the rapid release of a 60-kDa IL-1 binding molecule. The rate of IL-1R internalization was not affected by LPS. The 60-kDa IL-1 binding molecule was identified as the IL-1 decoy receptor, which represents the main IL-1R in myelomonocytic cells and an anti-IL-1 pathway (3, 11). Since TNF-α is a potent inducer of decoy IL-1RII release in monocytes and polymorphonuclear neutrophils (30) and LPS induces production of TNF-α in monocytes, it was important to investigate whether the effect of LPS on release of decoy IL-1RII was mediated by TNF-α. Using a blocking mAb specific for TNF-α, we found that the action of LPS was not mediated by TNF-α, indicating that LPS directly induced release of the decoy IL-1RII.

We recently showed that release of decoy IL-1RII mediated by TNF, chemoattractant molecules, and phorbol ester is mediated by metalloprotease(s) (31). Therefore, we investigated whether the LPS-induced release of decoy IL-1RII was also mediated by metalloprotease(s). BB-94, a classical metalloprotease inhibitor, blocked the LPS-induced release of the receptor, indicating that a common mechanism is responsible for decoy IL-1RII shedding.

LPS induces production of a number of mediators in mononuclear phagocytes, including the primary cytokines IL-1 and TNF. In preliminary experiments we found that IL-1 and, to some extent, TNF mimicked the action of LPS, having divergent effects on mRNA expression of IL-1Rs. However, IL-1Ra and anti-TNF mAb did not affect the regulation of IL-1R expression by LPS. Therefore, we conclude conclude that induction of IL-1 and TNF
does not account for the contrasting actions of LPS on IL-1R transcripts.

LPS induced expression of novel 2.4-kb IL-1RI transcripts. Others have noted the presence of similar IL-1RI-related mRNA and speculated that it might originate from usage of an alternative polyadenylation site located 518 bases 3’ of the termination codon (34). Using probes spanning the whole conventional IL-1R transcript, we determined that the 2.4-kb transcripts lack a portion of the 3’ coding region as well as 3’ untranslated sequences.

Using a combination of RT-PCR and cDNA cloning strategies, we determined that the 2.4-kb band contains polyadenylated transcripts, which all lack exon 12 (two cDNAs also lack exon 10 and 11) and contain intronic sequences. Since only three clones were sequenced, the relative abundance of different, incompletely spliced transcripts in the 2.4-kb band cannot be estimated based on these results. Based on Northern analysis with probes representative of different regions (Fig. 7), one would infer that most transcripts in the 2.4-kb band include exons 3–11. The occurrence of incompletely spliced transcripts with intronic sequences in mature polyadenylated mRNA has already been described (34–44). LPS-induced overexpression may override the capacity of the splicing machinery to generate conventional transcripts of IL-1RI.

The significance of these LPS-inducible anomalous IL-1RI 2.4-kb transcripts remains a matter of speculation. The membrane-bound, conventional IL-1RI is not susceptible to proteolytic shedding, unlike IL-1RII (Refs. 29–31 and 45 and our unpublished data). Soluble IL-1RI has been detected in normal serum (46) and in inflammatory conditions such as rheumatoid arthritis (47). One could therefore speculate that incompletely spliced transcripts may encode truncated, releasable isoforms of IL-1RI. Given the relative affinity of IL-1RI for different ligands and their relative concentration in biological fluids (1–3), soluble IL-1RI may primarily interact with and block IL-1Ra. Alternatively, the 2.4-kb band may not have physiological significance and may be a consequence of overriding of the splicing system under the pressure of LPS stimulation.

Available information is consistent with the view that IL-1RI and decoy IL-1RII have opposite roles in responses to IL-1, one being a signaling molecule in concert with IL-1AcP and the other a molecular trap for IL-1. LPS augments the expression of IL-1RI and IL-1AcP and decreases that of IL-1RII. We speculate that these effects on IL-1Rs play a role in the synergism between LPS and IL-1 observed in certain cellular systems (1, 2, 48).

Available information is consistent with a “decoy” model of function of the type II R, while the signal-transducing capacity of IL-1RI and IL-1RAcP is well established (3, 10, 11, 19). Consistently with this model, anti-inflammatory molecules, including glucocorticoid hormones and cytokines (e.g., IL-13) induce augmented expression of the decoy IL-1RII (32, 33). The observation of divergent effects of proinflammatory molecules on IL-1Rs, with stimulation of IL-1RI and inhibition of IL-1RII, is consistent with the view that these molecules subserve opposite functions in the pathophysiology of the IL-1 system. In particular, the rapid shedding of decoy IL-1RII by monocytes early in recruitment may serve to buffer the systemic action of IL-1 leaking from sites of inflammation. This early event, followed by prolonged inhibition of IL-1RII expression and up-regulation of IL-1RI and IL-1RAcP, may render monocytes more responsive to IL-1 at sites of inflammation.

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