The Differential Production of Three Forms of IL-1 Receptor Antagonist by Human Neutrophils and Monocytes

Mark Malyak, Michael F. Smith, Jr., Ashley A. Abel, Kenneth R. Hance and William P. Arend

*J Immunol* 1998; 161:2004-2010; ;
http://www.jimmunol.org/content/161/4/2004

References

This article cites 36 articles, 17 of which you can access for free at:
http://www.jimmunol.org/content/161/4/2004.full#ref-list-1

Why The JI? Submit online.

- Rapid Reviews! 30 days* from submission to initial decision
- No Triage! Every submission reviewed by practicing scientists
- Fast Publication! 4 weeks from acceptance to publication

*average

Subscription

Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

Permissions

Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts

Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts

*The Journal of Immunology* is published twice each month by The American Association of Immunologists, Inc., 1451 Rockville Pike, Suite 650, Rockville, MD 20852
Copyright © 1998 by The American Association of Immunologists All rights reserved.
Print ISSN: 0022-1767 Online ISSN: 1550-6606.
The Differential Production of Three Forms of IL-1 Receptor Antagonist by Human Neutrophils and Monocytes

Mark Malyak,* Michael F. Smith, Jr.,* Ashley A. Abel,* Kenneth R. Hance,* and William P. Arend2,©†

IL-1R antagonist (IL-1Ra) exists as three well-characterized isoforms. The 17-kDa secretory IL-1Ra (sIL-1Ra) and 18-kDa intracellular IL-1Ra (icIL-1RaI) arise by alternative transcription of the same IL-1Ra gene. The recently described 16-kDa intracellular IL-1Ra (icIL-1RaII) is formed by alternative translation initiation of sIL-1Ra mRNA. Transcription and translation of IL-1Ra isoforms were examined in LPS-stimulated human neutrophils and PBMC using RT-PCR, ELISA, and Western blot analysis. LPS stimulation of neutrophils resulted in elevated sIL-1Ra mRNA levels by 1 h, whereas icIL-1RaI mRNA remained undetectable through 22 h of culture. Extracellular glycosylated sIL-1Ra protein and intracellular icIL-1RaII were observed in LPS-stimulated neutrophils by 3 h of culture; no icIL-1RaI protein was detected by immunoblot. LPS stimulation of PBMC resulted in elevated sIL-1Ra mRNA levels by 1 h and detectable icIL-1RaI mRNA at 8 h of culture. LPS-stimulated PBMC demonstrated extracellular glycosylated sIL-1Ra protein and intracellular icIL-1RaII within 3 h of stimulation, whereas detection of icIL-1RaI protein was delayed until 15 h of culture. Subcellular localization experiments established that both icIL-1RaI and icIL-1RaII were present primarily within the cytoplasmic compartment, as expected by their lack of a signal peptide. These results demonstrate that although both LPS-stimulated neutrophils and PBMC synthesize sIL-1Ra and icIL-1RaII, only PBMC transcribe and translate icIL-1RaI. Furthermore, sIL-1Ra transcription and translation (and translation of icIL-1RaII) are early events, whereas icIL-1RaI transcription in PBMC is delayed. The Journal of Immunology, 1998, 161: 2004–2010.

The IL-1 family of cytokines includes two agonists, IL-1α and IL-1β, and a specific receptor antagonist, IL-1Ra (1–4). While IL-1α and IL-1β play proinflammatory roles in the pathophysiology of various human diseases, either endogenously produced or exogenously administered IL-1Ra is potentially anti-inflammatory (5–12). Three different isoforms of IL-1Ra have been described, one secreted and two intracellular (4, 13–16). Secretory IL-1Ra (sIL-1Ra) and the original intracellular isoform (icIL-1Ra) are transcribed from the same gene by alternative RNA splicing. The sIL-1Ra is a major secreted product of monocytes, macrophages, neutrophils, and other cells, whereas icIL-1Ra is found constitutively in large amounts in keratinocytes and other epithelial cells (14, 17).

A novel 16-kDa intracellular isoform of IL-1Ra has recently been described by our laboratory (15). We have proposed to name the 16-kDa isoform icIL-1RaII, and the earlier described 18-kDa icIL-Ra isoform icIL-1RaI. The icIL-1RaII is formed by alternative translation initiation from sIL-1Ra mRNA (15). The 5' AUG of sIL-1Ra is the translation start site for sIL-1Ra protein.

The ability of neutrophils and PBMC to transcribe and translate both IL-1α and IL-1β has been demonstrated (18–26). However, a complete profile of the size and species of IL-1Ra RNA and protein produced by neutrophils and PBMC has not been elucidated in detail. The present study used RT-PCR to examine sIL-1Ra and icIL-1RaI steady state mRNA levels in LPS-stimulated neutrophils and PBMC. Extracellular and cell-associated IL-1Ra protein production was quantified by ELISA, and the specific IL-1Ra protein species present were determined by Western blot analysis. The subcellular location of the intracellular isoforms was determined after nitrogen cavitation and differential centrifugation.

Materials and Methods

Isolation and culture of neutrophils and PBMC

Neutrophils were isolated from the peripheral blood of normal human donors using a previously described technique involving dextran sedimentation and Lymphoprep gradient centrifugation (20). All preparations were >99% neutrophils with <0.5% monocyte contamination as determined by light and non-specific esterase staining. PBMC were isolated from normal human donors using Lymphoprep gradient centrifugation as previously described (27). Preparations were approximately 25% monocytes and 75% lymphocytes as determined by modified Wright and non-specific esterase staining. Cell viability in both neutrophils and PBMC was >99% at the initiation of cell cultures as determined by trypan blue dye exclusion.

Neutrophils and PBMC were cultured at 37°C in 5% CO₂ at a concentration of 5 × 10⁶ and 3 × 10⁷ cells/ml respectively, in medium alone or with LPS (Escherichia coli 055:B5, Difco, Detroit, MI) at various concentrations for periods ranging from 1 to 66 h. In preliminary dose-response experiments, maximal stimulation of neutrophils at 22 h was observed with...
5 μg/ml LPS, and maximal stimulation of PBMC at 44 h was observed with 0.1 μg/ml LPS; these LPS concentrations were used in all subsequent experiments. All cultures contained RPMI 1640 medium (Mediatec, Herndon, VA), 1 mM L-glutamine, 100 μg/ml streptomycin, and 100 U/ml penicillin. Additionally, neutrophils and PBMC were cultured in 5% and 2% heat-inactivated, low endotoxin FCS (Summit Biotechnology, Greeley, CO), respectively. Cell cultures for IL-1Ra ELISA, Western blot analysis, and RT-PCR were performed in parallel from the same donors.

**IL-1Ra ELISA**

Cell-free supernatants were isolated from neutrophils and PBMC cultured in 1-ml volumes in 10 mm wells, and cell lysates were generated by adding 1 ml of fresh medium to the isolated cells followed by three freeze-thaw cycles. IL-1Ra protein concentrations were measured in culture supernatants and cell lysates using a modified standard sandwich ELISA (15, 28). ELISA was performed in triplicate for each supernatant and lysate sample; the range of triplicate values for each sample was <10%. Proteinase inhibitors added to cells before lysis did not alter levels within cell lysates (cell-associated IL-1Ra) and culture supernatants (extracellular IL-1Ra). Data are expressed as nanograms of cell-associated and total IL-1Ra per 10^6 cells (mean ± SEM) based on seven neutrophil and five PBMC experiments. Note the different x- and y-axis scales in A and B.

**IL-1Ra Western blot analysis**

IL-1Ra Western blot analyses, using a recently described anti-sIL-1Ra mAb, were performed on cell lysates and culture supernatants obtained from neutrophils and PBMC cultured in 5-ml volumes on 60-mm plates (29). Cell lysates were generated using a lysis buffer containing 0.5% Nonidet P-40 in 20 mM Tris, pH 7.5, and the following proteinase inhibitors: 1 μg/ml leupeptin, 1 μg/ml pepstatin, 1 mM PMSF, and 1 mM EDTA. Iodoacetamide (1 mM) and 0.2 mM N-methoxy succinyl-Ala-Ala-Pro-Val (Sigma, St. Louis, MO) were used as additional proteinase inhibitors in some experiments without any effect on the amount or size of IL-1Ra produced. In some experiments, cell lysates and culture supernatants were treated with N-glycanase according to the manufacturer’s instructions (Genzyme Institute, Cambridge, MA). Cell lysates (15 μg of total protein) and culture supernatants (undiluted) were heated to 95°C for 5 min in the presence of a reducing loading buffer (0.0625 M Tris base, 1.5% SDS, 5% glycerol, 2.5% β-ME, and 0.05% bromophenol blue, final pH 6.8). Samples were then electrophoresed on 15% polyacrylamide gels with 0.1% SDS followed by electrophoretic transfer to nitrocellulose paper, and the procedure was completed as previously described (29).

**RT-PCR of sIL-1Ra and icIL-1Ra mRNA**

Total cellular RNA was obtained from neutrophils and PBMC cultured in 10-ml volumes on 100-mm plates by guanidinium isothiocyanate extraction and CsCl gradient centrifugation (30). RT-PCR of sIL-1Ra and icIL-1Ra mRNA was performed as previously described (20). Amplification of the synthesized cDNA for sIL-1Ra and icIL-1Ra was performed separately for each sample. As a control, glyceraldehyde-3-phosphate dehydrogenase (G3PDH) cDNA was amplified simultaneously within the same reactions as sIL-1Ra and icIL-1Ra cDNA amplification. PCR amplification was performed with the following primers (5′ to 3′): 5′ sIL-1Ra, GCC CTC CGC AGT CAC CTA ATC ACT CT; 5′ icIL-1Ra, CAG GTA CTT CCC GGG TGC TAC TTT AT; 3′ common IL-1Ra, TAC TAC TCG TCC TCC TGG AAG TAG AA; 5′ G3PDH, AAG GTG AAG GTC GGA GTC GGA GTC AAG G; and 3′ G3PDH, CCT TCT CCA TGG TGA AGA C.

Thirty cycles of PCR were performed, each cycle consisting of 94°C for 90 s, 45°C for 90 s, and 72°C for 90 s. The amplified products were then electrophoresed within a 1% agarose gel containing ethidium bromide. DNA bands were visualized by UV fluorescence and photographed. The amplified cDNA products of icIL-1Ra, sIL-1Ra, and G3PDH were 577, 521, and 316 bp in size, respectively.

**Subcellular localization of IL-1Ra in neutrophils and PBMC**

Subcellular localization of IL-1Ra was performed in freshly isolated and LPS-stimulated neutrophils and PBMC using a modification of a previously described technique (31). Plasma membrane disruption was performed by nitrogen cavitation, which was modified to allow disruption of plasma membranes while leaving nuclear membranes intact. Upon release from the cell disruption bomb (Kontes, Vineland, NJ), the cavitate was collected, and an aliquot was examined by phase contrast microscopy, trypsin blue dye stain, and modified Wright stain for efficiency of plasma membrane disruption in the absence of disruption of nuclear membranes. Nuclei, granules, plasma membrane/endoplasmic reticulum, and cytoplasmic compartments were isolated by differential centrifugation (31). To determine the efficiency of subcellular separation, assays for lactate dehydrogenase and β-glucuronidase (Sigma) content were performed within each compartment as markers for the cytoplasm and granule compartments, respectively. Assays were performed according to the manufacturer’s recommendations. Additionally, an assay for DNA content was performed in each subcellular compartment as a marker for the nuclear compartment.

**FIGURE 1.** Effects of LPS stimulation on neutrophil (A) and PBMC (B) production of cell-associated and extracellular IL-1Ra protein. Freshly isolated (0 h) neutrophils and PBMC were cultured in the presence or the absence of LPS for the indicated intervals. Upon completion of culture, IL-1Ra protein concentrations were measured in culture supernatants and cell lysates by ELISA. Total IL-1Ra was determined by adding the amounts of IL-1Ra present within cell lysates (cell-associated IL-1Ra) and culture supernatants (extracellular IL-1Ra). Data are expressed as nanograms of cell-associated and total IL-1Ra per 10^6 cells (mean ± SEM) based on seven neutrophil and five PBMC experiments. Note the different x- and y-axis scales in A and B.
IL-1Ra protein was examined within each compartment by ELISA and Western blot analysis.

**Results**

**Neutrophil and PBMC production of cell-associated and extracellular IL-1Ra**

The effects of culture in LPS or medium alone on neutrophil and PBMC production of cell-associated and extracellular IL-1Ra as determined by ELISA of cell lysates and supernatants are shown in Figure 1. Freshly isolated neutrophils contained 0.19 ± 0.04 ng/10⁶ cells (mean ± SEM; n = 7) of cell-associated IL-1Ra (Fig. 1A). Total IL-1Ra levels (sum of supernatants and lysates) did not appreciably change in neutrophils cultured in medium alone for intervals up to 22 h, although there were small decreases in cell-associated levels with reciprocal increases in extracellular IL-1Ra levels. LPS stimulation (5 μg/ml) of neutrophils resulted in a 5.1-fold increase in total IL-1Ra by 22 h of culture, with minimal further change through 44 h. Culture in medium alone through 22 h led to no increase in cell-associated IL-1Ra compared with freshly isolated neutrophils. A small amount of approximately 23-kDa IL-1Ra was also present in lysates from neutrophils stimulated for 3 and 8 h with LPS and was determined by N-glycanase treatment to be glycosylated sIL-1Ra (data not shown). There were no bands detected within neutrophil lysates that aligned with recombinant 17-kDa sIL-1Ra or 18-kDa icIL-1Ra.

Freshly isolated PBMC also contained small amounts (0.20 ± 0.06 ng/10⁶ cells) of cell-associated IL-1Ra (n = 8), and this quantity did not appreciably change when PBMC were cultured in medium alone through 44 h of culture (Fig. 1B). LPS stimulation (100 ng/ml) of PBMC resulted in a 22-fold increase in total IL-1Ra by 44 h of culture, approximately 47% cell associated. Neutrophil and PBMC viability were >90% at the end of 22 and 44 h of culture, respectively, as determined by trypan blue dye exclusion.

**Neutrophil and PBMC production of sIL-1Ra, icIL-1RaI, and icIL-1RaII**

To determine which isoforms accounted for cell-associated and extracellular IL-1Ra in neutrophils and PBMC as determined by ELISA, Western blot analyses of lysates and supernatants from neutrophils and PBMC cultured in the absence or the presence of LPS were performed (Fig. 2). Regardless of culture conditions, the predominant band present within neutrophil lysates was 16 kDa icIL-1RaII (Fig. 2A, upper). The icIL-1RaII was present in freshly isolated neutrophils, and LPS stimulation resulted in substantial increases in icIL-1RaII in neutrophil lysates by 22 h of culture, with minimal further change through 22 h. Culture in medium alone through 22 h led to no increase in icIL-1RaII compared with freshly isolated neutrophils. A small amount of approximately 23-kDa IL-1Ra was also present in lysates from neutrophils stimulated for 3 and 8 h with LPS and was determined by N-glycanase treatment to be glycosylated sIL-1Ra (data not shown). There were no bands detected within neutrophil lysates that aligned with recombinant 17-kDa sIL-1Ra or 18-kDa icIL-1Ra. To determine
whether inadvertent proteolysis of sIL-1Ra peptide during preparation of neutrophils for Western blot analysis was responsible for the observed smaller species, multiple proteinase inhibitors were added to cells before lysis; no differences were observed in the amount or size of the IL-1Ra present.

Western blot analysis of culture supernatant from LPS-stimulated neutrophils revealed a band approximately 23 kDa in size (Fig. 2A, lower). This 23-kDa IL-1Ra species was determined to be glycosylated sIL-1Ra by N-glycanase treatment, as previously described (20). The 16-kDa icIL-1RaI protein was not observed in culture supernatants. Although the IL-1Ra ELISA cannot distinguish among the three species of IL-1Ra, these results demonstrate that the species measured by ELISA in neutrophil supernatants and lysates, respectively, are predominantly sIL-1Ra and icIL-1RaI.

Although 16-kDa icIL-1RaI protein was not present in all samples of freshly isolated PBMC by Western blot analysis (Fig. 2B, upper), LPS stimulation resulted in detectable icIL-1RaI in PBMC lysates by 8 h of culture, which further increased by 44 h in every experiment (n = 5). A small amount of approximately 23-kDa IL-1Ra was also present in lysates from PBMC stimulated for 8 to 22 h with LPS and was determined by N-glycanase treatment to be glycosylated sIL-1Ra (Fig. 3). An 18-kDa band in PBMC lysates became evident by 15 h of LPS stimulation and increased in amount by 44 h. Western blot analysis of culture supernatants from LPS-stimulated PBMC revealed a predominant band of approximately 23 kDa in size with a faint band at 18 kDa (Fig. 2B, lower). The larger IL-1Ra species in PBMC supernatants was previously determined to be glycosylated sIL-1Ra by N-glycanase studies (33). These results suggest that the IL-1Ra measured by ELISA in PBMC lysates primarily consists of both intracellular forms, whereas PBMC supernatants contain mainly glycosylated sIL-1Ra.

Secretory IL-1Ra and icIL-1RaI steady state mRNA levels in neutrophils and PBMC

The sIL-1Ra and icIL-1RaI steady state mRNA levels in neutrophils and PBMC cultured in the absence or presence of LPS were determined by RT-PCR (Fig. 4). Freshly isolated neutrophils contained relatively low levels of sIL-1Ra mRNA (Fig. 4A, upper). The sIL-1Ra steady state mRNA levels in neutrophils cultured in medium alone declined by 1 h and remained low through 44 h of culture. LPS stimulation of neutrophils resulted in substantial increases in sIL-1Ra steady state mRNA levels by 1 h of culture, which were maintained through 8 h. By 44 h of culture, LPS-induced sIL-1Ra mRNA levels had decreased substantially, yet remained greater than those in neutrophils cultured in medium alone.

The icIL-1RaI mRNA was undetectable in freshly isolated neutrophils and in cells cultured in medium alone (Fig. 4A, bottom). Furthermore, LPS stimulation of neutrophils for intervals ranging from 1 to 44 h failed to generate detectable levels of icIL-1RaI mRNA. To determine whether icIL-1RaI transcription was dependent upon LPS concentration, neutrophils were cultured for 24 h in medium alone declined by 1 h and remained low through 44 h of culture. LPS stimulation of neutrophils resulted in substantial increases in sIL-1Ra steady state mRNA levels by 1 h of culture, which were maintained through 8 h.
LPS concentrations ranging from 10 ng/ml to 100 µg/ml; icIL-1Ra mRNA remained undetectable in each of these conditions. As a control, the keratinocyte cell line A431 contained readily detectable levels of icIL-1Ra mRNA.

Freshly isolated PBMC also contained relatively low levels of sIL-1Ra mRNA (Fig. 4B, upper). The sIL-1Ra steady state mRNA levels increased slightly by 1 h of culture in medium alone, falling to baseline levels by 22 h of culture. LPS stimulation resulted in substantial increases in sIL-1Ra mRNA by 1 h that remained elevated through 22 h of culture, after which levels decreased substantially yet remained greater than those in PBMC cultured in medium alone. The icIL-1Ra mRNA was undetectable in freshly isolated PBMC and in cells cultured in medium alone (Fig. 4B, lower). However, LPS stimulation of PBMC resulted in detectable icIL-1Ra mRNA by 8 h of culture, increasing to maximal levels by 15 h and subsequently falling to low but still detectable levels by 66 h.

Subcellular localization of IL-1Ra peptide in neutrophils and PBMC

Subcellular localization of cell-associated IL-1Ra in neutrophils and PBMC was determined by ELISA and Western blot analysis of subcellular compartments generated by nitrogen cavitation and differential centrifugation. The subcellular compartments generated by this procedure were 1) intact nuclei, 2) granules, 3) plasma membrane/endoplasmic reticulum, and 4) cytoplasm. Phase contrast microscopic evaluation of nitrogen bomb cavitations revealed <10% intact cells. Lactate dehydrogenase (cytoplasm), β-glucuronidase (granules), and DNA (nuclei and intact cells) assays performed on each subcellular compartment demonstrated minimal contamination between compartments (Table I), with the exception of β-glucuronidase. This marker was present in the nuclear as well as the granular compartments, a result also obtained by the investigators who described this technique (31). The most important point for our results was that the cytoplasmic compartment demonstrated low levels of markers for nuclei and granules.

In freshly isolated unstimulated neutrophils, 94% of the total cell-associated IL-1Ra was present within the cytoplasm, with very small amounts observed in other compartments (Table II). When neutrophils were cultured in the absence or the presence of LPS (5 µg/ml) for 4 h, the percentage of IL-1Ra present within the granules increased slightly, but did not change appreciably in other compartments.

Because of the demonstrated late appearance of icIL-1Ra protein in LPS-stimulated PBMC, subcellular localization experiments were performed with PBMC cultured in the presence or the absence of LPS (100 ng/ml) for intervals of 0, 3, and 22 h (Table III). In freshly isolated PBMC, 100% of the intracellular IL-1Ra was present within the cytoplasm. Regardless of PBMC culture condition or duration, >94% of IL-1Ra remained within the cytoplasmic compartment, with only small amounts (<5%) appearing within the other compartments.

**Discussion**

These studies characterized production of the multiple isoforms of IL-1Ra by LPS-stimulated human neutrophils and PBMC. Both neutrophils and PBMC transcribed and translated sIL-1Ra within 3 h of LPS stimulation. The icIL-1RaII was demonstrated to be an inducible cytoplasmic protein that followed the kinetics of sIL-1Ra in both neutrophils and PBMC, consistent with the finding that icIL-1RaII is a product of alternative translation initiation from the

### Table I. Markers for subcellular compartments

<table>
<thead>
<tr>
<th>Subcellular Compartment</th>
<th>Neutrophils</th>
<th>PBMC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DNA β-glucuronidase LDH</td>
<td>DNA β-glucuronidase LDH</td>
</tr>
<tr>
<td>Nuclei</td>
<td>1.5 ± 9.8</td>
<td>6.3 ± 2.8</td>
</tr>
<tr>
<td>Granules</td>
<td>3.4 ± 12.5</td>
<td>1.4 ± 3.8</td>
</tr>
<tr>
<td>Plasma memb/ER</td>
<td>1.3 ± 1.3</td>
<td>4.2 ± 3.4</td>
</tr>
<tr>
<td>Cytoplasm</td>
<td>3.3 ± 3.4</td>
<td>89 ± 1.8</td>
</tr>
</tbody>
</table>

### Table II. Subcellular localization of IL-1Ra in neutrophils

<table>
<thead>
<tr>
<th>Subcellular Compartment</th>
<th>0 h cells, IL-1Ra</th>
<th>4 h medium, IL-1Ra</th>
<th>4 h LPS, IL-1Ra</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ng/10^6 cells</td>
<td>% of total</td>
<td>ng/10^6 cells</td>
</tr>
<tr>
<td>Nuclei</td>
<td>0.85</td>
<td>3.3</td>
<td>1.54</td>
</tr>
<tr>
<td>Granules</td>
<td>0.65</td>
<td>2.6</td>
<td>0.38</td>
</tr>
<tr>
<td>Plasma memb/ER</td>
<td>0.12</td>
<td>0.5</td>
<td>0.08</td>
</tr>
<tr>
<td>Cytoplasm</td>
<td>23.8</td>
<td>93.6</td>
<td>34.6</td>
</tr>
</tbody>
</table>

*Neutrophils were isolated and cultured in the presence or absence of LPS as described in Materials and Methods. Time 0 h represents the point of completion of the neutrophil isolation process. IL-1Ra peptide concentrations were measured in each subcellular compartment by ELISA. The data are expressed as ng IL-1Ra/10^8 neutrophils, representing the mean of triplicate determinations. The data are representative results from one experiment of five performed.*
sIL-1Ra mRNA (15). The other major findings reported herein included that icIL-1RaI mRNA and protein were not produced by LPS-stimulated neutrophils, and that icIL-1Rai was present in LPS-stimulated PBMC as a result of delayed transcription. Finally, both icIL-1RaI and icIL-1RaiII were present predominantly within the cytoplasmic compartment, as expected by their lack of a signal peptide.

The biologic role of icIL-1RaiI (and icIL-1Ral) is speculative at this time. icIL-1RaiI may function as an intracellular store of IL-1Ra, being released into the extracellular space upon necrotic cell death where it may then bind to cell surface receptors and block the stimulatory effects of IL-1. Alternatively, icIL-1RaiI may have intracellular effects separate from binding to IL-1R. Intracellular IL-1α has been demonstrated to promote senescence in human endothelial cells after transport to the nucleus (34, 35). Conceivably, icIL-1RaiI may play a role counter-regulatory to the intracellular effects of IL-1. Alternatively, icIL-1RaiI may also play an intracellular role similar to that observed for icIL-1RaiI involving alterations in IL-1-induced gene expression (36).

Although the capacity of monocytes to produce sIL-1Ra and icIL-1Rai has previously been demonstrated, this is the first study examining differential regulation of sIL-1Ra and icIL-1Rai transcription and translation in LPS-stimulated PBMC (3, 25). Whereas sIL-1Ra mRNA levels increased within 1 h upon LPS stimulation, icIL-1Rai mRNA was not detectable until 8 h of stimulation. Corresponding with the mRNA results, extracellular glycosylated sIL-1Ra and intracellular icIL-1Rai were detectable at 3 and 15 h of LPS stimulation, respectively. Small quantities of glycosylated sIL-1Ra were also noted within cell lysates at 8 to 22 h of LPS stimulation and probably represent newly translated and glycosylated sIL-1Ra within the secretory pathway. These observations extend earlier morphologic findings of a later cytoplasmic appearance of IL-1Ra protein in monocytes (37). The icIL-1Rai mRNA may be a delayed gene product, requiring de novo synthesis of transcription factors. Alternatively, LPS may be an indirect inducer of icIL-1Rai in monocytes, analogous to TGF-β induction of IL-1Ra in monocytes through IL-1 (38).

Neutrophils have previously been demonstrated to produce IL-1Ra protein, and our results indicate that these cells transcribe only the sIL-1Ra mRNA. Although no production of icIL-1Rai mRNA or protein by neutrophils was observed in the present study, Muzio et al. concluded that human neutrophils stimulated with TGF-β1 or IL-13 transcribed and translated icIL-1Rai, as determined by RT-PCR and ELISA of cell lysates (24, 25). Since the IL-1Ra ELISA cannot distinguish among the three forms of IL-1Ra, it is possible that the IL-1Ra assayed by ELISA in neutrophil lysates in the previous studies was the newly described icIL-1RaiI and not icIL-1RaiII. Furthermore, the degree of monocyte contamination in the previously reported studies was probably greater than that in the present study. Since monocytes produce 100-fold more IL-1Ra per cell than neutrophils, it is possible that the observed icIL-1Rai mRNA levels in the neutrophil preparations in the previous studies may be secondary to monocyte contamination.

Muzio et al. also cloned a unique IL-1Ra cDNA in neutrophils that was larger than icIL-1Rai cDNA and that was concluded to arise by inclusion of a 63-bp segment present within the first intron of the IL-1Ra gene (39). In the present experiments, no IL-1Ra mRNA or intracellular protein corresponding to this species was observed in LPS-stimulated neutrophils or PBMC. In a pattern similar to that in PBMC, small quantities of glycosylated sIL-1Ra were noted within neutrophil lysates at 3 to 8 h of LPS stimulation, probably representing newly transcribed and glycosylated sIL-1Ra within the secretory pathway.

In summary, the results of this study demonstrate a differential production of the multiple isoforms of IL-1Ra by LPS-stimulated neutrophils and PBMC. The early transcription and translation of sIL-1Ra by both neutrophils and PBMC may play an important role in regulating the cytokine effects of IL-1 in the extracellular environment. Late transcription and translation of icIL-1Rai in PBMC conceivably play an intracellular regulatory role as acute inflammation resolves or becomes chronic. Finally, icIL-1RaiI is translated by both neutrophils and PBMC, and potentially may also play an intracellular regulatory role. Further studies are necessary to determine the possible biologic role of both intracellular forms of IL-1Ra in normal cell physiology and in pathophysiologic conditions.

Acknowledgments

We thank Dr. Peter Ralph of the Chiron Corp. (Emeryville, CA) for his generous contribution of human recombinant icIL-1Rai.

References


