Vol. 209, No. 1, 1995 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
April 6, 1995 Pages 279-285

GONADAL STEROIDS MODULATE INTERLEUKIN-1 RECEPTOR ANTAGONIST mRNA EXPRESSION IN CULTURED HUMAN MONOCYTES

Bo Yon Lee‡, Tri Huynh§, Lynn E. Prichard‡, Joseph McGuire§, and Mary Lake Polan‡*

‡Department of Gynecology and Obstetrics, § Department of Dermatology
Stanford University School of Medicine, Stanford, CA 94305

Received March 1, 1995

This study investigated whether gonadal steroids modulate the expression of the cytokine Interleukin-1 receptor antagonist in monocytes. Human male peripheral monocytes were isolated and cultured in serum free media with serially diluted concentrations of estradiol and progesterone. mRNA expressions with increasing steroid concentrations were compared by reverse transcription-polymerase chain reaction for intracellular and secretory interleukin-1 receptor antagonist specific primers and glyceraldehyde 3-phosphate dehydrogenase primers. Monocyte expression of secretory Interleukin-1 receptor antagonist mRNA was significantly elevated in the presence of normal physiological levels of estradiol (10^{-11} M) and progesterone (10^{-8} M), while expression was suppressed by higher concentrations of steroids. Intracellular receptor antagonist was also detected . This study is the first to describe the dose related response of cytokine interleukin-1 receptor antagonist to gonadal steroids.

Interleukin-1 receptor antagonist (IL-1ra) is a member of interleukin-1 (IL-1) family and inhibits the biological action of IL-1 by binding competitively to IL-1 receptors and preventing IL-1 signal transduction. Complementary DNA cloning has revealed the presence of two mRNA sequences, an intracellular IL-1ra (icIL-1ra) form which is produced by many epithelial cells and a secretory IL-1ra (sIL-1ra) produced by circulating immune cells such as monocytes and neutrophils (1,2) . The effects of IL-1ra expression by monocytes and keratinocytes and their biological sequelae have been described (3,4). Although the definitive role of IL-1ra in human reproduction is not clearly understood, recent reports have documented the involvement of the immune cytokine IL-1ra in implantation, parturition and postmenopausal bone loss (5-7). The involvement of IL-1ra in these processes, which are closely linked

* To whom correspondence should be sent. FAX: 415-723-7737.

Abbreviations : IL-1 (Interleukin-1), IL-1ra (Interleukin-1 receptor antagonist), icIL-1ra (intracellular interleukin-1 receptor antagonist), sIL-1ra (secretory interleukin-1 receptor antagonist), GAPDH (glyceraldehyde 3-phosphate dehydrogenase).

0006-291X/95 $5.00

to changes in gonadal steroid levels, prompted us to investigate the effect of estrogen and progesterone on IL-1ra expression in human peripheral monocytes.

Materials and Methods

Materials : RPMI-1640 medium, L-glutamine and gentamicin were obtained from GIBCO Laboratories, Grand Island NY. Penicillin-streptomycin, nystatin, BSA (Fraction V), progesterone and estradiol were from Sigma Chemical Co., St. Louis, MO. Lymphocyte Separation Media (LSM) was obtained from Organon Teknika Corporation, Durham, NC. RNA isolation media (RNAzol) was from Tel Test, Friendswood, TX. Reagents for reverse transcription-polymerase chain reaction (RT-PCR) were obtained from Perkin Elmer Cetus, Norwalk, CT. A SR1 enzyme immunoassay kit from Serono Diagnostic S.A. was used to check estradiol and progesterone levels in serum and culture media. Based on the published cDNA sequence (1,2), icIL-1ra, sIL-1ra specific primer pairs and human glyceraldehyde 3-phosphate dehydrogenase (GAPDH) specific primer pair were synthesized at the Genset Corp. La Jolla, CA. The specific sequence of the primers were described (Table 1).

Monocyte isolation and culture : Monocytes were isolated as described (8) Peripheral blood 100-150 ml was obtained from each four healthy male volunteers with no recent history of infection or illness. Written consents from volunteers were obtained and approved by the Institutional Committee on the Right of Human Subjects at Stanford University. Isolation procedures were carried out at < 4 °C. Isolated monocytes were diluted to a density 1 x 10^6 cells/ml in complete RPMI medium, pH 7.4 containing 25 mM HEPES buffer, 0.1% BSA, 2mM L-glutamine, 1% penicillin streptomycin, 0.05 mg/ml gentamicin and 0.05 ng/ml nystatin. Two milliliters of medium containing monocytes were plated in 35x10 mm culture dishes and incubated at 37 °C in 5 % CO_2, with the indicated concentration of either progesterone or estradiol. After 6 h, cells were pelleted in preparation for RNA extraction. Estradiol and progesterone concentrations in complete RPMI medium containing 0.1% BSA were checked by enzyme immunoassay and found to be < 10^{-11} M estradiol and < 10^{-8} M progesterone. As a 0 h control, cells were centrifuged at 4 °C immediately after isolation, the pellet was snap frozen and stored at -80 °C.

RNA preparation : Total cellular RNA was obtained by the RNAzol method as described (9). The RNA pellet was washed twice in 75% ethanol, dried and resuspended in 10 µl diethylpyrocarbonate treated water. The purity and yield of total RNA was determined spectrophotometrically.

Reverse Transcription-Polymerase Chain reaction : For RT-PCR, the GeneAmp RNA PCR kit was used. Reactions were started from 0.25 µg of total RNA in a total volume of 50 µl containing 1 mM each of dNTPs, 2.5µM oligo d(T)16, 50 U RNAase inhibitor and 125 U reverse transcriptase in reaction buffer containing 5 mM $MgCl_2$ and 10 x PCR buffer (500 mM KCl, 100 mM TrisHCl, pH 8.3). First strand cDNA were obtained after one cycle of 10 min at 25 °C, 15 min at 42 °C, 5 min 95 ° in the Perkin Elmer Cetus thermal cycler 480. Aliquots of the RT product were subjected to PCR in the presence of a master mix containing PCR buffer, 2mM $MgCl_2$, 2.5 U AmpliTaq DNA polymerase and paired primers for either sIL-1ra or icIL-1ra to a total volume of 50 ul. Final concentrations of each primers were 2.5 pmole/ul. Human GAPDH specific primers were also used to normalize the starting amount of cDNA for each sample. For comparison among samples, PCR was performed simultaneously from a single

Table I. Oligonucleotide primers for mRNA amplification

mRNA	Product size (bp)	Primers 5'-3'
icIL-1ra	575	GTCCATGACGGGCCCACGATGAAATA
		CTACTCGTCCTCCTGGAAGTA
sIL-1ra	519	CCGGAGGCGTCAGTGGATTAGTGAGA
		CTACTCGTCCTCCTGGAAGTA
GAPDH	581	CTACTCGTCCTCCTGGAAGTA
		GAGTGGGTGTCGTTGAAGTCAGA

master mix in the different tubes with each primer pair. After the first denaturation at 95 °C for 2 min, PCR cycles were 95 °C for 25 s, 60 °C for 30 s and 72 °C for 25 s. Final extension was at 72 °C for 3 min. In the study to assess the optimal amplification cycle for each primers, each sets of serially diluted samples were taken at the end of a determined number of cycles and immediately incubated at 72 °C for 3 min. Whole sets of products in each determined cycles were stored at -20 °C and electrophoresed simultaneously.

Comparison of mRNA expression by RT-PCR :Equal volume of each PCR samples were analyzed electrophoretically in parallel with size markers on a 1% agarose gel. Gels were stained with ethidium bromide and photographed with Polaroid 665 film under uv light. Densitometric analysis was done using a Scanning Densitometer GS-300 from Hoefer Scientific Instruments.

The optimal number of cycles of PCR for each pair of primers was assessed as described in results. Because the amount of amplified PCR product showed variance in each experiment, the intensity of secretory and intracellular IL-1ra product bands were normalized using the intensity of simultaneously amplified GAPDH product by loading an equal volume of product from GAPDH and sIL-1ra primers simultaneously on the gel.

Statistics : Group means for steroid dose response study were compared by paired t-test and ANOVA.

Results

The functional integrity of sIL-1ra and icIL-1ra specific primers were confirmed using RNA samples extracted from cultured monocytes and keratinocytes as controls (figure 1).

To determine the optimal number of amplification cycles for each primer pair, 4 μg of total RNA from a 6 h cultured control was serially diluted (4 μg, 1 μg, 250 ng, 60 ng) and an equal volume of dilutant was reverse transcribed. The RT products from each dilution were amplified simultaneously with 20, 25, 30, 35, 40 and 45 cycles of PCR for each primer pair. GAPDH specific products showed a 581 bp size band on 1% agarose gel (figure 2A). On densitometry of GAPDH production cycles, 25 and 30 cycles of amplification showed a linear increase in density with the initial concentration of total RNA (figure 2B). Secretory IL-1ra specific products showed no band at 20 cycles of PCR, but a 519 bp sIL-1ra specific band was visualized at all dilutions of RNA after 25 cycles of PCR (figure 3A). The quantity of sIL-1ra specific product increased directly with initial concentration of total RNA only at 25 cycles of PCR

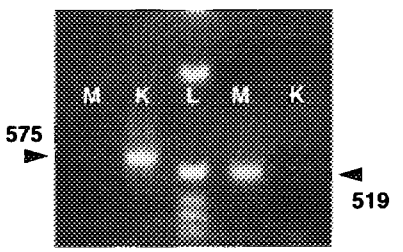

Figure 1. Integrity of IL-1ra specific primers. As the controls, extracted RNAs from cultured monocytes and keratinocytes were reverse transcribed and amplified with icIL-1ra (left two lanes) and sIL-1ra (right two lanes) specific primers. After 35 cycles of PCR, keratinocyte produced a 575-bp band on the 1% agarose gel corresponding icIL-1ra. Similarly, Monocytes produced a 519-bp band corresponding to sIL-1ra. M, monocytes; K, keratinocytes; and L, DNA ladder.

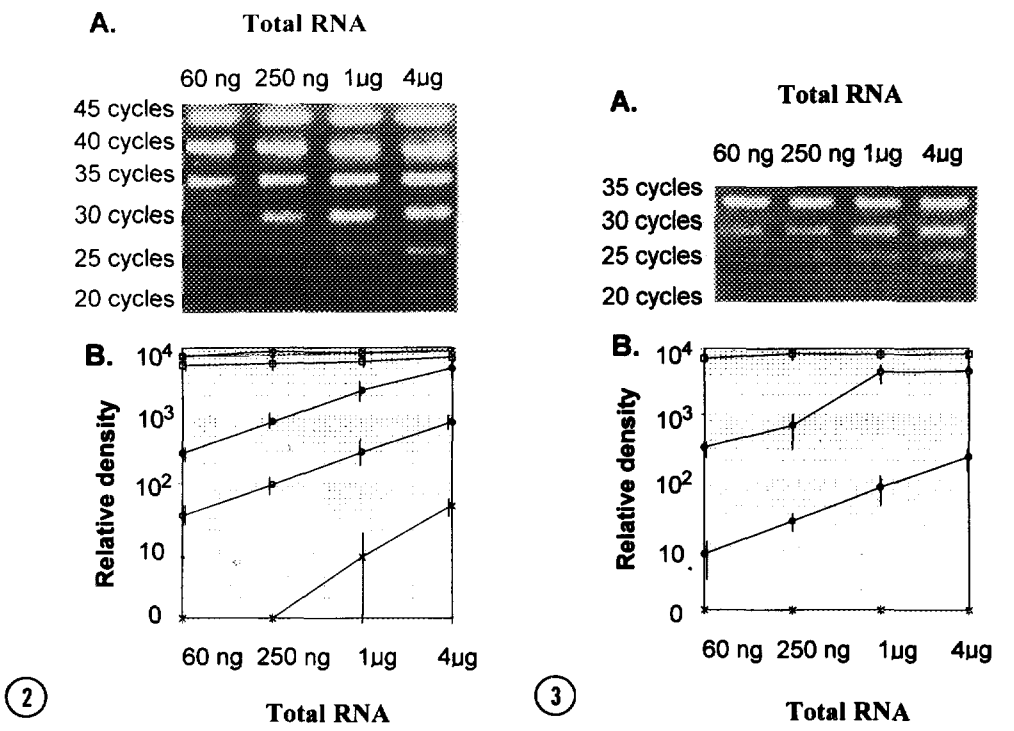

Figure 2. Optimal amplification cycle for GAPDH primers. Serially diluted (60 ng, 250 ng, 1µg, 4µg) total RNA samples were reverse transcribed and amplified with GAPDH primers. Equal volumes of amplified products were electrophoresed at 1% agarose gel and stained with ethidium bromide (A). On densitometry, 25 and 30 cycles of amplification showed linear increase in density with the initial concentrations of total RNA (B). 20 (-X-); 25 (-O-); 30 (-●-); 35 (-■-); 40 (-□-) and 45 (-◆-) cycles. Values are the mean ± STD of 6 experiments.

Figure 3. Optimal amplification cycle for sIL-1ra primers. With the same method as GAPDH primers, amplified products from sIL-1ra primers were electrophoresed (A). On densitometry, 25 cycles of amplification showed linear increase in density with initial concentrations of total RNA (B). 20 (-X-), 25 (-O-), 30 (-●-)and 35 (-□-) cycles. Values are the mean ± STD of 6 experiments.

(figure 3B). A specific icIL-1ra band of 575 bp was detected at 45 cycles of PCR ,also demonstrating linear increase related to the starting amount of total RNA (figure 4A,B). Because the expression of mRNA was optimally comparable at 25 cycles of PCR for both sIL-1ra and GAPDH primers, a steroid dose response study for sIL-1ra was performed at 25 cycles for both progesterone and estradiol. and sIL-1ra levels were normalized to GAPDH. Intracellular IL-1ra production was assessed at 45 cycles of PCR without normalization.

After 25 cycles of PCR, the level of monocyte sIL-1ra mRNA expression was modulated by steroid concentration. The 0 h control sample from freshly isolated monocytes did not demonstrate a visible band. (figure 5,6). Monocyte sIL-1ra mRNA demonstrated maximal expression at the lowest experimental concentration of progesterone (10^{-8} M) and estrogen (10^{-11} M). Expression gradually decreased with increasing steroids concentrations. At the concentration of 10^{-6} M progesterone, the

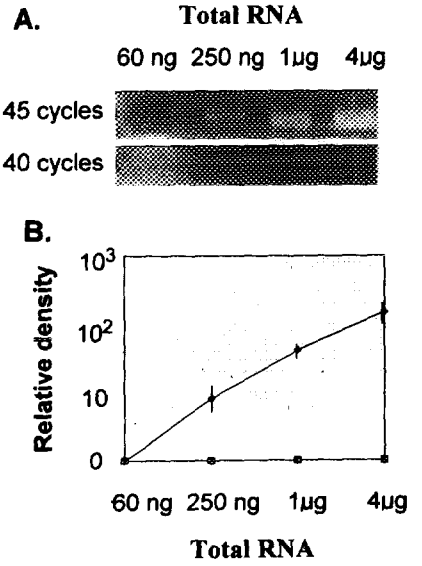

Figure 4. Optimal amplification cycle for icIL-1ra primers. With the same method as GAPDH primers, amplified products from icIL-1ra primers were electrophoresed (A). On densitometry, 45 cycles of amplification showed visible band on agarose gel and linear increase in density with initial concentrations of total RNA (B). 40 (-■-) and 45 (-◆-) cycles. Values are the mean ± STD of 3 experiments.

expression was significantly reduced to 30% of maximal production ($p<0.05$) (figure 5B). At the highest experimental concentration (10^{-8} M) of estradiol, mRNA expression was 20.4% of maximal production ($p<0.05$) (figure 6B).

Intracellular IL-1ra mRNA expression was detected in the cultured monocytes at the 45 cycles of PCR and showed a similar pattern of steroid modulation as did sIL-1ra.(Fig.7). This pattern of IL-1ra mRNA expression with gonadal steroid concentrations was consistent through all experiments.

Discussion

The communication between the immune and endocrine systems has long been of interest. The IL-1 system has been reported to be involved in many reproductive processes linked to gonadal steroid action. IL-1ra has been demonstrated to have an inhibitory effect on IL-1 action. Exogeneously administered IL-1ra prevents preterm delivery induced by IL-1β (6). Monocyte secretions of IL-1 and IL-1ra have been reported to change after menopause (10), such that the action of IL-1ra may be responsible for decreasing IL-1 induced bone resorption (7). IL-1β has been found in embryo culture media, and its level is closely related to pregnancy rate (11). Furthermore, according to previous reports from this laboratory, exogeneously administered IL-1ra is capable of blocking embryonic implantation in mice (5). Because the biologic action of IL-1 in these processes results from balance of IL-1 and IL-1ra, the investigation of factors modulating IL-1ra expression is crucial.

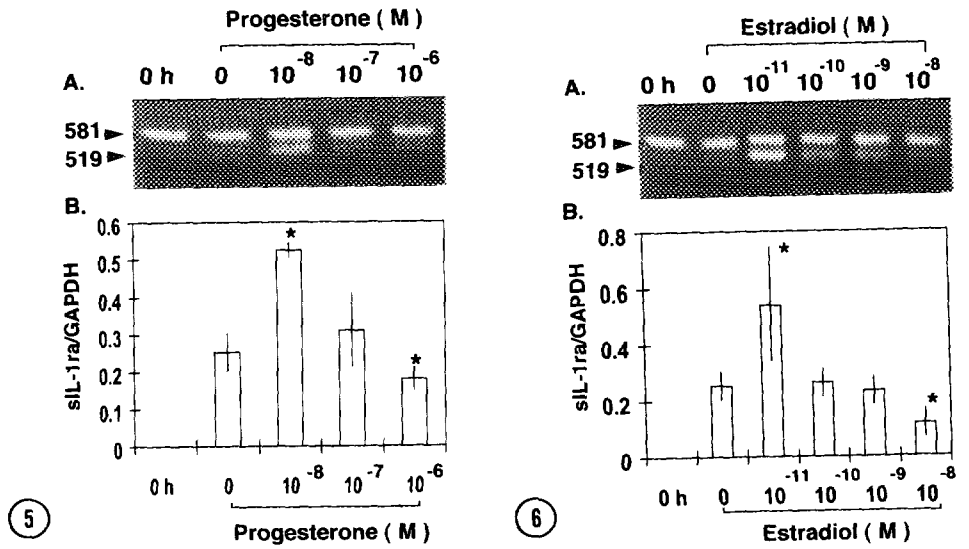

Figure 5. Progesterone modulation of monocyte sIL-1ra mRNA. Extracted RNAs from monocytes cultured with serially diluted progesterone (0-10^{-6} M) were reverse transcribed and amplified with sIL-1ra primers and GAPDH primers. After 25 cycles of PCR, equal volume of products from both sIL-1ra primers (519 bp) and GAPDH primers (581 bp) were simultaneously electrophoresed on 1% agarose gel and stained with ethidium bromide(A). On densitometric analysis, monocyte sIL-1ra showed maximal expression at 10^{-8} M progesterone (B). * Secretory IL-1ra mRNA expression gradually decreased to 30% of maximum at 10^{-6} M progesterone ($p<0.05$). Values are the mean ± SEM of four different experiments with four different samples. 0h, Control mRNA expression was measured in uncultured, freshly isolated monocytes.

Figure 6. Estradiol modulation of monocyte sIL-1ra mRNA. Extracted RNAs from monocytes cultured with serially diluted estradiol (0-10^{-8} M) were reverse transcribed and amplified with sIL-1ra primers and GAPDH primers. With the same methods as progesterone, products from both sIL-1ra primers and GAPDH primers were simultaneously electrophoresed (A). On densitometric analysis, monocyte sIL-1ra mRNA showed maximal expression at 10^{-11} M estradiol (B). * Secretory IL-1ra mRNA expression gradually decreased to 20.4% of maximum at 10^{-8} M estradiol ($p<0.05$). Values are the mean ± SE of four different experiments with four different samples. 0h, mRNA expression was measured in uncultured, freshly isolated monocytes.

Our results suggest that normal physiologic levels of progesterone and estradiol stimulate sIL-1ra mRNA expression in cultured monocytes, while high doses of steroids inhibit expression. The pattern of modulation of IL-1ra expression by gonadal steroid level is similar to the patterns previously reported in monocytes for both IL-1β and tumor necrosis factor-α (8,12). Given the similar response pattern of IL-1ra and IL-1 mRNA expression in response to gonadal steroid levels, it is reasonable that IL-1ra acts as a biological buffer to prevent the abrupt activation of a potent immune response by IL-1, a response which could disrupt normal cell functions.

Although we have not examined the expression of monocyte IL-1 receptor in respond to gonadal steroid, such receptors have been shown to be inhibited by high levels of gonadal steroids in other cells and tissues such as endometrium (13). Thus, the stimulatory effect of gonadal steroids on monocyte expression of cytokines may be reduced because of lower levels of steroid receptors in the presence of elevated concentrations of progesterone and estradiol.

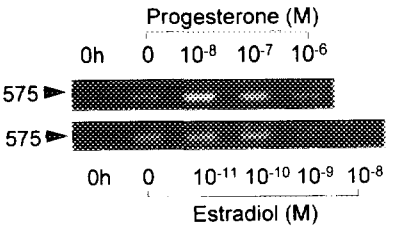

Figure 7. Progesterone and estradiol modulation of monocyte icIL-1ra mRNA. Extracted RNA from monocytes cultured with serially diluted estradiol and progesterone were reverse transcribed and amplified with icIL-1ra primers. After 45 cycles of PCR, icIL-1ra demonstrated similar pattern of expression as sIL-1ra, showing maximal production at 10^{-11} M progesterone and 10^{-8} M estradiol.

This is the first report of the expression of small amount of icIL-1ra mRNA in cultured monocytes at levels well below the level of sIL-1ra mRNA. Many epithelial cells such as keratinocytes are known to produce icIL-1ra, however, the functional role of icIL-1ra is unknown.

In summary, cultured human male monocytes predominately express sIL-1ra mRNA at the physiologic concentrations of progesterone and estradiol, while higher steroid concentrations suppress sIL-1ra expression. The physiologic role of this steroid modulation of sIL-1ra remains to be described.

Acknowledgments

This work was supported by Johnson and Johnson Focused Giving Program and by NIH Grant HD31575.

References

1. Haskill S., Martin G., Le L. V., Morris J., Peace A., Bigler C. F., Jaffe G. J., Hammerberg C., Sarah A., Sporn A., Fong S., and Arend W. P. (1991) Proc. Natl. Acad. Sci. USA. 88, 3681-3685.
2. Eisenberg S.P., Evans R. J., Arend W. P., Verderber E., Brewer M.T., Hannum C. H., and Thompson R. C. (1990) Nature. 343, 341-346.
3. Arend W. P., Welgus H. G., Thompson R. C., and Eisenberg S. P. (1990) J. Clin. Invest. 85, 1694- 1697.
4. Bigler C. F., Norris D. A., Weston L. W., and Arend W. P. (1992) J. Invest. Dermatol. 98, 38-43.
5. Simon C., Frances A., Piquette G. N., E. Danasouri I, Zurawski G., Dang W., and Polan M. L. (1994) Endocrinology. 134, 521-528.
6. Romero R., and Tartakovsky B. (1992) Am. J. Obstet. Gynecol. 167, 1041-1045.
7. Kimble R.B., Vannice J. L., Bloedow D. C., Tompson R. C., Hopfer W., Kung V. T., Brownfeld C., and Pacifici R. (1994) J. Clin. Invest. 93, 1959-1967.
8. Polan M. L., Loukides J., Nelson P., Carding S., Diamond M., Walsh A., and Bottomly K. (1989) J. Clin. Endocrinol. Metab. 69,1200-1206.
9. Chomczynski P., and Sacchi N. (1987) Ana. Biochem. 162, 156-161.
10. Pacifici R., Vannice J. L., Rifas L., and Kimble R. B. (1993) J. Clin. Endocrinol. Metab. 77, 1135- 1141.
11. Sheth K. V., Roca G. L., al-Sedairy S. T., Parhar R. S., Hamilton C. J., and al Abdul Jabbar F. (1991). Fertil. Steril. 55, 952-957.
12. Loy R. A., Loukides J. A., amd Polan M. L. (1992) Fertil. Steril. 58, 733-739.
13. Asribekova M. K., Karpova S. K., Murashko L. E., Sidelinikova V. M., and Zheleznov B. I. (1986) Problemy Endocrinologii. 32, 30-33.