Acta Medica Okayama

Volume 44, *Issue* 1 1990 *Article* 1

FEBRUARY 1990

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Abstract

We studied the effect of endogenous prostaglandin E2 (PGE2) on interleukin 1 (IL-1) production by peripheral blood monocytes from patients with rheumatoid arthritis (RA). IL-1 production by RA monocytes was not different from that of monocytes from normal controls, when the cells were either unstimulated or stimulated with lipopolysaccharide (LPS, 20 micrograms/ml), as measured by two different bioassays (thymocyte or fibroblast proliferation assay) and enzyme-linked immunosorbent assay. However, IL-1 production by LPS-stimulated monocytes from RA patients cultured in medium containing indomethacin, an inhibitor of PGE2 synthesis, was significantly greater than that of monocytes from normal controls. In addition, the levels of PGE2 in culture supernatants of unstimulated or LPS-stimulated monocytes from RA patients were higher than in culture supernatants of monocytes from normal controls. Moreover, the increase of in vitro IL-2 production by RA T cells stimulated by phytohemagglutinin (PHA) was observed when monocytes were removed from peripheral blood mononuclear cells. These results indicated that peripheral blood monocytes from RA patients could produce IL-1 in excess in vitro, but that in vivo IL-1 production by RA monocytes and IL-2 induction by RA T cells might be negatively regulated by endogenous PGE2.

KEYWORDS: rheumatoid arthritis, monocytes, interleulin 1, prostaglandin E2, interleukin 2

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*PMID: 2330842 [PubMed - indexed for MEDLINE]

Acta Med Okayama 44 (1) 13-20 (1990)

Role of Endogenous Prostaglandin E_2 in Interleukin 1 Production by Peripheral Blood Monocytes from Patients with Rheumatoid Arthritis

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We studied the effect of endogenous prostaglandin E_2 (PGE_2) on interleukin 1 (IL-1) production by peripheral blood monocytes from patients with rheumatoid arthritis (RA). IL-1 production by RA monocytes was not different from that of monocytes from normal controls, when the cells were either unstimulated or stimulated with lipopolysaccharide (LPS, 20μg/ml), as measured by two different bioassays (thymocyte or fibroblast proliferation assay) and enzyme-linked immunosorbent assay. However, IL-1 production by LPS-stimulated monocytes from RA patients cultured in medium containing indomethacin, an inhibitor of PGE_2 synthesis, was significantly greater than that of monocytes from normal controls. In addition, the levels of PGE_2 in culture supernatants of unstimulated or LPS-stimulated monocytes from RA patients were higher than in culture supernatants of monocytes from normal controls. Moreover, the increase of *in vitro* IL-2 production by RA T cells stimulated by phytohemagglutinin (PHA) was observed when monocytes were removed from peripheral blood mononuclear cells. These results indicated that peripheral blood monocytes from RA patients could produce IL-1 in excess *in vitro*, but that *in vivo* IL-1 production by RA monocytes and IL-2 induction by RA T cells might be negatively regulated by endogenous PGE_2.

Key words : rheumatoid arthritis, monocytes, interleukin 1, prostaglandin E_2, interleukin 2

Rheumatoid arthritis (RA) is a chronic inflammatory joint disease of unknown etiology that mainly affects synovial tissue. Immunological mechanisms are involved in the pathogenesis of this disease. A number of studies have demonstrated that monocytes of RA patients circulate in a functionally activated state (1-5). Of many different monocyte products, interleukin-1 (IL-1) and prostaglandin E_2 (PGE_2) are well established as potent factors leading to joint destruction (6-8).

IL-1 was originally designated as a lymphocyte activating factor (LAF) produced by monocytes (9, 10), but it is now evident that IL-1 exhibits a wide variety of biological activities (11). IL-1 appears to be stimulatory to different target cells. On the other hand, these stimulatory effects are regulated by the induction of PGE_2 production by many different cells. For instance, IL-1 stimulates both IL-2 production by T cells and IL-2 receptor expression (11), leading to T cell growth. However, PGE_2 directly suppresses the

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effect of IL-1 by inhibiting IL-2 production (12), as well as acting as an autoregulatory factor on IL-1 production by monocytes (13, 14). These opposite effects are believed to be important to feedback regulation in immunoregulation and inflammation.

Since PGE_2 production has previously been shown to be increased in monocytes of RA patients (5), we studied IL-1 production by peripheral blood monocytes cultured with or without lipopolysaccharide (LPS) and the role of endogenous PGE_2 in IL-1 production in patients with RA, using thymocyte and fibroblast proliferation assays and the enzyme-linked immunosorbent assay (ELISA).

Materials and Methods

Subjects. Twenty-two patients with classical or definite RA (3 males and 19 females) as defined by the American Rheumatism Association criteria (15) were studied. Their mean age was 51 (range 30–76) years. Nineteen patients were seropositive and 3 were seronegative by the latex particle agglutination test. Eighteen patients were treated with nonsteroidal anti-inflammatory drugs, 7 with gold, 4 with D-penicillamine, and 10 with predonisolone (< 10mg/day). Twenty healthy subjects (3 males and 17 females) with a mean age of 44 (range 27–79) years served as normal controls.

Materials. Four-to 8-week-old C3H/HeJ mice were sacrificed to obtain thymus tissue for the assay of IL-1 biological activity. Plastic dishes (Falcon #3003), culture plates (Falcon #3047) and microtiter plates for cell culture and for the ELISA assay were purchased from Becton Dickinson Labware, Lincoln Park, NJ, USA or Dynatech Labs., Inc., Chantilly, VA, USA. The following reagents were purchased : RPMI 1640 medium, Hepes buffer, Dulbecco's minimum essential medium (DMEM) and trypsin from GIBCO Labs., Grand Island, NY, USA ; 2-aminoethylisothiouronium bromide (AET), indomethacin, PHA and DNase from Sigma Chemical Co., St. Louis, MO, USA ; PHA and lipopolysaccharide (LPS) from Difco Labs., Detroit, MI, USA ; staphylococcal protein A (SpA) from Pharmarcia, Uppsala, Sweden ; ^{3}H-thymidine from Amersham plc, UK ; collagenase from Worthington Biochemical Corp., Freehold, NJ, USA and ^{125}I-PGE_2 kit from Du Pont Co., Boston, MA, USA. Recombinant human IL-1β (rIL-1β) was kindly supplied by Otsuka Pharmaceutical Co., Tokushima, Japan.

Cell preparations. Peripheral blood mononuclear cells (PBMC) were separated from heparinized venous blood (20ml) on Ficoll-Hypaque gradients. Monocytes were obtained according to the method of Kumagai *et al.* (16). Briefly, PBMC in complete medium, RPMI 1640 containing 10% (v/v) fetal calf serum (FCS ; heat-inactivated), 2mM L-glutamine, penicillin (100IU/ml), streptomycin (100μg/ml), and 25mM Hepes buffer were incubated for 60min in FCS-coated plastic dishes (Falcon #3003) at 37°C. After nonadherent cells were removed, adherent cells were incubated for 20 min at 4°C with phosphate buffered saline (PBS) containing 0.2% ethylenediamine tetraacetate (EDTA) and 5% (v/v) FCS, and then collected by vigorous pipetting with RPMI 1640. The monocyte fraction contained more than 85% of nonspecific esterase staining cells. T cells were obtained from nonadherent cells by rosette formation with AET-treated sheep red blood cells (17) followed by separation of rosette forming cells on Ficoll-Hypaque gradients and treatment with 0.83% NH_4Cl.

Cell cultures. Monocytes, at a density of 1×10^6 cells/ml in complete medium, were cultured with or without 1 or 20μg/ml LPS in 24-well tissue culture plates at 37°C in humidified 5% CO_2. After 48h, culture supernatants (SN) were collected, sterilized by filtration, and stocked at −20°C for assay. The culture SN of 20 μg/ml LPS-stimulated monocytes with 1μg/ml indomethacin were also obtained. T cells, at a density of 1×10^6 cells/ml, were cultured for 48 h with 0.1% PHA or 10μg/ml SpA, and culture SN were assayed for IL-2. In some experiments PBMC culture SN with 0.1% PHA were also assayed for IL-2.

Assay for IL-1.

Thymocyte proliferation assay (9, 10). Single-cell thymocyte suspensions from C3H/HeJ mice, at a density of 1.5×10^6 cells/200μ/well in complete medium with 5×10^{-5}M 2-mercaptoethanol and 2μg/ml PHA, were cultured for 72h with serially diluted test samples. Cultures were pulsed with 0.5μCi of [^{3}H] thymidine (^{3}H-TdR) per well for 16h before harvesting onto glass fiber filters. The incorporated radioactivity was measured using a liquid scintillation counter. The activity of IL-1 was quantified by a probit analysis (18) using rIL-1β as a standard in every assay. rIL-1β was arbitrarily assigned to have 2×10^7U of biological activity per 1mg protein. All test samples were established to be free of IL-2 activity.

Fibroblast proliferation assay (19). Synovial tissues from RA patients were digested with 1 mg/ml collagenase and 25 μg/ml DNAase in complete medium (20). Nonadherent cells were removed from the isolated synovial cells after a 2-h incubation at 37 °C in plastic dishes and synovial fibroblasts were grown in DMEM with 10 % (v/v) FCS. These fibroblasts were cultured overnight at a density of 1×10^4 cells/100 μl/well in DMEM with 10 % (v/v) FCS and 10^{-5} M indomethacin, and then were cultured further for 72 h with serially diluted test samples with a pulse of 0.5 μCi of ^{3}H-TdR for 16 h. After treatment with 0.5 mg/ml trypsin and 0.5 mM EDTA in PBS for 10 min, detached cells were harvested, and the radioactivity was measured. The activity of IL-1 was determined as described above.

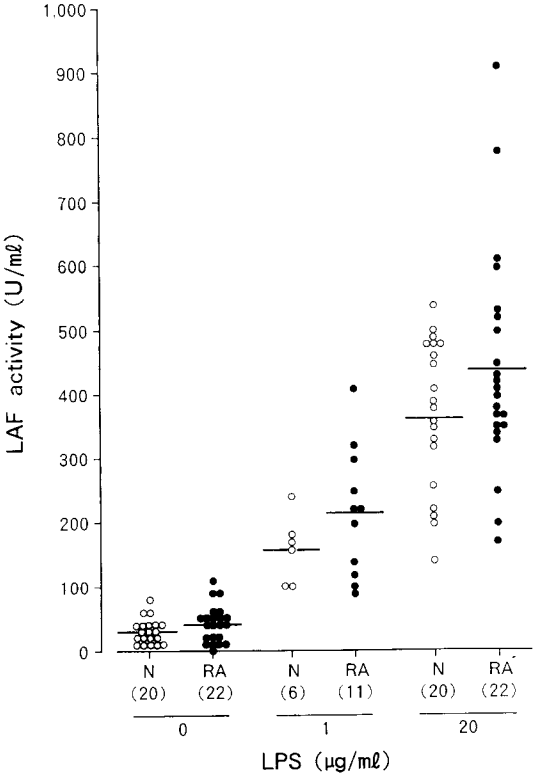

Fig. 1 Interleukin 1 (IL-1) production by monocytes from patients with rheumatoid arthritis (RA) and normal controls (N). Monocytes were cultured for 48 h with or without lipopolysaccharide (LPS) (1 or 20 μg/ml), and IL-1 biological activity (U/ml) in culture supernatants was assayed by the thymocyte proliferation assay as lymphocyte activating factor (LAF) activity (see Materials and Methods). Horizontal bars indicate the group mean. The numbers of subjects are shown in parentheses.

ELISA for IL-1α and β. Microtiter plates were coated with monoclonal anti-human IL-1 overnight at 4 °C and blocked with 0.1 % skim milk in PBS. Test samples and standards (rIL-1α and β) were added to each well and incubated overnight at 4 °C. After washing with 0.05 % (v/v) Tween 20 in PBS (PBS-T), plates were incubated for 2 h at 37 °C with polyclonal rabbit anti-human IL-1. After washing with PBS-T, horse radish peroxidase-conjugated anti-rabbit immunoglobulin was added, followed by a 2-h incubation at 37 °C. Plates were then washed with PBS-T, and 0.25 mg/ml o-phenylenediamine in citratephosphate buffer with 0.05 % (v/v) H_2O_2 was added to the wells. After 5 to 15 min, the reaction was quenched with 4 M sulphuric acid, and the absorbance at 492 nm was read on an automatic ELISA reader. The amount of IL-1 was expressed in ng/ml calculated from the standard curve.

Assay for IL-2. IL-2 was measured using an IL-2 dependent murine cell line (CTLL2) as described by Gillis *et al.* (21).

Assay for PGE_2. The PGE_2 concentration was measured by radioimmunoassay (RIA) with [^{125}I]-PGE_2.

Statistical methods. Differences between two groups were analyzed using the nonparametric Student's t-test except for paired samples which were tested by the parametric Welch's t-test.

Results

IL-1 production by monocytes from RA patients. IL-1 production by monocytes cultured with or without LPS was measured by the thymocyte proliferation assay (Fig. 1). Monocytes from both RA patients and normal controls produced only small amounts of IL-1 without any stimulation. LPS stimulation induced a dose dependent increase in IL-1 production. As shown in Fig. 1, the mean IL-1 production by monocytes from RA patients was higher than that of monocytes from normal controls, although the difference was not statistically significant.

To eliminate the inhibitory effect of endogenous PGE_2 on IL-1 production, monocytes were cultured with 20 μg/ml LPS and indomethacin to block the synthesis of PGE_2 (Fig. 2). The

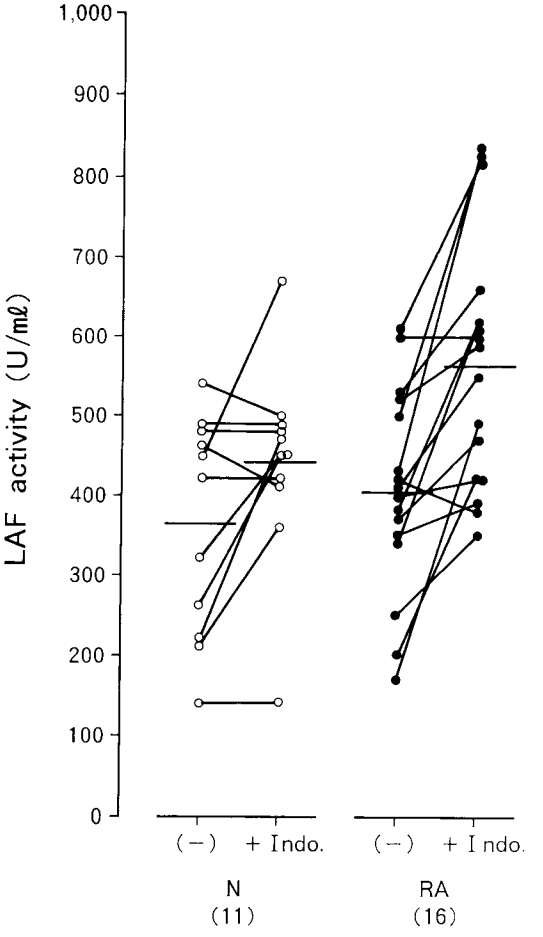

Fig. 2 Effect of indomethacin (Indo) on interleukin 1 (IL-1) production by monocytes from patients with rheumatoid arthritis (RA) and normal controls (N). Monocytes were cultured for 48h with 20μg/ml lipopolysaccharide, with or without Indo, and IL-1 production was measured by the thymocyte proliferation assay as lymphocyte activity factor (LAF) activity (U/ml). Horizontal bars indicate the group mean. The numbers of subjects are shown in parentheses.

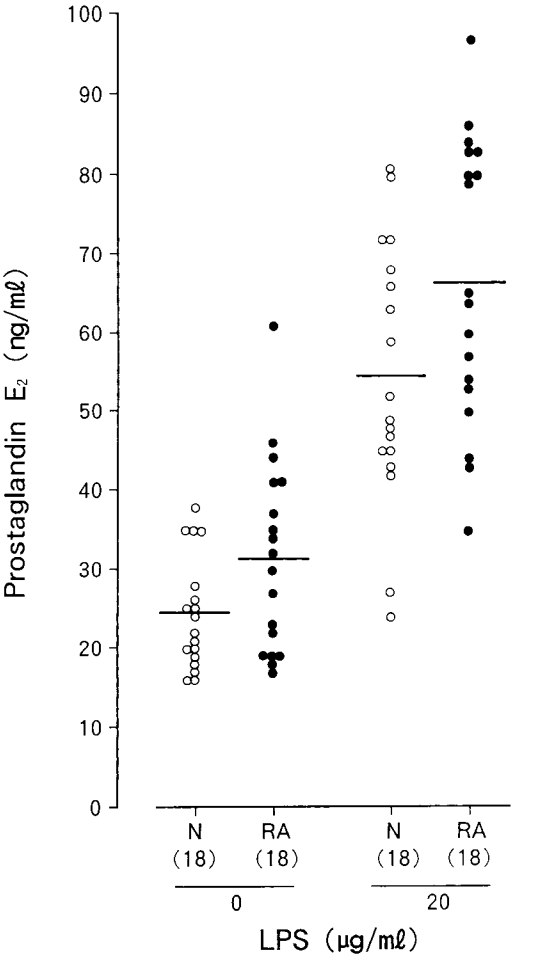

Fig. 3 Prostaglandin E_2 (PGE_2) production by monocytes from patients with rheumatoid arthritis (RA) and normal controls (N). Culture supernatants of monocytes for 48 h with or without 20μg/ml lipopolysaccharide (LPS) were assayed for PGE_2 using the radioimmunoassay (ng/ml). Horizontal bars indicate the group mean. The numbers of subjects are shown in parentheses.

addition of indomethacin resulted in a significant increase in IL-1 production in monocytes from both RA patients ($p < 0.01$) and normal controls ($p < 0.05$). The mean percent enhancement of IL-1 production in monocytes from RA patients was greater than that in monocytes from normal controls (22.8 % *vs.* 8.7 %), and IL-1 production by LPS-stimulated monocytes from RA patients was significantly higher than that of monocytes from normal controls when treated with indomethacin ($p < 0.05$) as shown in Fig. 2.

Fibroblast proliferation assay and ELISA. The fibroblast assay was over 15-fold more sensitive than the thymocyte assay, but was more variable ($r = 0.47$). The values in the fibroblast assay were larger than those in the thymocyte assay, and IL-1 production by LPS-stimulated, indomethacin-treated monocytes from RA patients was much higher than that in those from normal controls in this assay (Table 1).

Table 1 Interleukin 1 (IL-1) production by monocytes from patients with rheumatoid arthritis (RA) and normal controls[a]

Subjects	Assay method	LPS(−)		LPS(20μg/ml)		LPS(20μg/ml) +Indomethacin	
RA patients	(a)	42±6	(22)	440±36	(22)	565±39*	(16)
	(b)	76.3±6.7	(19)	488.5±39.7	(21)	835.6±78.9**	(16)
	(c)	0.28±0.02*	(22)	1.67±0.14	(22)	2.11±0.26	(16)
	(d)	4.4±0.7	(22)	22.4±1.3	(22)	30.8±2.1*	(16)
Normal controls	(a)	31±4	(20)	373±25	(20)	440±36	(11)
	(b)	58.9±6.6	(19)	405.0±36.8	(19)	524.7±42.9	(11)
	(c)	0.21±0.02	(20)	1.55±0.09	(20)	1.65±0.16	(11)
	(d)	3.3±0.3	(20)	19.2±1.2	(20)	23.4±2.3	(11)

a : IL-1 production was measured by thymocyte proliferation assay (a) (mean U/ml ± SE), fibroblast proliferation assay (b) (U/ml), and ELISA for IL-1α(c) and β (d) (ng/ml). The numbers of subjects are shown in parentheses. Statistical significance : *, $p < 0.05$; **, $p < 0.01$. LPS, lipopolysaccharide.

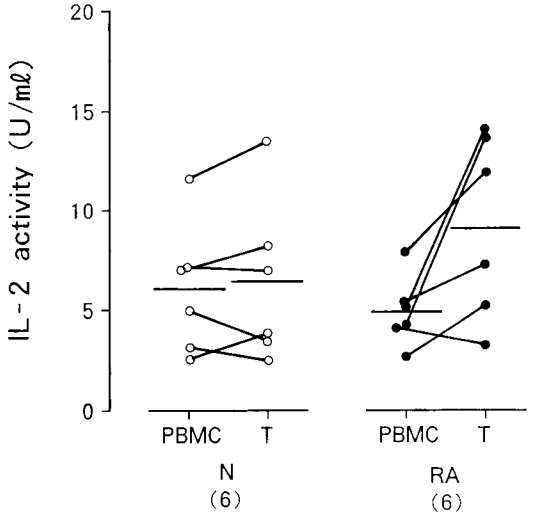

Fig. 4 Influence of monocytes on interleukin-2 (IL-2) production by T cells from patients with rheumatoid arthritis (RA) and normal controls (N). Peripheral blood mononuclear cells (PBMC) and monocyte-depleted T cells (T) were cultured for 48 h with 0.1 % PHA, and culture supernatants were assayed for IL-2 activity using CTLL2 cells. Horizontal bars indicate the group mean. The numbers of subjects are shown in parentheses.

Table 2 Interleukin 2 (IL-2) production by T cells from patients with rheumatoid arthritis (RA) and normal controls[a]

Subjects	PHA(0.1 %)	SpA(10μg/ml)
RA patients (15)	8.7±1.5	10.5±1.5
Normal controls (13)	6.8±1.3	7.7±0.8

a : T cells were stimulated for 48 h with phytohemagglutinin (PHA) or staphylococcal protein A (SpA), and culture supernatants were assayed for IL-2 activity (mean U/ml ± SE) using CTLL2 cells. The numbers of subjects are shown in parentheses.

The ELISA was sensitive to IL-1 at concentrations of about 0.01–0.04 ng/ml for IL-1β and 0.1–0.4 ng/ml for IL-1α, respectively. The ELISA showed that monocytes produced in culture 15-fold more IL-1β than IL-1α (Table 1). The values in the ELISA (IL-1α plus β) correlated well with those in the thymocyte assay ($r = 0.86$, $p < 0.01$), and thus similar results were obtained at the protein level.

PGE_2 production by monocytes from RA patients. The levels of PGE_2 in monocytes culture SN with or without LPS were measured by the RIA (Fig. 3). PGE_2 production was significantly higher in monocytes from RA patients than in monocytes from normal controls, either with or without LPS stimulation ($p < 0.05$). When IL-1 and PGE_2 levels in the same SN were compared, there was a slight, negative correlation ($r = -0.40$) between IL-1 and PGE_2 production in monocytes from RA patients (data not shown).

Effect of monocytes on IL-2 production by T cells in RA patients. PHA stimulated IL-2 production by monocyte-depleted T cells was compared with that by PBMC (Fig. 4). The removal of monocytes from cultures of T cells from RA patients caused a significant increase in IL-2 production ($p < 0.05$), although IL-2 production remained unchanged in normal controls. PGE_2 production was found to be higher in PBMC from RA patients than in PBMC from

normal controls (mean ng/ml ± SE: 28.7 ± 6.2 *vs.* 21.5 ± 3.3).

When T cells were stimulated with PHA or SpA, there was no difference between IL-2 production of T cells from RA patients and from normal controls (Table 2).

Discussion

We measured IL-1 production by two established bioassays, thymocyte and fibroblast proliferation assay, and a recently developed immunoassay, since the levels of IL-1 determined by an assay may be more or less than the actual amount of IL-1 in crude monocyte culture SN. It has been shown that stimulated monocytes produce not only IL-1 but also many other factors, including IL-1 inhibitors specific for LAF activity (22) and fibroblast growth factors (23). Thus, the presence of such factors has a great influence on the sensitivity and specificity of the bioassay. Even in the immunoassay, molecules with epitopes of IL-1, such as IL-1 fragments (24) and IL-1 precursors (25), may not necessarily be relevant to biological activity. In our study, analogous results were obtained by three different assays, although there were minor differences among the levels of IL-1 measured by the three assays. Moreover, the ELISA showed that IL-1β was the dominant form in monocyte culture, corresponding with the published data (25).

IL-1 production by peripheral blood monocytes from RA patients after LPS stimulation was increased when endogenous PGE_2 production was blocked by adding indomethacin to the cultures. Their IL-1 production without indomethacin was, however, similar to that of monocytes from normal controls. This result could be explained by coincidental PGE_2 production in monocyte cultures. It has been shown that IL-1 stimulates monocytes to produce PGE_2 (26), and that PGE_2 inhibits IL-1 production in monocytes by increasing intracellular cyclic adenosine monophosphatase (13), indicating an autoregulatory role of PGE_2 in IL-1 production. The phospholipase activity that enhances the release of arachidonic acid metabolites has been shown to be increased in monocytes from RA patients (27). Indeed, monocytes from RA patients produced higher levels of PGE_2 than those of normal controls, as reported previously (5). Thus, IL-1 production by monocytes without indomethacin might be more suppressed in RA patients via PGE_2 induction, resulting in the same levels as in normal controls. A slight but negative correlation between the levels of IL-1 and PGE_2 in monocyte culture SN from RA patients (data not shown) supports this possibility. Moreover, it has recently become evident that IL-1 production by monocytes is amplified by its own stimulation (28), suggesting that this positive feedback process could operate more strongly in RA patients without the negative influence of PGE_2 . Taken together, our results indicate that monocytes from RA patients can produce IL-1 in excess, but that IL-1 production at inflammatory sites of RA may be modulated, at least in part, by its own induction of PGE_2. Indeed, IL-1 production was rather impaired in monocytes when they were stimulated with higher levels of LPS ($> 50\mu g/ml$) which would induce greater PGE_2 production (data not shown).

Previous studies have shown that peripheral blood monocytes from RA patients (29), or only from recently active patients (30), spontaneously produce higher levels of IL-1. In our study, spontaneous IL-1 production *in vitro*, which could be induced by some stimulation during separation or by contact with plastic (31), tended to be increased in RA patients, although not significantly, except in the ELISA for IL-1. This may be due to the difference in the patient population studied or in the culture conditions which would influence PGE_2 production. In any event, the previous studies also seem to indicate increased IL-1 production by *in vivo* activated peripheral monocytes from RA patients, which has been well documented by other studies (1–5).

Our results also showed that monocytes from

RA patients suppressed IL-2 production by PHA-stimulated T cells. This inhibitory effect may be mediated by PGE_2 production, since the levels of PGE_2 were increased in culture SN of PBMC from RA patients. It has been shown that PGE_2 directly inhibits IL-2 production by T cells (12). Therefore, in RA, IL-1 might have an inhibitory influence on IL-2 production by stimulating monocytes to produce PGE_2 rather than just its direct stimulating influence. The cell-mediated immunity of RA has been shown to be depressed (32). This finding could be explained, at least in part, by increased PGE_2 induction by IL-1 in monocytes of RA, since T cell growth response represents the overall consequence of the opposite effects of IL-1 and PGE_2 on IL-2 production.

Acknowledgments. We thank Dr. K. Hirai for the gift of rIL-1β and monoclonal and polyclonal antibodies to IL-1.

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Received August 4, 1989 ; accepted November 7, 1989.