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Modulation of interleukin-1β production by cyclic AMP in human monocytes

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Elevation of cAMP has been considered to be an important downregulative signal in the production of interleukin-1(IL-1). This study demonstrates that this phenomenon is dependent on the signal used to activate the IL-1 production. The IL-1β production of lipopolysaccharide activated human monocytes was readily inhibited by dibutyryl cAMP. This took place without a significant change in the steady-state levels of IL-1β mRNA. By contrast, in PMA activated monocytes 100 μM dibutyryl cAMP increased in IL-1β production ca. 4-fold. The steady-statelevels of IL-1β mRNA were also simultaneously increased.

Interleukin-1; AMP, 3',5'-cyclic; Monocyte; (Human)

1. INTRODUCTION

Interleukin 1 (IL-1) is a family of related polypeptides, which are efficiently produced by cells of the monocyte/macrophage lineage. Until now two distinct forms, IL-1α and IL-1β, have been characterized, and their genes cloned and sequenced [1]. IL-1 exhibits a very broad range of biological activities in immune and inflammatory reactions as well as in normal hematopoiesis (reviewed in [2,3]). It may also be involved in the malignant growth of myeloid leukemia cells [4–6]. Because of these activities, it is obvious that the precise control of IL-1 is of vital importance in vivo. IL-1 production, at least in vitro, is often associated with production of inhibitory proteins, but the exact nature and significance of these are still largely unknown (reviewed in [7]). A better characterized negative feedback control mechanism is that exerted by prostaglandin-induced elevation of cyclic AMP. This has been demonstrated both in bacteriotoxin-activated normal macrophages and monocytic leukemia cells [8–10]. Increased intracellular cyclic AMP levels did not alter the steady-state levels of IL-1β mRNA [8], suggesting that this control operates at the post-transcriptional level. It has been previously demonstrated that in monocytic cells IL-1β gene expression can be induced via two distinct, functionally independent pathways [11]. The one pathway is activated by protein kinase C activating phorbol esters, e.g. PMA, and the other by bacterial lipopolysaccharide, LPS. In this report, we show that elevated cAMP levels are suppressive only in the LPS-induced pathway, while in PMA-stimulated human monocytes elevated cAMP levels significantly increase the IL-1β amounts produced.

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2. MATERIALS AND METHODS

2.1. *Cell cultures*

Leukocyte-rich buffy coats were obtained from the Finnish Red Cross Blood Transfusion Service (Helsinki, Finland) and mononuclear cells were isolated from them by centrifugation on Ficoll-Isopaque (Pharmacia, Uppsala, Sweden). After being washed, the cells were resuspended, 10^7 cells/ml, in RPMI-1640 medium (Flow, Irvine, UK) containing 10% heat-inactivated human AB serum (The Finnish Red Cross Blood Transfusion Service), 10 mM Hepes, 2 mM L-glutamine, and antibiotics (complete medium). 10 ml aliquots of this cell suspension were then incubated in 90 mm diameter Petri dishes (Costar, Cambridge, MA) at 37°C, 5% CO_2, for 1 h. After incubation the nonadherent cells were removed by vigorous pipetting with warm complete medium, and then the adherent cells were harvested by incubating the cells in cold PBS (Ca^{2+} and Mg^{2+} free) and using a rubber policeman. 80–90% of the adherent cells were monocytes, as demonstrated by standard morphological criteria or with monoclonal, monocyte-specific antibodies. 1×10^6 adherent cells (in 1 ml of complete medium) were cultured (at 37°C, 5% CO_2) in wells of tissue culture plates (24-well, Costar) and the cultures were stimulated with the indicated concentrations of the following agents: phorbol 12-myristate 13-acetate (PMA, Sigma Chemical Co., St. Louis, MO) or bacterial lipopolysaccharide (LPS, *E. coli* 026:B6, Difco Laboratories, Detroit, MI). When indicated, *N*-2-*O*-dibutyryladenosine 3',5'-cyclic monophosphate (dbcAMP, Sigma), *N*-2-*O*-dibutyrylguanosine 3',5'-cyclic monophosphate (dbcGMP, Sigma), prostaglandin E2 (PGE2, Sigma) or forskolin (Sigma) were added to the cultures. After 24 h, the cultures were harvested after two cycles of freezing and thawing. Thus the samples represent the total amount of IL-1 protein produced (intracellular, membrane fragments and that released to the culture medium).

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2.2. *Assay for IL-1*

The IL-1β content of the cultures was measured by using the IL-1β specific ELISA kit (Cistron, Pine Brook, NJ). The assays were performed according to the manufacturer's instructions.

2.3. *RNA isolation and analysis*

For RNA isolations, mononuclear cells from each buffy coat were directly divided to the experimental groups indicated (in 90 mm Petri dishes), nonadherent cells were removed as described above, and then 10 ml of complete medium and the indicated stimulators were added. To avoid interindividual variations between the buffy coats, each experimental group consisted of cells derived from 5–6 buffy coats. At the times indicated, the adherent cells were harvested as described above and total cellular RNA was isolated by guanidinium isothiocyanate lysis and CsCl centrifugation [12,13]. RNA isolated was quantitated spectrophotometrically and 15 μg samples were size-fractionated on 0.8% formaldehyde-agarose gels, transferred to a nylon membrane (Pall, Glen Cove, NY), dried and baked at 80°C.

Table I

The effect of dibutyryl cyclic AMP (dbcAMP) on the IL-1β production of human monocytes stimulated either with LPS or PMA

Stimulator	IL-1β (pg/ml)
–	<20
LPS 100 ng/ml	33671
LPS 100 ng/ml + dbcAMP 100 μM	11428
LPS 100 ng/ml + dbcAMP 10 μM	19642
PMA 10 ng/ml	3359
PMA 10 ng/ml + dbcAMP 100 μM	23035
PMA 10 ng/ml + dbcAMP 10 μM	7173
dbcAMP 100 μM	136
dbcAMP 10 μM	45

1×10^6 human monocytes (in 1 ml) were cultured with the stimulators indicated and after 24 h the cells and supernatants were harvested after two cycles of freezing and thawing (thus the samples represent the total amount of IL-1β protein produced). IL-1β was measured by IL-1β specific ELISA

Table II

The effect of dibutyryl cyclic GMP (dbcGMP), prostaglandin E2 (PGE2) and forskolin on the IL-1β production of human monocytes stimulated with LPS or PMA

Stimulator	IL-1β (pg/ml)
–	60
LPS 100 ng/ml	36383
LPS 100 ng/ml + dbcAMP 100 μM	17358
LPS 100 ng/ml + dbcGMP 100 μM	35937
LPS 100 ng/ml + PGE2 1 μM	20377
LPS 100 ng/ml + PGE2 0.1 μM	22264
LPS 100 ng/ml + PGE2 0.01 μM	29575
LPS 100 ng/ml + forskolin 10 μM	30468
LPS 100 ng/ml + forskolin 1 μM	36941
PMA 10 ng/ml	9436
PMA 10 ng/ml + dbcAMP 100 μM	26227
PMA 10 ng/ml + dbcGMP 100 μM	7816
PMA 10 ng/ml + PGE2 1 μM	9084
PMA 10 ng/ml + PGE2 0.1 μM	9014
PMA 10 ng/ml + PGE2 0.01 μM	8802
PMA 10 ng/ml + forskolin 10 μM	19150
PMA 10 ng/ml + forskolin 1 μM	22830

For explanation see Table I

The IL-1β cDNA probe used (HU-IL-1β, pcDSRα) was kindly provided by Dr Kari Varkila (DNAX Research Institute, Palo Alto, CA). The RNA levels in the nylon membranes were also quantitated (data not shown) by using a constant probe, glyceraldehyde phosphate dehydrogenase (pRGAPDH-13). This was obtained from Dr Kari Alitalo (Dept. Pathology, University of Helsinki). The cDNA probes were labeled with ^{32}P by nick-translation. Prehybridizations and hybridizations were performed in a solution containing 50% formamide, 1 × Denhardt's solution, 3 × SSC, 50 mM Hepes and 150 μg/ml of salmon sperm DNA. Filters were washed in 1 × SSC plus 0.1% SDS, 2 times for 30 min at room temperature and then at 60°C for 30 min. Then the filters were exposed to Kodak AR X-Omat films at −70°C with intensifying screens.

3. RESULTS

Monocyte cultures contained 3–10 times more IL-1β protein after the optimal LPS stimulation (100 ng/ml) than after the optimal PMA stimulation (10 ng/ml). The cell permeable structural analog of cyclic AMP, dbcAMP, had a clear dose-dependent suppressive effect on the LPS-induced IL-1β production (Table I), thus being in accordance with the previous results [8,9]. By contrast, dbcAMP clearly enhanced the PMA induced IL-1β production (Table I). In monocytes derived from different cell donors, the increasing effect of 100 μM dbcAMP varied between 1.96 and 6.86 fold

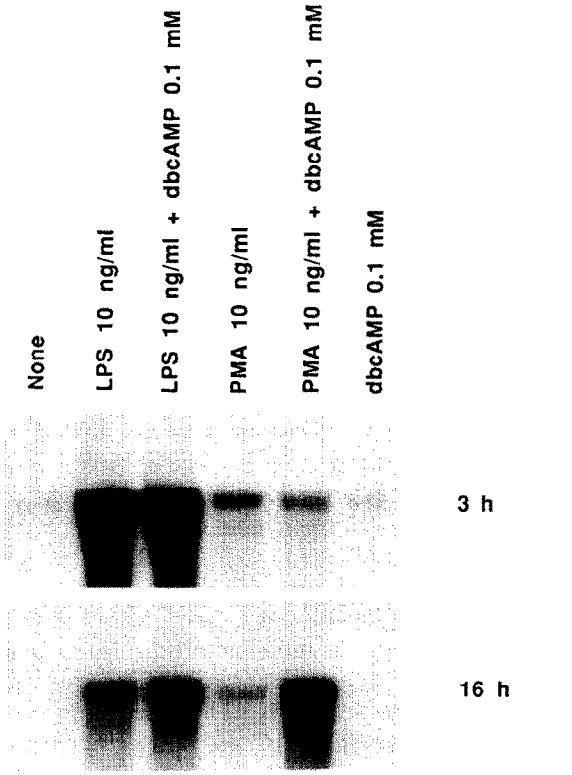

Fig. 1. The effect of dbcAMP on the IL-1β mRNA expression induced either by LPS or PMA. Monocytes were cultured for 3 h or 16 h in the presence of the agents indicated, and then total cellular RNA was isolated, fractionated on agarose gels, transferred to a nylon membrane and then hybridized to ^{32}P-labelled IL-1β cDNA probe.

(mean 4.13, $n = 5$), 100 μM dbcAMP alone only slightly increased the IL-1β production (maximally up to 400 pg/ml). The butyrate moiety of the dbcAMP molecule was not responsible for the enhancing effect, because dbcGMP was entirely inactive (Table II). The agonist (PGE2 or forskolin)-induced cAMP elevation clearly suppressed the LPS-induced IL-1β production, PGE2 being more effective, while in PMA stimulated cultures forskolin enhanced the IL-1β production, but PGE2 was without any effect (Table II).

It has been reported that elevated intracellular cAMP levels do not have any influence on the IL-1β mRNA levels in LPS activated macrophages or in bacteriotoxin activated U937 cells [8,14]. The same was true also in LPS-activated human monocytes (Fig. 1). By contrast, the enhancing effect of dbcAMP in the PMA induced IL-1β production could be seen at the transcriptional level: IL-1β mRNA levels were clearly higher in the cells stimulated for 16 h with PMA plus 100 μM dbcAMP than in cells stimulated with PMA alone (Fig. 1). Earlier after the stimulation (3 h), the enhancing effect was not yet visible (Fig. 1).

4. DISCUSSION

The data shown in this report demonstrate that elevation of intracellular cAMP can either down- or upregulate the IL-1β production of human monocytes and, additionally, that these opposite regulatory effects are mediated via different mechanisms.

Downregulation of the IL-1β production was observed after LPS stimulation. Elevated cAMP levels did not change the steady-state levels of IL-1β mRNA in this case. In PMA-stimulated cells cAMP had a clear enhancing effect on the mRNA levels, and consequently, on the IL-1β protein production. Fenton et al. [11] have shown that PMA induces a stable ($T_{1/2}$ = 12 h) IL-1β mRNA, while after LPS stimulation the predominant mRNA is short-lived ($T_{1/2}$ = 4 h). Thus, it is possible that the cAMP mediated upregulatory mechanisms are working only on this more stable mRNA form. We are currently analyzing the effects of cAMP on the rate of transcription and stability of the IL-1β mRNA.

The differences between the LPS- and PMA-induced activation pathways are still poorly characterized. It is evident that protein kinase C is also involved in the LPS-induced pathway, because inhibitors of this kinase are able to block the IL-1β mRNA accumulation and protein production in LPS activated macrophages [15].

PMA-induced IL-1β production was also enhanced by a receptor agonist, forskolin, which is able to elevate the endogenous cAMP levels. However, another agonist, PGE2, was ineffective, although it was clearly decreasing the LPS-induced IL-1β production. The reason for this is unknown, but it is possible that PMA treatment selectively downregulates the agonist-induced cAMP responses. This kind of phenomenon has been observed for example in B lymphocytes [16].

The two signal transduction systems, one activated by e.g. phorbol esters and involving protein kinase C, and the other activated by cAMP and involving protein kinase A, can interact in various ways [17]. The effect of cAMP on the PMA induced IL-1β production described here is an example of the synergistic interactions, which are operating at the levels of gene transcription. It is interesting to note that the production of another cytokine, interleukin-6, which is also mainly produced by cells of the monocyte/macrophage lineage, can also be activated by protein kinase C or cAMP dependent pathways [18]. In that case, however, these pathways do not interact with each other [18].

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REFERENCES

[1] March, C.J., Mosley, B., Larsen, A., Cerretti, D.B., Braedt, G., Price, V., Gillis, S., Henney, C.S., Kronheim, S.R., Grabstein, K., Conlon, P.J., Hopp, T.P. and Cosman, D. (1985) Nature 315, 641–647.
[2] Oppenheim, J.J., Kovacs, E.J., Matsushima, K. and Durum, S.C. (1986) Immunol. Today 7, 45–56.
[3] Dinarello, C.A. (1988) FASEB J. 2, 108–115.
[4] Griffin, J.D., Rambaldi, A., Vellenga, E., Young, D.C., Ostapovicz, D. and Cannistra, S.A. (1987) Blood 70, 1218–1221.
[5] Erroi, A., Specchia, G., Liso, V., Colotta, F., Bersani, L., Polentarutti, N., Zhen-Guo, C., Allavena, P. and Mantovani, A. (1989) Eur. J. Hematol. 42, 16–23.
[6] Cozzolino, F., Rubatelli, A., Aldinucci, D., Sitia, R., Torcia, M., Shaw, A. and Di Guglielmo, A. (1989) Proc. Natl. Acad. Sci. USA 86, 2369–2373.
[7] Larrick, J.W. (1989) Immunol. Today 10, 61–65.
[8] Knudsen, P.J., Dinarello, C.A. and Strom, T.B. (1986) J. Immunol. 137, 3189–3194.
[9] Brandwein, S.R. (1986) J. Biol. Chem. 261, 8624–8632.
[10] Kunkel, S.L., Chensue, S.W. and Phan, S.M. (1986) J. Immunol. 136, 186–192.
[11] Fenton, M.J., Vermeulen, M.W., Clark, B.D., Webb, A.C. and Auron, P.E. (1988) J. Immunol. 140, 2267–2273.
[12] Chirgwin, J.M., Pryzbyla, A.E., MacDonald, R.J. and Rutter, W.J. (1979) Biochemistry 18, 5294–5299.
[13] Glisin, V., Crkvenjakov, R. and Byus, C. (1974) Biochemistry 13, 2633–2637.
[14] Tannenbaum, C.S. and Hamilton, T.A. (1989) J. Immunol. 142, 1274–1280.
[15] Kovacs, E.J., Radzioch, D., Young, H.A. and Varesio, L. (1988) J. Immunol. 141, 3101–3105.
[16] Wiener, E. and Scarpa, A. (1989) J. Biol. Chem. 264, 4324–4328.
[17] Nishizuka, Y. (1986) Science 233, 305–312.
[18] Zhang, Y., Xin-Lin, J. and Vilcek, J. (1988) J. Biol. Chem. 263, 6177–6182.