

Increase of poly(ADP-ribose) polymerase mRNA levels during TPA-induced differentiation of human lymphocytes

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The non-mitogenic stimulation of human peripheral blood mononuclear cells (PBMC) with low concentrations of the phorbol ester 12-*O*-tetradecanoylphorbol 13-acetate (TPA) caused a progressive increase in the percent fraction of the cells that were positive for the early activating antigen CD69. At the same time, it caused a progressive increase in the steady-state levels of poly(ADP-ribose) polymerase (pADPRP) transcripts. A further increase in TPA concentration, while inducing the maximal expression of the levels of CD69 activating surface antigen, both in the presence or in the absence of proliferative activity, did not evoke any additional heightening of pADPRP mRNA levels. Time course of PBMC stimulation with a non-mitogenic dose of TPA showed an early increase in the accumulation of pADPRP mRNA, which changed at 8–16 h, and remained high for several days thereafter. On the basis of these data, we suggest that the increase in pADPRP mRNA may be associated with the commitment of human lymphocytes from a quiescent (G_0) to an activated (G_1) state.

Poly(ADP-ribose) polymerase; Lymphocyte; TPA; Differentiation

1. INTRODUCTION

Considerable interest has been focused on the differences between quiescent and activated or actively cycling cells and the processes responsible for the transitions between these various states [1]. As for lymphocytes, these transitions can be triggered *in vitro* by lectins, by monoclonal antibodies to cell surface receptors, and by phorbol esters, a known class of activators of protein kinase C [2]. After stimulation of proliferatively resting (G_0 phase) lymphocytes, T cells enter the G_1 phase of the mitotic cell cycle, during which they become able to express newly synthesized antigens, such as the CD69 early activation antigen [3–4] and an interleukin-2 receptor (IL-2R). Thereafter, they start to produce interleukin 2 (IL-2), which then will bind to its own receptors to make possible the intra-cycle progression of T cells leading to the G_1 –S transition [5].

Poly(ADP-ribose) is synthesized from NAD^+ inside the nuclei of eukaryotic cells by the enzyme poly(ADP-ribose) polymerase (EC 2.4.2.30), which is thought to be involved in eukaryotic differentiation and in mitogenic events including the activation of quiescent lymphocytes [6]. Several reports have hitherto appeared, indicating that: (i) pADPRP activity increased with

DNA synthesis in phytohemagglutinin (PHA)-stimulated lymphocytes [6–7]; (ii) mitogenic stimulation of purified T lymphocytes with the phorbol ester TPA and with a monoclonal antibody to the T3 cell surface antigen caused an increase in pADPRP activity [8]; and (iii) competitive inhibitors of pADPRP prevented the proliferation of PHA-stimulated T lymphocytes, whereas the stimulation of DNA synthesis by TPA was more resistant to the action of pADPRP inhibitors [9]. On the other hand, we recently observed that pADPRP steady-state mRNA levels promptly increased starting 4 h after PHA stimulation of human T lymphocytes, remaining high until the end of the S phase of the cell cycle [10].

In the present study, the expression of the pADPRP gene was studied during the activation of human peripheral blood mononuclear cells (PBMC) with TPA. This phorbol ester activated lymphocytes, by inducing the *de novo* synthesis and expression of nuclear proto-oncogenes and surface activation antigens. However, differently from PHA, TPA itself did not induce a significant IL-2 production [11]. Therefore, the mitogenic activity of TPA was much weaker than that of PHA.

The aim of our work was to investigate whether the increase in pADPRP mRNA level is exclusively linked to stimuli, such as PHA, operating on the cell surface receptors, or may represent a general phenomenon that follows activation of lymphocytes by various agents. Moreover, we tried to understand whether non-mitogenic doses of TPA could increase pADPRP mRNA

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levels or the phenomenon was only the consequence of the full mitogenic response.

2. MATERIALS AND METHODS

2.1. Isolation and culture of lymphocytes

Human PBMC were isolated from blood of normal donors by centrifugation on Ficoll-Hypaque density gradients. Cells were cultured for different periods in macro wells at 2×10^6 /ml in RPMI 1640 medium containing 10% (v/v) fetal calf serum in the presence of different concentrations of TPA (Sigma) or PHA (Gibco, 1% v/v).

2.2. Immunofluorescence and flow cytometry

PBMC, cultured for 24 h, were stained by indirect immunofluorescence using C1.18 (anti-CD69) monoclonal antibody (mAb) [4] and fluoresceinated goat anti-mouse Ig (Becton Dickinson). The background fluorescence was assayed on cells stained with the fluoresceinated anti-mouse Ig reagent alone. Samples were analyzed with a flow cytometer (Elite, Coulter, Hialeah, FL).

2.3. Proliferative activity

To evaluate the proliferative activity of PBMC after 3 days in culture, $0.5 \mu\text{Ci}$ of tritiated thymidine (^3H]TdR) was added 18 h before harvesting the cells and the macromolecular incorporation of this precursor was next measured by a liquid scintillation counter.

2.4. Northern hybridization analysis

Total cellular RNA was extracted from 10^8 cells with guanidinium isothiocyanate and purified by centrifugation through 5.7 M CsCl [12]. The RNA ($20 \mu\text{g}$ per track) was then electrophoresed on a 1% agarose denaturing gel, transferred to a Hybond N membrane and hybridized to ^{32}P -labeled human pADPRP cDNA [13].

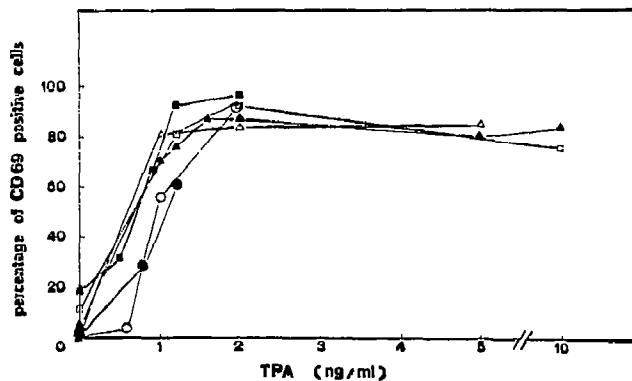


Fig. 1. The expression of CD69 surface activating antigen in PBMC treated for 24 h with different concentrations of TPA. Each curve refers to data obtained in PBMC from a single donor.

3. RESULTS

As a first step, we analyzed the ability of different concentrations of TPA to increase pADPRP mRNA levels in relation both to PBMC activation and the proliferative response.

In different individual donors, low doses of TPA, in the absence of other concomitant stimuli, induced the expression of the CD69 surface antigen in a dose-dependent fashion (Fig. 1). PBMC activated for 24 h by TPA (dose range, 0.5–1.2 ng/ml) showed increases in pADPRP mRNA levels (Fig. 2). In this experiment, it

Table I
Effect of low concentrations of TPA on the CD69 antigen expression and the proliferation of PBMC

	TPA (ng/ml)				PHA (1%)
	0	0.5	0.8	1.2	
CD69 antigen expression	20(1.6)*	32(1.9)	67(2.2)	92(5.8)	78(3.5)
^3H]TdR incorporation	300**	100	1,900	7,000	35,000

*The results, evaluated after 24 h of culture, are expressed as percentages of CD69⁺ cells. The figures within brackets are mean fluorescence intensities of CD69⁺ cells.

**The results (cpm), evaluated after 72 h of culture, are expressed as means from quadruplicate samples.

Table II
Effect of different concentrations of TPA on the CD69 antigen expression and the proliferation of PBMC

	TPA (ng/ml)					PHA (1%)
	0	1.2	1.6	2	5	
CD69 antigen expression	6(5.4)*	76(26)	87(29)	87(30)	80(18)	65(13)
^3H]TdR incorporation	200**	2,500	3,000	3,300	2,500	49,000

*The results, evaluated after 24 h of culture, are expressed as percentage of CD69⁺ cells. The figures within brackets are mean fluorescence intensities of CD69⁺ cells.

**The results (cpm), evaluated after 72 h of culture, are expressed as means from quadruplicate samples.

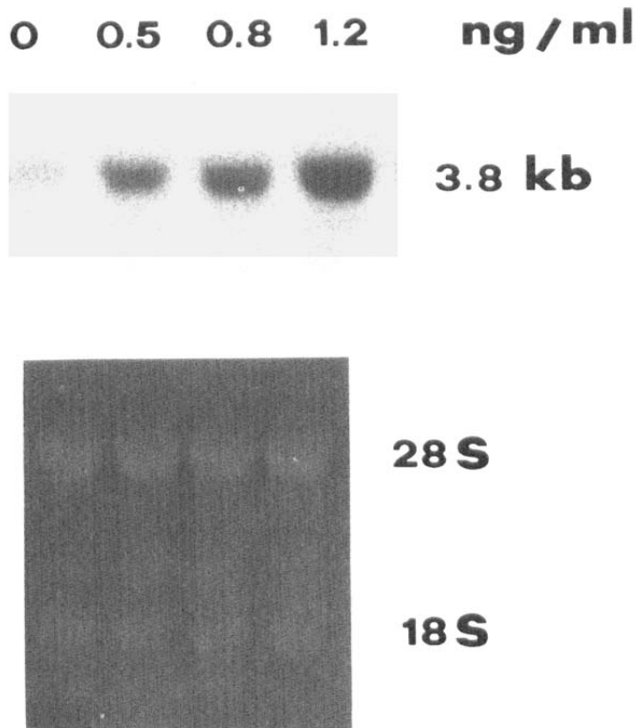


Fig. 2. The steady-state mRNA levels for pADPRP gene in PBMC cultured with low concentrations of TPA. The mRNA level of pADPRP (3.8 kb) augments with the increase in concentration of TPA (0.5, 0.8, 1.2 ng/ml). The amount of ribosomal RNAs in the agarose gel quantified by ethidium bromide staining is shown in the lower part of the panel. The position of the ribosomal RNAs (28 S and 18 S) are indicated on the right. The data concerning the expression of CD69 surface activating antigen and the $[^3\text{H}]\text{TdR}$ incorporation for the same experiment are reported in Table I.

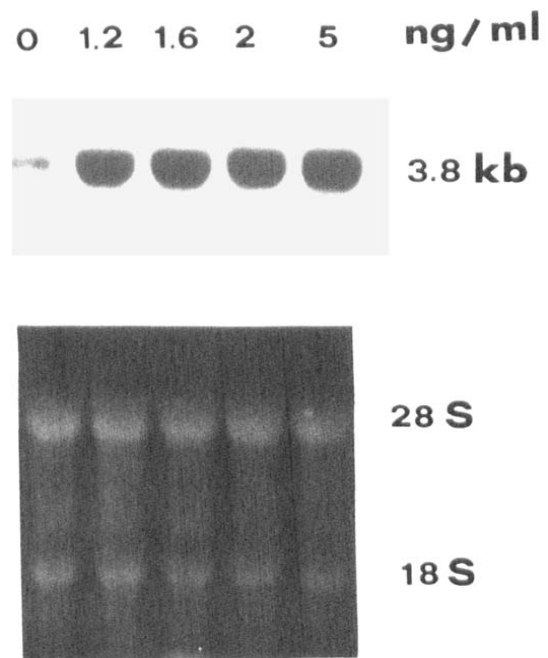


Fig. 3. The steady-state mRNA levels for pADPRP gene in PBMC cultured with different concentrations of TPA. The mRNA level of pADPRP (3.8 kb) does not augment with the increase in concentration of TPA (from 1.2 to 5 ng/ml). The amount of ribosomal RNAs in the agarose gel quantified by ethidium bromide staining is shown in the lower part of the panel. The position of the ribosomal RNAs (28 S and 18 S) are indicated on the right. The data related to the expression of CD69 surface activating antigen and the $[^3\text{H}]\text{TdR}$ incorporation for the same experiment are reported in Table II.

appears that the increase of the level of pADPRP mRNA was accompanied by an enhanced expression of the CD69 antigen (Table I). Whereas, the increase in pADPRP mRNA was not linked to a significant proliferative activity (Table I). Concentrations of TPA above 1.2 ng/ml, while providing the maximal level of CD69 antigen expression (Table II), did not produce a further increase of the levels of pADPRP mRNA, which was already and remained steadily high (Fig. 3). When TPA, at the highest concentrations used, acted as an effective mitogen, the pADPRP mRNA levels were similar to those induced by the G_0/G_1 activating, yet non-mitogenic doses (data not shown).

Figure 4 shows a time-course of the steady-state accumulation of pADPRP mRNA after the activation with a non-mitogenic dose of TPA (1.2 ng/ml). The increase in the accumulation of pADPRP transcript was detectable after 8–16 h exposure to the phorbol ester, and peaked at 48 h not declining appreciably until 4 days had elapsed. Hence, the time course of pADPRP mRNA accumulation was similar to that observed by us in T lymphocytes [10] and in PBMC (unpublished data) after stimulation with a mitogenic dose of PHA.

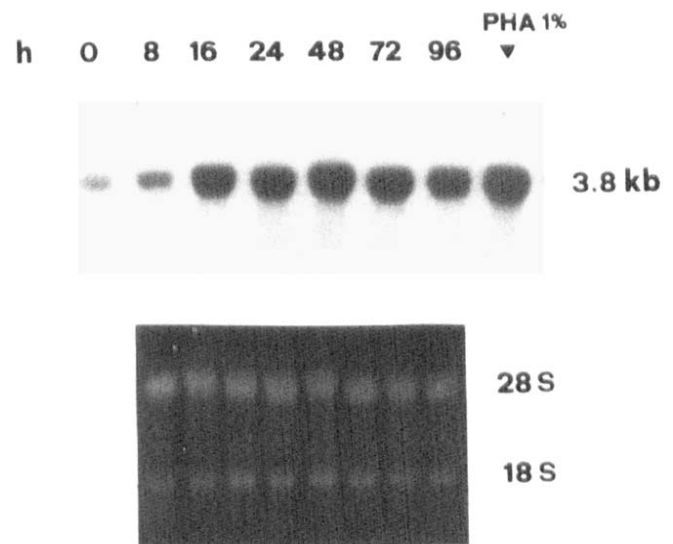


Fig. 4. The time course of steady-state mRNA levels for pADPRP gene in PBMC cultured with a non-mitogenic dose of TPA. PBMC were cultured with 1.2 ng/ml of TPA for different times (h), as indicated at the top of the panel. PBMC were cultured with 1% of PHA for 24 h as a positive control. The figure shows the mRNA level for the pADPRP gene (3.8 kb). The amount of the ribosomal RNAs in the agarose gel quantified by ethidium bromide staining is shown in the lower part of the panel. The positions of ribosomal RNAs (28 S and 18 S) are indicated on the right.

4. DISCUSSION

The study of the transition from the quiescent to the activated state of human lymphocytes and the identification of the enzymes involved in these changes is interesting since the information thus obtained may help to find control points and clarify processes regulating differentiation and/or proliferation.

In the present work the effects of TPA on PBMC differentiation and pADPRP gene expression were examined.

Several authors have claimed that pADPRP activity in lymphocytes increases 3–10-fold over a period of 24–72 h following the stimulation with PHA [6–7]. This was recently confirmed by us in PBMC using activity gel methods [14]. We suggested that this amplified activity depended on the *de novo* enzyme synthesis, since a remarkable surge of the steady-state mRNA levels for pADPRP was shown to occur in purified T lymphocytes [10] and in PBMC (unpublished data) 4 h and 8 h, respectively, after PHA stimulation.

King et al. [9] suggested that ADP-ribosylation plays a different role in the response of lymphocytes to various stimuli, because the proliferative response to TPA was found to be much less affected by pADPRP inhibitors than was that to PHA. We could not reject this suggestion, but we demonstrated that PBMC stimulated by PHA or TPA did not exhibit marked differences in the amounts or in the time courses of the transcript for the pADPRP gene.

A previous report [8] showed that the increase in pADPRP activity occurred in association with the proliferative response in purified T-cell suspensions treated with TPA and the anti-T3 antibody. The two signals acted synergistically to stimulate the maximum increase in pADPRP activity. We used TPA alone to measure the level of pADPRP mRNA in activated, yet non-proliferating PBMC. In fact, in the majority of our experiments carried out with human PBMC, TPA was not mitogenic; nevertheless, the phorbol ester was found to induce the cell-surface expression of the early activation antigen CD69. In all experiments, lymphocyte activation by non-mitogenic doses of TPA was accompanied by the induction of increased steady-state levels of pADPRP mRNA. Hence, we suggest that this increase in pADPRP mRNA is specifically associated with the transition from the quiescent to the activated state of human PBMC, i.e. with the commitment of such cells to grow, but not with their subsequent intra-cycle progression. In fact, when the mitogenic response occurred, no further increase in the steady-state pADPRP mRNA levels could be detected. This finding is in keeping with reports from several authors [15–17] which show that various competitive inhibitors of the nuclear enzyme pADPRP do block the differentiation of several

eukaryotic cell types without affecting their proliferation.

In conclusion, our data imply that the expression of the pADPRP gene is an early event, important for the response of PBMC to different stimuli, be they fully mitogenic (PHA) or simply activatory (TPA). Therefore, the observed increase in the mRNA levels for pADPRP is not in itself sufficient to cause the progression through G₁ phase to DNA synthesis, an event possibly modulated by other processes in human lymphocytes.

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REFERENCES

- [1] Baserga, R. (1984) *Exp. Cell Res.* 151, 1–5.
- [2] Hara, T. and Fu, S.M. (1985) *J. Exp. Med.* 161, 641–656.
- [3] Cosulich, M.E., Rubartelli, A., Risso, A., Cozzolino, F. and Bargellesi, A. (1987) *Proc. Natl. Acad. Sci. USA* 84, 4205–4209.
- [4] Gerosa, F., Tommasi, M., Scardoni, M., Accolla, R.S., Pozzan, T., Libonati, M., Tridente, G. and Carra, G. (1991) *Mol. Immunol.* 28, 159–168.
- [5] Reed, J.C., Alpers, J.D., Nowell, P.C. and Hoover, R.G. (1986) *Proc. Natl. Acad. Sci. USA* 83, 3982–3986.
- [6] Lehmann, A.R., Kirk-Gell, S., Shall, S. and Whish, W.J.D. (1974) *Exp. Cell Res.* 83, 63–72.
- [7] Rochette-Egly, C., Ittel, M.E., Bilen, J. and Mandel, P. (1980) *FEBS Lett.* 120, 7–11.
- [8] Berger, S.J., Manory, I., Sudar, D.C. and Berger, N.A. (1987) *Exp. Cell Res.* 169, 149–157.
- [9] King, S.L., Mc Nerney, R., Whitley, G.St.J. and Johnstone, A.P. (1989) *Immunology* 67, 258–262.
- [10] Menegazzi, M., Gerosa, F., Tommasi, M., Uchida, K., Miwa, M., Sugimura, T. and Suzuki, H. (1988) *Biochem. Biophys. Res. Commun.* 156, 995–999.
- [11] Stadler, B.M., Dougherty, S.F., Farrar, J.J. and Oppenheim, J.J. (1981) *J. Immunol.* 127, 1936–1940.
- [12] Chirgwin, J.M., Przybyla, A.E., MacDonald, R.J. and Rutter, W.J. (1979) *Biochemistry* 18, 5294–5299.
- [13] Uchida, K., Morita, T., Sato, T., Ogura, T., Yamashita, R., Noguchi, S., Suzuki, H., Nyunoya, H., Miwa, M. and Sugimura, T. (1987) *Biochem. Biophys. Res. Commun.* 148, 617–622.
- [14] Menegazzi, M., Carcereri de Prati, A., Ogura, T., Columbano, A., Ledda-Columbano, M.G., Poltronieri, P., Miwa, M., Libonati, M. and Suzuki, H., in: *ADP-Ribosylation Reactions* (Poirier, G.G. and Moreau, P. eds.) Springer Verlag, New York, in press.
- [15] Farzaneh, F., Zalin, R., Brill, D. and Shall, S. (1982) *Nature* 300, 362–366.
- [16] Green, W.L. and Kaplan, J.G. (1986) *Exp. Cell Res.* 166, 399–415.
- [17] Johnstone, A.P. (1984) *Eur. J. Biochem.* 140, 401–440.