Journal of Cellular Biochemistry 47:242–250 (1991)

ARTICLES

Platelet Release Products Modulate Some Aspects of Polymorphonuclear Leukocyte Activation

A. Allegrezza-Giulietti, R. Serretti, E. Beccerica, S. Muti, G. Ferretti, and C. Cervini

Institutes of Rheumatology (A.A.-G., R.S., E.B., S.M., C.C.) and Biochemistry (G.F.), Faculty of Medicine, University of Ancona, Ancona, Italy

Abstract The aim of this research was to evaluate in vitro interactions between platelets and polymorphonuclear leukocytes. The effects of supernatant from thrombin-activated platelets and two platelet release products (adenosine triphosphate and beta-thromboglobulin) were tested on the following features of polymorphonuclear leukocytes activation: opsonized zymosan and phorbol myristate acetate stimulated chemiluminescence, release of membrane bound calcium, NADPH-oxidase activity, and membrane fluidity (fluorescent polarization). The results showed that the addition of platelet supernatant to polymorphonuclear leukocytes induces a significant activation of cells. On the other hand, after three hours of preincubation of polymorphonuclear leukocytes with platelet supernatant, a decreased response of polymorphonuclear leukocytes to stimulation with phorbol myristate acetate, a significant decrease in NADPH-oxidase activity, and a lowered membrane fluidity were observed. Adenosine triphosphate modulated only opsonized zymosan stimulated chemiluminescence, with and without preincubation with polymorphonuclear leukocytes. Beta-thromboglobulin caused a decrease of the chemiluminescent response of polymorphonuclear leukocytes, using both agonists, with and without preincubation with polymorphonuclear leukocytes. Moreover beta-thromboglobulin only caused a decrease of the polymorphonuclear leukocytes membrane fluidity without preincubation with the cells. These results support the thesis that platelets have a "time-related" modulating activity on polymorphonuclear leukocytes.

Key words: chemiluminescence, NADPH-oxidase, membrane fluidity, membrane bound calcium, adenosine triphosphate, beta-thromboglobulin

Polymorphonuclear leukocytes (PMNLs) play a fundamental role in inflammation mechanisms since they release lysosomal enzymes, leukotrienes, complement components, and several reactive oxygen species [1–3]. Reactive oxygen species production is triggered by the activation of the NADPH-oxidase, which generates superoxide anion, essential for the production of other reactive oxygen species [4,5]. The mechanism of PMNLs activation, in particular the process of signal transduction through the plasma membrane, has been only partially clarified. However, it has been established that the plasma membrane plays a crucial role during PMNLs activation. The binding of appropriate stimuli to receptors of the cell surface induces a series of metabolic modifications, activation of phospholipases, and production of inositol triphosphate and diacylglycerol. Diacylglycerol activates protein kinase C, and inositol triphosphate causes membrane bound calcium mobilization with a subsequent increase in intracellular calcium concentration. These twin signals account for the activation of PMNLs, expressed as aggregation, lysosomal enzyme release, and superoxide anion generation [3].

Platelets have also been shown to play an active role in the inflammation process, and potential modulatory interactions between platelets and PMNLs during the inflammatory response have been observed [6,7]. The ability of intact platelets and/or their products to alter PMNLs response has been observed by several authors, but contrasting findings have been reported [8–13]. To further investigate the ability of platelet release products to modulate PMNLs activation, we studied the effect of supernatant from thrombin-activated platelets on chemiluminescence (CL) of stimulated and unstimulated

Received December 12, 1990; accepted June 17, 1991.

Address reprint requests to Dr. A. Allegrezza-Giulietti, Clinica Reumatologica, Ospedale "Murri," Via dei Colli, 52, 60035 Iesi (AN), Italy.

PMNLs, NADPH-oxidase activity, mobilization of membrane bound calcium, and PMNLs' membrane fluidity. To obtain further information regarding platelet supernatant action on PMNLs, the effects of one non-specific component of platelet supernatant, adenosine triphosphate (ATP), and one specific platelet release product, beta-thromboglobulin (β-TG) were studied.

MATERIALS AND METHODS

Chemicals

N-formyl-methionyl-leucyl-phenylalanine (FMLP), β-nicotinamide adenine dinucleotide phosphate reduced (NADPH), cytochrome c, chlorotetracycline (CTC), phorbol myristate acetate (PMA), ATP, and luminol were obtained from Sigma Chemical Co. (St. Louis, MO).

FMLP (10^{-2} M), PMA (5 mg/ml), and luminol (10^{-3} M) were dissolved in dimethylsulfoxide (DMSO). For basal CL determinations (PMNLs untreated with opsonized zymosan or PMA), a more highly concentrated solution of luminol (3×10^{-3} M) was used in order to enhance luminometer response. Before use, the agonists were diluted with modified Krebs-Ringer phosphate buffer (KRP). NADPH (1.1×10^{-3} M) and cytochrome c (10^{-4} M) were dissolved according to Markert et al. [14]. Opsonized zymosan (2.5×10^{-2} g/ml) was prepared as described previously [15,16]. CTC (10^{-2} M) was freshly prepared each time. 1-(4-trimethylaminophenyl)-6-phenyl-1,3,5-hexatriene (TMA-DPH) (Molecular Probes, Inc., Junction City, OR) was dissolved in ethanol at a concentration of 2×10^{-3} M. A stock solution of β-TG (100 ng/ml) (Boehringer Mannheim, Federal Republic of Germany) was prepared in KRP. All of the stock solutions were stored at −30°C, except luminol, which was stored at room temperature and protected from light. The amounts of DMSO added did not alter PMNLs viability evaluated using trypan blue exclusion test (data not shown).

Chemiluminescent Response of PMNLs

Preparation of cells. Platelets were obtained from the venous blood of healthy donors using EDTA 5% (300 μl/10 ml of blood) as an anticoagulant. 10 milliliters of each sample were centrifuged at 200g for 15 minutes to obtain the platelet rich plasma. This was then added to the following buffer: EDTA 10^{-3} M, NaCl 15×10^{-2} M, glucose 5×10^{-3} M, Tris-HCl 10^{-2} M (pH = 7.4). Platelets were isolated by centrifugation (500g for 10 minutes), washed twice at 500g for 10 minutes, and finally resuspended in KRP at a final concentration of 10^6 cells/μl. Following this process, PMNLs were separated from the remaining blood as reported in our previous studies [15,16]. Cells were finally resuspended in KRP (10^4 cells/μl); 3-5 platelets/100 PMNLs were usually observed. The trypan blue test showed that 97 ± 1.8% of the PMNLs were vital after each separation.

Preparation of platelet release products. Platelets (10^6 cells/μl) were stimulated with thrombin (Behringwerke AG, Marburg, Federal Republic of Germany) (final concentration 0.50 UI/ml) at 37°C for 5 minutes with gentle shaking. In our experimental conditions, this amount of thrombin induces aggregation of platelets and the release of granule content. The mixture was then centrifuged (1,000g for 10 minutes) and the cell-free supernatant obtained, containing platelet release products, was used for subsequent experiments. Thrombin, at the concentration used to activate platelets, did not modify PMNLs activity (data not shown).

Measurements of CL. CL was measured as previously described [15,16]. Basal CL was measured simultaneously on four samples from each donor. The first sample, used as reference, contained: PMNLs in KRP (5×10^5 cells/ml) and luminol (6×10^{-5} M). Platelet supernatant (50 μl), β-TG (1 ng/ml), or ATP (3×10^{-5} M) were added to the other samples. A final volume of 1 ml was used for all samples. Basal values have not been subtracted from stimulated CL values, as the former are very small. In fact, to obtain basal CL values the signal was amplified using a much more sensitive (100-fold) range of the recorder, and a more highly concentrated solution of luminol was used. In stimulated CL measurements, PMNLs were treated with opsonized zymosan (final concentration 5×10^{-4} g/ml) or PMA (final concentration 2 ng/ml) and diluted luminol was used (final concentration 2×10^{-5} M). Two samples were prepared for each donor. Platelet supernatant (50 μl) was added to the second. Moreover, opsonized symosan and PMA stimulated CL were measured after 1, 2, and 3 hours (at 37°C with gentle shaking) of incubation of PMNLs with platelet supernatant. Stimulated CL was also measured, in the same experimental conditions, after the addition of ATP (3×10^{-5} M) and β-TG (1 ng/ml) to PMNLs.

NADPH-Oxidase Assay

NADPH-oxidase was obtained from opsonized zymosan-activated PMNLs by a modified Markert's method [14]. PMNLs (18,000 ± 2,000 cells/μl) were activated with opsonized zymosan (25 μl/10^6 cells) and preincubated for 7 minutes at 37°C with gentle shaking. Cells were then disrupted by homogenization at 0°C for 10 minutes and the mixture centrifuged at 200g for 5 minutes at 4°C to remove zymosan, nuclei, and unbroken cells. The supernatant, containing membrane particulate NADPH-oxidase (diluted to reach a concentration of protein of 0.5 mg/ml), was employed for measurements of superoxide anion production at 25°C, by the reduction of cytochrome c (at 550 nm) [17]. An LKB 4050 spectrophotometer equipped with a constant temperature sample holder and connected to a personal computer was used for measuring. All reagents were suspended in phosphate buffer (5×10^{-3} M; pH = 7.8) [17]. Two samples (final volume 2 ml for both samples) were prepared from each donor. The first, used as reference, contained supernatant from the processed PMNLs (500 μl), cytochrome c (final concentration 3×10^{-5} M), and Triton X-100 0.2% w/v in water (200 μl). One hundred microliters of the supernatant from thrombin-activated platelets was added to the second sample. The reaction was started by adding NADPH (final concentration 2.2×10^{-4} M). Enzyme activity was also measured after the addition of ATP (3×10^{-5} M) and β-TG (1 ng/ml) to the membrane fragments.

Moreover a suspension of intact PMNLs (18,000 ± 2,000 cells/μl) was preincubated with platelet supernatant for 10 minutes, 1, 2, and 3 hours at 37°C with gentle shaking. Two samples were prepared for each experiment: the first, used as reference, contained only PMNLs; platelet supernatant was added to the second. After each preincubation PMNLs were activated with opsonized zymosan, then processed, and NADPH-oxidase activity was evaluated.

Measurements of Membrane Bound Calcium Release

Membrane bound calcium release was measured using the fluorescence of CTC, as described by Smolen and Weissmann [18]; however, in this case KRP was used. CTC, from the stock solution, was added to 1 ml of PMNLs (4×10^6 cells/ml) to reach the final concentration of 10^{-4} M. After preincubation of 30 minutes at 37°C [18], PMNLs were washed once and then resuspended in 2 ml of KRP to obtain a concentration of 2×10^6 cells/ml. A Perkin-Elmer MPF-66 fluorescence spectrofluorometer equipped with a constant temperature (37°C) sample holder was used for measuring. Excitation and emission wavelengths were 380 nm and 560 nm. Slitwiths were 10 nm for excitation and 5 nm for emission. Baseline levels of CTC fluorescence in PMNLs were monitored for 2 minutes, followed by the addition of 100 μl of platelet supernatant. The same amount of KRP did not modify the CTC fluorescence intensity. CTC fluorescence was also studied after the addition of FMLP (10^{-6} M), PMA (2 ng/ml), ATP (3×10^{-5} M), and β-TG (1 ng/ml) to PMNLs.

Fluorescent Polarization Measurements

The effect of platelet release products on PMNLs plasma membrane fluidity was studied using TMA-DPH fluorescence polarization [19]. In these experiments PMNLs were isolated by dextran sedimentation and Hypaque-Ficoll density gradient centrifugation to eliminate lymphocyte contamination. A suspension of PMNLs (2×10^6 cells/ml; final volume 2 ml) was used immediately to assess membrane fluidity of PMNLs in the absence of platelet release products. The other suspensions were preincubated in absence or in presence of 100 μl of platelet supernatant at 37°C with gentle shaking for 1, 2, and 3 hours. After preincubation, the samples were washed in KRP and cells were labelled with TMA-DPH at a final concentration of 10^{-6} M [20]. At the cell concentration used the effect of light scattering was not significant. A Perkin-Elmer spectrofluorometer MPF-66 (xenon lamp 150 W, filter 390 nm) linked to a Perkin-Elmer model 7300 computer, with PECLS-III software was used to measure TMA-DPH fluorescence polarization. The excitation and emission wavelengths were 360 nm and 430 nm respectively. The results of steady-state polarization experiments are expressed as fluorescence polarization (P). $P = (Ia - Ibxg)/(Ia + Ibxg)$, where Ia and Ib are the fluorescence intensities, parallel and perpendicular to the sample, and the g (0.7) represents a factor used to correct the unequal transmission of differently polarized light. Membrane fluidity was also measured after the addition of ATP (3×10^{-5} M) and β-TG (1 ng/ml) to PMNLs in two different experimental conditions. In the first case, chemicals were added to

PMNLs and measurements were immediately taken. In the second case, measurements were carried out after 1, 2, or 3 hours of preincubation.

Statistical Analysis

The signed rank test for paired data was used to perform the statistical analysis of the results. In order to confirm the significant differences obtained by the signed rank test, the student t-test for paired data was also employed. To calculate the mean of percentage values, data were normalized by arcsin transformation, as percentage values are not normally distributed [21].

RESULTS

Chemiluminescence Measurements

The addition of platelet supernatant to PMNLs induced a statistically significant increase of 83.3 ± 15% of basal CL (Table I). The addition of platelet supernatant to activated PMNLs induced also a statistically significant increase of opsonized zymosan stimulated CL ($P < 0.05$) and PMA stimulated CL ($P < 0.05$) (Table II). The percentage increase were 17.26 ± 6% and 30 ± 6% respectively. PMNLs preincubated with platelet supernatant showed a lower PMA stimulated CL response compared with PMNLs preincubated in the absence of platelet supernatant. After a preincubation of 1, 2, and 3 hours, percentage decreases of PMA stimulated CL values were respectively: 19.96 ± 8.9%, 35 ± 7%, and 25.8 ± 24.8% ($P < 0.05$) (Fig. 1). On the contrary, PMNLs preincubated with platelet supernatant did not show any significant modification of opsonized zymosan stimulated CL (Table II).

ATP inhibited the CL response only in PMNLs stimulated with opsonized zymosan, both with and without preincubation of 3 hours, percentage decreases were respectively 30.6 ± 3.5% ($P < 0.05$); and 31.7 ± 13% ($P < 0.05$) (Fig. 2a). However, ATP did not induce significant modifications of basal and PMA stimulated CL response (Fig. 2b) (Table I).

The addition of β-TG to PMNLs induced a considerable decrease in both opsonized zymosan and PMA stimulated CL (Fig. 3a), the percentage decreases were, respectively, 82.7 ± 8.3% ($P < 0.01$) and 47.9 ± 9.5% ($P < 0.01$). After 3 hours of preincubation, the percentage decreases were 99.8 ± 0.0% for opsonized zymosan ($P < 0.01$) and 98.7 ± 4.7% for PMA ($P < 0.01$) (Fig. 3b). The analysis of β-TG action on PMNLs over a complete time course showed a time-dependent increase of its inhibitory effect on opsonized zymosan and PMA stimulated CL (Fig. 4). However, β-TG failed to produce a significant decrease of basal CL (Table I). In order to evaluate if the inhibitory effect shown by β-TG was due to cellular damage during preincubation, the trypan blue test was performed. It showed that 98.1 ± 1.5% of cells were vital. Furthermore, the strong inhibitory action of β-TG was not due to its function as oxygen radical scavenger. In fact, the addition of β-TG to a cell-free system, in which superoxide anion was generated by the aerobic action of xanthine oxidase on xanthine, failed to produce a significant change in the rate of cytochrome c reduction (data not shown).

TABLE I. Effect of Platelet Supernatant, ATP, and β-TG on Basal CL†

	Basal chemiluminescent units (bCLU)
Reference	1.81 ± 1.3
PMNLs + platelet supernatant	2.9 ± 1.9*
PMNLs + ATP (3×10^{-5} M)	1.53 ± 0.9
PMNLs + β-TG (1 ng/ml)	1.76 ± 0.5

†Measurements of basal CL were performed for 50 minutes at 37°C. Data are expressed as mean values ± SD of 13 experiments; bCLU = (area of CL curves/number of PMNLs) × 10^5.
*$P < 0.05$.

Assay of NADPH-Oxidase

The ability of membrane vesicles containing NADPH-oxidase to produce superoxide anion was enhanced by the addition of platelet supernatant. Mean values of nmoles of superoxide anion produced were: 10.6 ± 4.2 without platelet supernatant and 12.6 ± 5.2 with platelet supernatant, the difference was statistically significant ($P < 0.05$; confidence limits = 0–4.1; n = 13). ATP and β-TG failed to produce changes of production of superoxide anion by membrane fragments (data not shown).

We also studied the activity of NADPH-oxidase in membrane vesicles prepared from intact PMNLs preincubated with platelet supernatant. No change in enzyme activity was shown when PMNLs were preincubated for a period of 10 minutes or 1 hour. On the contrary, a signifi-

TABLE II. Effects of Platelet Supernatant on PMNLs Stimulated CL

	A[a]			
	Chemiluminescent units (CLU)		Confidence limits	Significance
	Without platelet supernatant	With platelet supernatant		
PMA	2.27 (± 1.65)	3.14 (± 1.93)	0–1.74	$P < 0.05$
O. Zymosan	10.94 (± 7.86)	13.45 (± 10.02)	0–5.02	$P < 0.05$
	B[b]			
	Chemiluminescent units (CLU)		Confidence limits	Significance
PMA	2.75 (± 1.62)	2.17 (± 1.60)	0–1.16	$P < 0.05$
O. Zymosan	3.16 (± 1.70)	6.01 (± 5.29)	0–5.71	n.s.

[a]Platelet supernatant was added to PMNLs immediately before the addition of agonists.
[b]PMNLs were preincubated with platelet supernatant (3 hours at 37°C), then followed the addition of agonists. Data are expressed as mean values ± SD of 13 experiments; CLU = (area of CL curves/number of PMNLs) × 10^3. n.s., not significant.

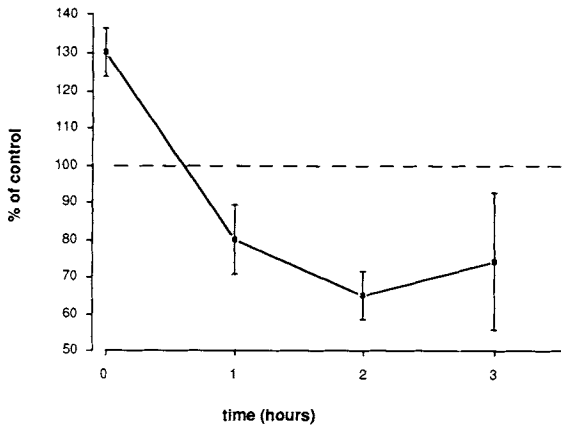

Fig. 1. Effect of platelet supernatant on PMA stimulated CL of PMNLs. Measurements were performed both without and with different periods of preincubation of PMNLs with platelet supernatant (1, 2, and 3 hours at 37°C). Data, mean ± SD of 3 experiments, are expressed as a percentage of control.

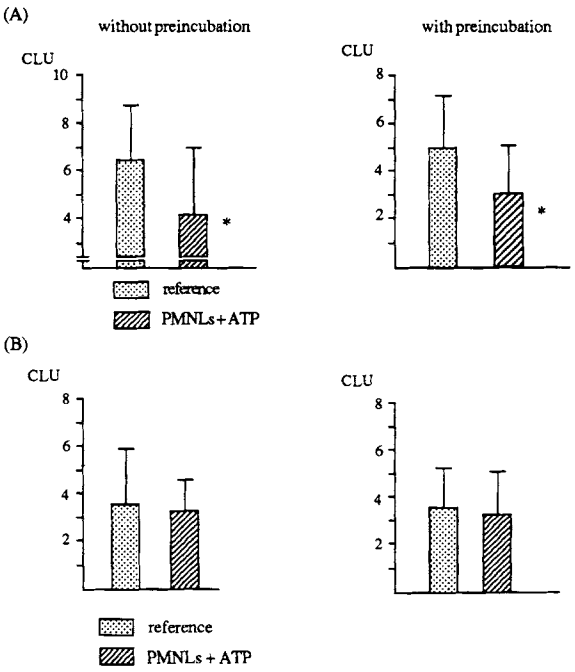

Fig. 2. Effect of ATP on PMNLs CL response stimulated by opsonized zymosan (A) or PMA (B). Measurements were performed both without and with a preincubation of cells with ATP (3 hours at 37°C). Data are expressed as chemiluminescent units (CLU); CLU = (area of CL curves/number of PMNLs) × 10^3. Results are mean ± SD of 13 experiments. *$P < 0.05$ with respect to the control sample.

cant decrease of enzyme activity was observed when PMNLs were preincubated for 2 or 3 hours with platelet supernatant (Fig. 5).

Release of Membrane Bound Calcium

A rapid decrease of CTC fluorescence was observed after the addition of platelet supernatant to CTC preloaded PMNLs, which indicates a release of membrane bound calcium (Fig. 6) [18].

ATP and β-TG produced only a small decrease of CTC fluorescence (Fig. 6). PMA and FMLP were used to test the method; results obtained were in accordance with those of other authors [18].

Fluorescent Polarization Measurements

The mean value of TMA-DPH fluorescence polarization (P) in non-preincubated PMNLs was 0.358 ± 0.017, in conformity with results obtained by other authors [22]. Platelet supernatant did not modify plasma membrane fluidity after 1 or 2 hours of preincubation (data not

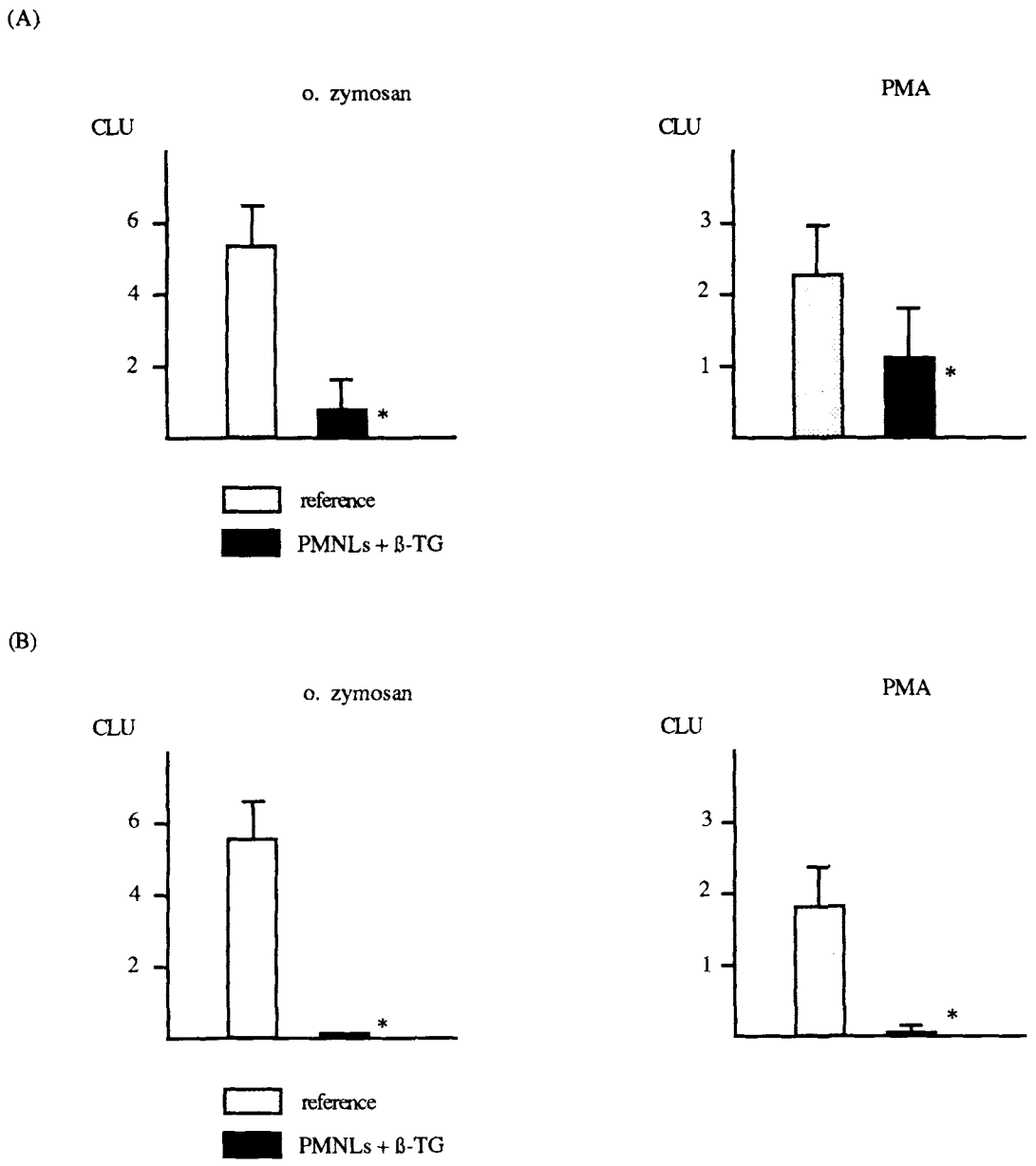

Fig. 3. Effect of β-TG on PMNLs CL response. Measurements were performed both without (**A**) and with (**B**) a preincubation of cells with β-TG (3 hours at 37°C). Data are expressed as chemiluminescent units (CLU); CLU = (area of CL curves/number of PMNLs) × 10^3. Results are mean ± SD of 13 experiments. *$P < 0.01$ with respect to the control sample.

shown). PMNLs preincubated for 3 hours in the absence of platelet supernatant did not show significant changes of P value. However, in the same experimental conditions, but in the presence of platelet supernatant, PMNLs showed a significant increase of P value ($P < 0.05$), which indicates a decrease in plasma membrane fluidity (Table III).

ATP did not modify the P values both with or without preincubation (Fig. 7).

The addition of β-TG to PMNLs induced an instantaneous significant increase of P values ($P < 0.05$) (Fig. 7). No relevant changes of P values were observed for longer incubations (Fig. 7).

DISCUSSION

Using CL, the addition of platelet supernatant to PMNLs induced an immediate increase both of basal and stimulated CL, in line with the findings of other authors [8,10].

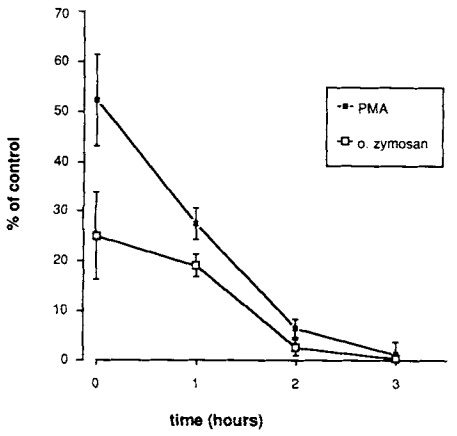

Fig. 4. Effect of β-TG on PMA or opsonized zymosan stimulated CL of PMNLs. Measurements were performed both without and with different periods of preincubation of PMNLs with β-TG (1, 2, and 3 hours at 37°C). Data, mean ± SD of 3 experiments, are expressed as a percentage of control.

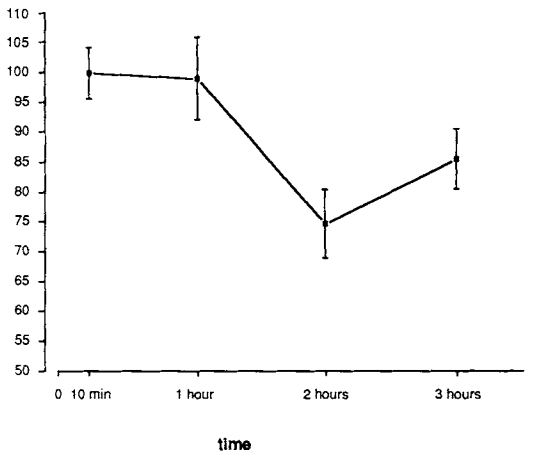

Fig. 5. Effect of platelet supernatant on superoxide anion production by NADPH-oxidase. Measurements were performed after different periods of preincubation of PMNLs with platelet supernatant (10 minutes, and 1, 2, and 3 hours at 37°C). Data, mean ± SD of 3 experiments, are expressed as a percentage of control.

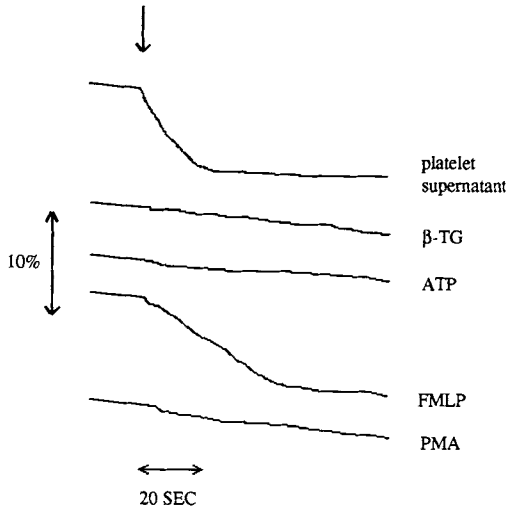

Fig. 6. Effect of platelet supernatant, FMLP, ATP, and PMA on membrane bound calcium release expressed by the fluorescence of CTC-loaded PMNLs. Platelet supernatant, FMLP, ATP, and PMA were added to PMNLs after 2 minutes from the beginning of measurements. The stimuli were added at the time indicated by the arrow.

TABLE III. Fluorescent Polarization Values (P) of TMA-DPH in PMNLs With and Without Platelet Supernatant†

Samples	Time of preincubation	P
Reference		0.358 (± 0.017)
Reference	3 hours	0.357 (± 0.032)
+ platelet supernatant	3 hours	0.370* (± 0.017)

†Preincubation was carried out at 37°C. Results are expressed as mean values (± SD) of P of 13 experiments.
*$P < 0.05$.

The stimulating effect on CL could be related to the increase of NADPH-oxidase activity induced by platelet release products. In fact, the stimulation of NADPH-oxidase produces superoxide anion and other reactive oxygen species formed indirectly [5]. The stimulatory effect of platelet release products on reactive oxygen species production is also confirmed by the increase of membrane bound calcium mobilization after the addition of platelet supernatant. It is known that intracellular calcium is important for the mechanism of PMNLs activation. In fact, superoxide anion generation and lysosomal enzyme release can be eliminated by an inhibition of membrane bound calcium mobilization [23,24]. In order to investigate the time-dependent effect of platelet release products on PMNLs activity, we tested PMNLs response after different periods of preincubation with platelet supernatant. With reference to the CL experiments, we observed that after 3 hours of preincubation with platelet supernatant, the PMA stimulated CL was significantly decreased, while opsonized zymosan stimulated CL was unchanged. NADPH-oxidase superoxide anion production was decreased after 2 or 3 hours of preincubation of PMNLs with platelet supernatant.

A hypothesis can be advanced to explain these inhibitory effects. During preincubation with platelet supernatant, PMNLs are continuously

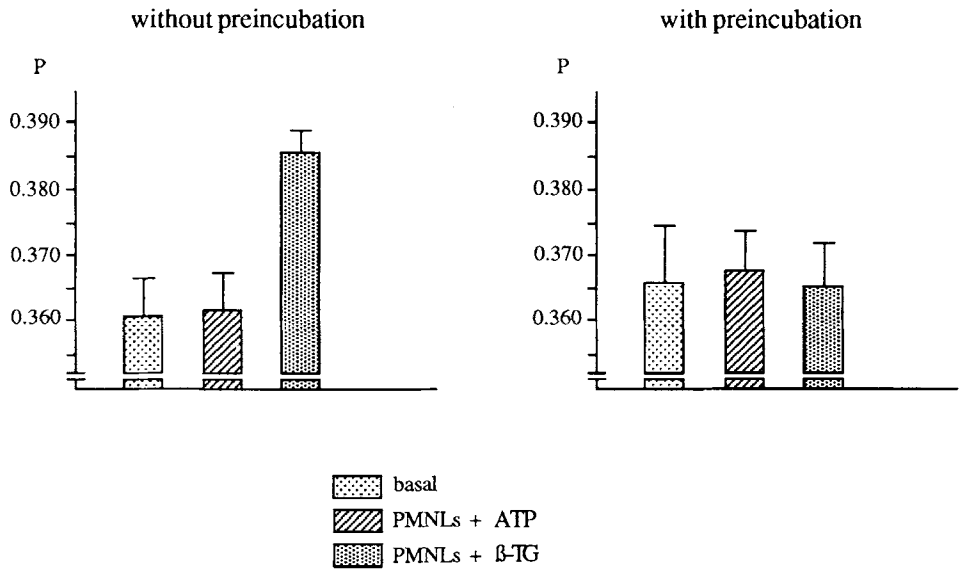

Fig. 7. Effect of ATP and β-TG on membrane fluidity of PMNLs. Measurements were performed both without and with preincubation of cells with ATP or β-TG (3 hours at 37°C). Results are expressed as fluorescent polarization values (P) of TMA-DPH. Results are expressed as mean ± SD of 13 experiments.

stimulated, as demonstrated by the increase of basal CL. Various experimental evidence suggests that the release of reactive oxygen species could induce the peroxidation of membrane lipids [25–29]. In model and natural membranes, an increase of lipid peroxidation is associated with a decrease of membrane fluidity that causes a loss of balance in the expression of the receptor surfaces [30–32]. During PMNLs activation, the preformed receptors for the components of complement (C3b and C3bi) are translocated from the cytoplasm to the cell surface through the membrane [33–35]. In our opinion a decrease of membrane fluidity, probably mediated by lipid peroxidation, could modify the receptors' exposition and/or prevent signal transduction following the binding of agonist molecules with their membrane receptors.

It is nevertheless possible that the platelet release products act directly on PMNLs membrane and not by the mediation of reactive oxygen species.

The reduced response of PMNLs after a long period of preincubation with platelet supernatant could also be related to receptor saturation [36]. However, this possibility could be excluded due to the fact that the same decreases of cell activity were observed in PMNLs, washed and unwashed, after preincubation (data not shown).

Our results indicate that molecules present in the platelet supernatant modify the PMNLs response. It has to be stressed that some of these products exert stimulatory effects and their action can be evaluated immediately; others exert an inhibitory effect only after a period of contact with PMNLs. In order to investigate if the changes of PMNLs activity observed are due to specific molecules released by platelets, we studied the effects of ATP and β-TG on PMNLs. McGarrity et al. have earlier suggested that adenine nucleotides could play an inhibitory effect on PMNLs activity [12]. We found that ATP exerts an inhibitory effect on PMNLs, both immediately and after a long incubation, but only when cells were stimulated with opsonized zymosan. On the other hand, using PMA as an agonist, ATP caused no modification of the PMNLs CL response. Opsonized zymosan activates the phosphoinositide turnover, with subsequent calcium mobilization and protein kinase C phosphorilation, while PMA directly activates protein kinase C and bypasses these cellular mechanisms. Therefore, our results suggest that ATP acts before protein kinase C activation. In this manner, the PMNLs activation pathway due to opsonized zymosan is prevented; this results in diminished CL response. On the other hand, PMNLs stimulation by PMA is not influenced by ATP, because the agonist acts directly on protein kinase C. These results, and the fact that ATP does not influence NADPH-oxidase activity, membrane-bound calcium release and

membrane fluidity, could be explained by the direct action of the nucleotide on specific receptors (opsonized zymosan receptors).

Our findings showed that without preincubation, β-TG induces a considerable decrease of the PMNLs CL response using either opsonized zymosan or PMA as an agonist. This inhibition could be related to the decrease of PMNLs membrane fluidity observed after the addition of β-TG. The inhibitory effect of β-TG on PMNLs CL response was time-related, the highest value of inhibition was reached after 2 hours (Fig. 5). Membrane fluidity, on the contrary, was not modified after preincubation. An explanation of these conflicting findings can be found in a possible cleavage action by PMNLs on β-TG during preincubation. It has been demonstrated that during preincubation of β-TG with human blood mononuclear cells, these cells generate a neutrophil-activating peptide corresponding to a carboxy-terminal fragment of β-TG [37]. In our opinion PMNLs, during a long period of preincubation, could produce one or more peptide(s) from β-TG cleavage. The derivated peptide(s) could modify the PMNLs CL response by mechanisms as yet unknown and not related to changes in membrane fluidity. This possibility is currently under investigation.

Although further work is needed to elucidate the nature of platelet factors involved, as well as their target site(s) on the PMNLs, these findings are consistent with the participation of platelets in the modulation of PMNLs activity in vitro and, possibly, in vivo at the site of inflammation.

ACKNOWLEDGMENTS

We are grateful to Mrs. Frances Chetcuti, B.A. Hons., who very constructively helped to prepare the manuscript.

REFERENCES

1. Allegrezza Giulietti A, Muti S, Piergiacomi G: Clin Ter 122:445–457, 1987.
2. Root RK, Metcalf JA: J Clin Invest 60:1266–1279, 1977.
3. Weissmann G, Smolen JE, Korchak HM: N Engl J Med 303:27–34, 1980.
4. Deby C, Pincemail J: Presse Méd 15 (31):1468–1474, 1986.
5. Rossi F: Biochem Biophys Acta 853:65–89, 1986.
6. Marcus AJ, Safier LB, Ullman HL, Islam N, Broekman MJ, Falck JR, Fischer S, von Schacky C: J Biol Chem 263(5):2223–2229, 1988.
7. Ward PA, Cunningham TW, McCulloch KK, Phan SH, Powell J, Johnson KJ: Lab Invest 58:37–42, 1988.
8. Boogaerts MA, Vercellotti G, Roelant C, Malbrain S, Verwilghen RL, Jacob HS: Scand J Haematol 37:229–236, 1986.
9. Del Maschio A, Corvazier E, Maillet F, Kazatchkine MD, Maclouf J: Br J Haematol 72:329–335, 1989.
10. Del Maschio A, Maclouf J, Corvazier E, Grange MJ, Borgeat P: Nouv Rev Fr Haematol 27:275–278, 1985.
11. McGarrity ST, Hyers TM, Webster RO: J Leukoc Biol 44:93–100, 1988.
12. McGarrity ST, Stephenson AH, Hyers TM, Webster RO: J Leukoc Biol 44:411–421, 1988.
13. Tzeng DY, Deuel TF, Huang JS, Senior RM, Boxer LH, Baehner RL: Blood 64:1123–1128, 1984.
14. Markert M, Andrews PC, Babior BM: In Colowick SP, Kaplan NO, (eds): "Methods in Enzymology," Vol 105. New York: Academic Press, 1984, pp 358–365.
15. Piergiacomi G, Allegrezza Giulietti A, Muti S, Silveri F, Cervini C: Z Rheumatol 47:151–155, 1988.
16. Silveri F, Allegrezza Giulietti A, Muti S, Piergiacomi G: Clin Tri J 25:390–400, 1988.
17. Crapo JD, McCord JM, Fridowich I: In Colowick SP, Kaplan NO, (eds): "Methods in Enzymology," Vol 53. New York: Academic Press, 1978, pp 382–387.
18. Smolen JE, Weissmann G: Biochem Biophys Acta 720: 172–180, 1982.
19. Ferretti G, Offidani AM, Simonetti O, Valentino M, Curatola G, Bossi G: Biochem Med Metab Biol 41:132–138, 1989.
20. Kuhry JG, Duportail G, Bronner C, Laustriat G: Biochem Biophys Acta 845:60–68, 1985.
21. Armitage P: Blackwell Scientific Publication 493, 1971.
22. Valentino M, Governa M, Fiorini R, Curatola G: Biochem Biophys Res Commun 141:1151–1156, 1986.
23. Naccache PH, Molski TFP, Alobaidi T, Becker EL, Showell HJ, Sha'afi RI: Biochem Biophys Res Commun 97(1): 62–68, 1980.
24. Smolen JE, Korchack HM, Weissmann G: Biochem Biophys Acta 677:512–520, 1981.
25. Bonvini E, Bougnoux P, Stevenson HC, Miller P, Hoffman T: J Clin Invest 73:1629–1637, 1984.
26. Fong KL, McKay PB, Poyer JL, Keele BB, Misra H: J Biol Chem 248:7792–7797, 1973.
27. Hirata F, Axelrod J: Science 209:1082–1090, 1980.
28. Lunec J, Halloran SP, Withe AG, Dormandy TL: J Rheumatol 8:233–245, 1981.
29. Muus P, Bonta IL, Den Oudsten JA: Prostagl Med 2:63–68, 1979.
30. Heron DS, Shinitzky M, Hershkowitz M, Samuel D: Proc Natl Acad Sci USA 77:7463–7467, 1980.
31. Muller CP, Shinitzky M: Br J Haematol 42:355–362, 1979.
32. Shinitzky M, Henkart P: Int Rev Cytol 60:121–126, 1980.
33. Berger M, O'Shea J, Cross AS, Folles TM, Chused TM, Brown EJ, Frank MM: J Clin Invest 74:1566–1570, 1984.
34. Fearon DT, Collins LA: J Immunol 130:370–373, 1983.
35. Shalit M, von Allmen C, Atkins PC, Zweiman B: J Leukoc Biol 44:212–217, 1988.
36. English D, Roloff JS, Lukens JN: J Immunol 126:165–171, 1981.
37. Waltz A, Baggiolini M: Biochem Biophys Res Commun 3:969–975, 1989.