

## Neutrophil-endothelial cell interaction: evidence *in vitro* for a regulation by endothelial cells of neutrophil functions

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*Neutrophil-endothelial cell interaction: evidence in vitro for a regulation by endothelial cells of neutrophil functions. P. Lassalle, Y. Delneste, P. Gosset, B. Wallaert, A.B. Tonnel, J.P. Dessaint, A. Capron.*

**ABSTRACT:** The purpose of this study was to investigate a possible relationship between human umbilical vein endothelial cells (EC) triggered by ionophore A23187 at different doses (0.5–2.5  $\mu\text{M}$ ) and polymorphonuclear neutrophils (PMN). EC supernatants were shown to contain neutrophil chemoattractant activity (NCA) and in parallel a factor inducing an inhibition of PMN chemiluminescence (PMN CL). Supernatants obtained from EC triggered by A23187 exhibited a high level of NCA ( $73 \pm 5$  PMN·hpf<sup>-1</sup> compared to  $21 \pm 4$  PMN·hpf<sup>-1</sup> in untreated EC supernatants,  $p < 0.01$ ). This NCA was independent from arachidonic acid metabolites, since indomethacin and nordihydroguaiaretic acid failed to suppress the chemotactic activity. Using gel filtration chromatography (AcA 54) the NCA was recovered in a single peak of apparent molecular weight of  $37,000 \pm 4,000$  daltons. Checkerboard analysis indicated that NCA exhibited both chemotactic and chemokinetic activities. In addition, supernatants of A23187-stimulated EC, and at a lesser degree, supernatants of unstimulated EC, inhibited PMN CL induced by N-formyl-Methionyl-Leucyl-Phenylalanine (61% inhibition,  $p < 0.05$ ), and by A23187 itself (80% inhibition,  $p < 0.01$ ), but not that induced by phorbol-myristate-acetate. Indomethacin and protamine sulphate did not modulate this inhibitory activity. By contrast, EC-derived inhibitory activity was inhibited (50%) by an adenosine antagonist (8-phenyltheophylline), indicating a participation of adenosine in this inhibitory activity of PMN CL. These data suggest the possibility that activated endothelial cells could both enhance PMN migration and protect themselves against potential damaging effects of oxygen metabolites produced by PMN, particularly during transvascular migration.

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In lung diseases, a variety of disorders are known to involve influx of neutrophils into the alveolar structures [1, 2]. The stimulus for accumulation of neutrophils appears to be supported by alveolar macrophages as a source of chemotactic factors [3, 4]. More recently, endothelial cells have been proposed as an alternative source of recruiting factors. In this context, it has been shown that endothelial cells could release neutrophil recruiting factors either spontaneously [5] or in response to several stimuli such as hypoxia [6], thiourea [7], angiotensin II [8], and histamine [9]. Since activated neutrophils are able to generate toxic oxygen metabolites, it seems reasonable to hypothesize that endothelial cells, like other cells, employ a variety of scavenger mechanisms to protect themselves from oxidant attacks. Such defences include glutathione redox cycle, catalase [10, 11], prostacyclin which modulates aggregation and migration of activated neutrophils [12],

and adenosine which seems to inhibit  $\text{O}_2^-$  and  $\text{H}_2\text{O}_2$  release from stimulated neutrophils by engaging specific adenosine  $\text{A}_2$  receptors at their surface [13].

Because endothelial cells are potentially able to produce both neutrophil recruiting factors and potent inhibitors of oxygen radical production such as adenosine, we investigated whether these two activities could be simultaneously demonstrated in an *in vitro* model using ionophore A23187 as a stimulating agent of endothelial cells.

### Materials and methods

#### *Isolation and culture of endothelial cells*

Endothelial cells (EC) were obtained from human umbilical veins, using the method of JAFFEE *et al.* [14],

slightly modified. Briefly, EC were collected from umbilical cords treated by 0.2% (v:v) collagenase (Sigma Chemical Co., St Louis, MO) in Hanks balanced salt solution (HBSS) (M.A. Bioproducts, Walkerville, MD). EC were isolated, suspended at approximately  $1-2 \times 10^5$  cells·ml<sup>-1</sup> in RPMI 1640 (Gibco, Grand Island, NY) containing 25 mM HEPES (M.A. Bioproducts), 2 mM L-glutamine, 100 U·ml<sup>-1</sup> penicillin, 100 µg·ml<sup>-1</sup> streptomycin (all from Sigma), supplemented with 20% (v:v) heat-inactivated foetal calf serum, 100 µg·ml<sup>-1</sup> heparin, and 25 µg·ml<sup>-1</sup> endothelial cell growth supplement (Sigma). EC were then cultured in 35 mm diameter tissue culture wells (Falcon, Los Angeles, CA) and maintained in a humidified atmosphere of 5% CO<sub>2</sub> in air at 37°C. The medium was changed twice a week and the cultures reached confluency in 5 or 7 days. EC were then detached by incubation with 5 mM ethylene diamine tetraacetic acid (EDTA) (Sigma) in phosphate buffered saline (PBS) for 10 min at 37°C, centrifuged at 500 g for 10 min, resuspended in fresh medium and further cultured in 16 mm diameter tissue culture wells (NUNC, Denmark). The secondary culture reached confluency in 3-5 days. Only second passage EC cultures were used in these studies. Morphology and detection of factor VIII related antigen (FVIII-R:Ag) by indirect immunofluorescence were used to check purity of the cultures.

#### Isolation of human PMN

Granulocytes were collected from heparin-treated peripheral blood from healthy volunteers and purified by centrifugation over Ficoll-Hypaque (Pharmacia Fine Chemicals AB, Uppsala, Sweden) followed by dextran sedimentation and hypotonic lysis of the contaminating erythrocytes, as described by BOYUM [15]. The granulocyte pellet was washed in PBS and resuspended at  $1 \times 10^6$  cells·ml<sup>-1</sup> in HBSS. Cell preparations contained more than 95% PMN and >90% of viable cells as defined by trypan blue exclusion.

#### Preparation of activated EC supernatants

For the preparation of EC supernatants, all EC cultures were used after they reached confluency ( $1.6-2.2 \times 10^5$  cells·well<sup>-1</sup>). Each well was washed three times with HBSS. Ionophore A23187 (0.5-2.5 µM at final concentration) was added to cell cultures. Preliminary time-course kinetic experiments have shown a dual detectable neutrophil chemoattractant and PMN chemiluminescence inhibitory activity as early as 15 min, reaching a plateau within 30-90 min. For these reasons, an incubation time of 30 min was chosen for all the studies reported here. After 30 min incubation at 37°C, EC supernatants were recovered, centrifuged (500 g for 10 min) and kept on ice until tested. In some experiments, 1 mM dibutyryl cyclic adenosine monophosphate (dibutyryl-cAMP, Sigma), 10 µM 8-phenyltheophylline (Aldrich Chem. Corp.) were added

to EC supernatants before testing. To evaluate the participation of arachidonic acid metabolites, 10 µM indomethacin, or 10 µM nordihydroguaiaretic acid (NDGA) (all from Sigma) were added before EC A23187-triggering.

#### Studies of PMN motility

The chemotaxis assay was carried out with a 48-well microchemotaxis assembly (Neuroprobe, Cabin John, Md, USA) [16] in which the upper and lower compartments were separated by a 3 µm pore polycarbonate filter (Nucleopore Corp., Pleasanton). The lower chambers were filled with 24 µl of activated EC supernatants, A23187 alone in HBSS, HBSS alone as negative control, and 1 µM fMLP (N-formyl-MET-LEU-PHE, Sigma) in HBSS as positive control. The upper chambers were filled with 45 µl of PMN suspension ( $1 \times 10^6$  cells·ml<sup>-1</sup> in HBSS) and incubated for 30 min at 37°C in humidified air with 5% CO<sub>2</sub>. Following incubation, the filters were removed, fixed and stained in May Grünwald Giemsa. The number of neutrophils which have migrated through the filter was determined microscopically using an immersed oil objective. Four fields were examined and counted per well. Experiments were conducted in quadruplicate. Results were expressed as the difference between the mean number of cells per field in the experimental well and the mean number of cells per field in the control wells (migration towards medium). Statistical analysis was performed by using two way analysis of variance. Checkerboard analysis for chemotaxis versus chemokinesis of column effluents from activated EC supernatants was performed as described by ZIGMOND and HIRSCH [17].

#### Chemiluminescence

Chemiluminescence (CL) was assessed as described by DESCAMP-LATSCHA *et al.* [18]. Briefly, PMN suspensions ( $1 \times 10^6$  cells·ml<sup>-1</sup>) were diluted in HBSS and kept at 4°C before testing. Seventy microliters were transferred into a counting tube, previously filled with 40 µl of EC supernatants, and either 20 µl of control buffer, or stimulated agent solution, *i.e.* 0.5-2.5 µM A23187, 1 µM fMLP or 1-10 ng phorbol myristate acetate (PMA) diluted in dimethylsulphoxide (DMSO) and HBSS (less than 0.1% of DMSO in final dilution). Fifty microliters of dark-adapted 2 ng·ml<sup>-1</sup> luminol solution (5-amino-2,3 dihydro-1,4-phthalazinedione, Sigma) diluted 1:10 (v:v) in HBSS were added. The tubes were incubated at 37°C for 14 min and then counted in an automatic photometer (Nucleotimetre 107, Interbio CLV, Paris). Catalase (100 U·ml<sup>-1</sup>) and superoxide dismutase (100 U·ml<sup>-1</sup>) were used as controls in each experiments, and provoked respectively 68±10% and 75%±8% inhibition of PMN CL induced by 2.5 µM A23187. Light emission measurements were expressed in millivolts.

CL was also assessed by minor modifications of the method of WILLIAMS *et al.* [19], using lucigenin (Sigma) as light amplifier (final concentration of 100  $\mu\text{M}$ ) and gave an identical pattern of results.

#### Gel filtration chromatography

A 1.6  $\times$  100 cm Ultrogel AcA54 column (Pharmacia Fine Chemicals AB, Uppsala, Sweden) equilibrated with PBS was employed at a flow rate of 6  $\text{ml}\cdot\text{h}^{-1}$  for gel filtration chromatography. Three of EC supernatants from 6  $\times$  10<sup>6</sup> cells were applied to the column. Fractions of 3 ml were collected and tested for protein concentration by absorbance at 280 nm. All experiments were done at 4°C. The chemotactic activity of each fraction was then tested. Results were expressed as the difference between samples and control buffer (PBS).

#### Cell viability test

A spectrophotometric method, called MTT test, was employed to determine cell viability as described by MOSMANN [20]. Briefly, 5  $\text{mg}\cdot\text{ml}^{-1}$  dimethylthiazol-diphenyl-tetrazolium bromide (Sigma) was prepared as a stock solution in PBS. The viability of the neutrophil preparations were 92% $\pm$ 5% as judged by trypan blue exclusion (corresponding to the "100%" viability in the MTT test). The viability of endothelial cells and neutrophils were made as follows:

a) *Viability of endothelial cells.* In the 24-well culture plates, endothelial cells were incubated for 30 min with various doses of A23187 (ranging from 0.5–2.5  $\mu\text{M}$ ). Following the recovery of supernatants, EC were incubated in HBSS with a 10-fold diluted MTT stock solution, and then incubated for 4 h at 37°C. At this time, supernatants were removed and 300  $\mu\text{l}$  0.025N HCl-isopropanol solution was added. After homogenization, 200  $\mu\text{l}$  supernatants were transferred in a 96-well microtiter plate which was analysed by a multiwell scanning spectrophotometer at 620 nm. The percentages were calculated by comparison with untreated cells.

b) *Viability of neutrophils.* Forty five microlitres of PMN suspension were deposited in several wells of a 96-well microtitre plate. Twenty four microlitres of A23187 at final concentration ranging from 0.5–2.5  $\mu\text{M}$  were added in each well. The plate was incubated for 30 min at 37°C and then the MTT test started as described above.

#### Radioimmunoassay for prostacyclin

The non-enzymatic derivative of prostacyclin ( $\text{PGI}_2$ ), 6-keto-PGF<sub>1</sub>- $\alpha$  was quantified using a radioimmunoassay (New England Nucl., Boston, USA). The sensitivity was around 2 pg. Cross-reactivity studies showed less than 1% cross-reactivity with PGF<sub>2 $\alpha$</sub> , PGE<sub>2</sub>, PGE<sub>1</sub>, and TxB<sub>2</sub>.

## Results

### Effects of EC supernatants of PMN motility

Supernatants of EC stimulated by A23187 exerted a dose-dependent chemoattractant activity (fig. 1), with an optimal activity at 1.5  $\mu\text{M}$  of A23187. This activity was found to be nearly as high as that of 1  $\mu\text{M}$  fMLP used as a positive control for PMN motility. Negative control experiments were performed with A23187 added directly to PMN. Viability of PMN or EC were not significantly affected by exposure to A23187 (respectively, 83 and 89 $\pm$ 5%).

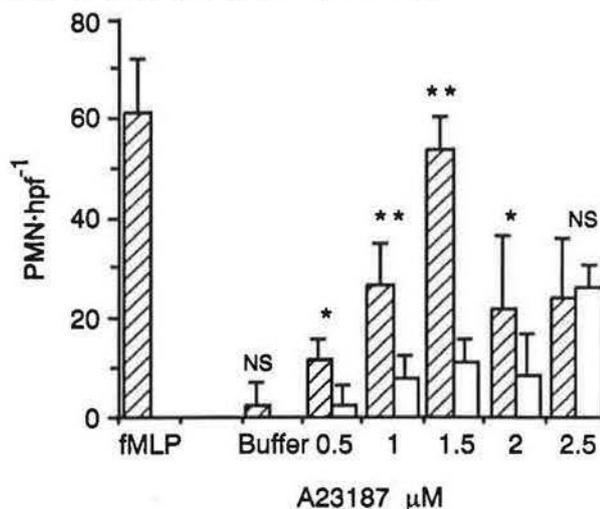


Fig. 1. - Presence of neutrophil chemoattractant activity (NCA) in endothelial cells (EC) supernatants. EC/A23187 denotes supernatants of EC stimulated with A23187. A23187 alone was used as a negative control to determine the direct effect of A23187 on polymorphonuclear neutrophils (PMN) motility in the absence of EC supernatants. EC were triggered with A23187 at various doses ranging from 0.5–2.5  $\mu\text{M}$ . The figure represents an average of five separate experiments. NCA was expressed as number of  $\text{PMN}\cdot\text{hpf}^{-1}$  (difference between the number of  $\text{PMN}\cdot\text{hpf}^{-1}$  towards EC supernatants and the control buffer). Differences from controls; \*:  $p<0.05$ ; \*\*:  $p<0.01$ ; ns: not significant.  $\square$  : EC/A23187;  $\square$  : A23187 alone.

Since EC are known to produce arachidonic acid metabolites, the possible eicosanoid nature of this neutrophil chemotactic activity (NCA) was investigated indirectly by using cyclooxygenase and lipooxygenase inhibitors. Pretreatment of EC cultures with indomethacin (cyclooxygenase inhibitor) or NDGA (lipooxygenase inhibitor) failed to modify the A23187-induced NCA (fig. 2). EC or PMN viability and the dose-response curve of NCA were not modified in the presence of these inhibitors (data not shown). A23187-activated EC supernatants were fractionated using gel filtration chromatography. One single peak of NCA was found with an estimated molecular weight between 34,000 and 41,000 daltons (fig. 3). Checkerboard analysis was performed with 10-fold concentrated column effluents, showing that PMN migration was stimulated when supernatants were placed simultaneously in the upper and lower compartments of the chemotactic chamber, indicating a chemokinetic property of the EC-released NCA (table 1).

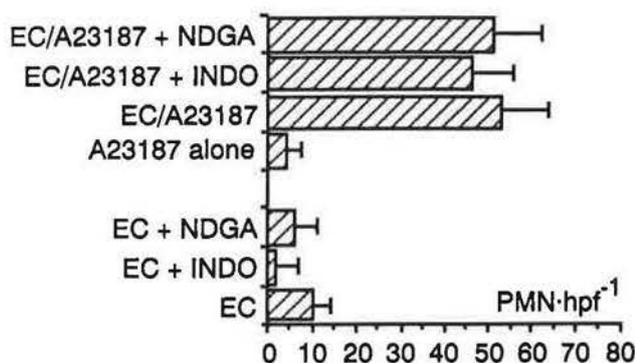


Fig. 2. - Failure of cyclooxygenase and lipoxygenase inhibitors to inhibit neutrophil chemotactic activity (NCA). Neither 10  $\mu$ M indomethacin (INDO) nor 10  $\mu$ M nordihydroguaiaretic acid (NDGA) induced any significant reduction of NCA. See legend to figure 1 for abbreviations.

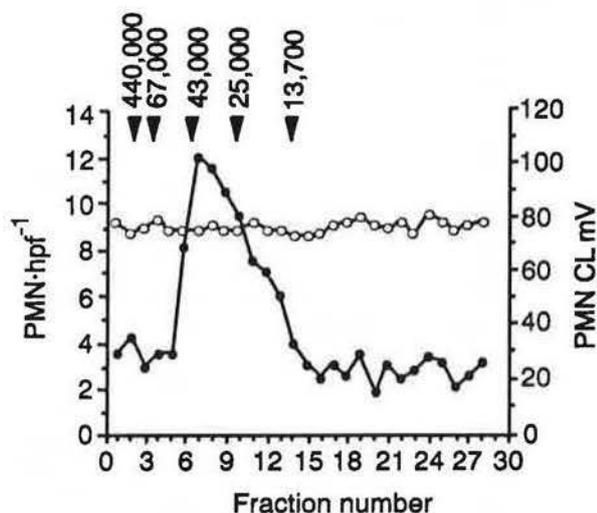


Fig. 3. - AcA54 gel chromatography of A23187-activated endothelial cells (EC) supernatants. Each fraction was tested for neutrophil chemotactic activity (NCA) and for inhibitory on polymorphonuclear neutrophil chemiluminescence (PMN CL). This figure represents a mean of 5 successive tests. For PMN CL, 2  $\mu$ M A23187 was added in each fraction and incubated with PMN suspension (as described in materials and methods) before CL evaluation.  $\bullet$  : PMN·hpf<sup>-1</sup>;  $\circ$  : PMN CL mV.

Table 1. - Checkerboard analysis of chemotaxis versus chemokinesis of EC-derived NCA

Lower compartments	Dilution in the upper compartments		
	1/5	1/10	1/20
1/5	39 (5)	27 (2)	39 (6)
1/10	28 (3)	29 (3)	32 (6)
1/20	19 (5)	30 (4)	23 (3)

Ratios indicate effluents fractions containing NCA (estimated m.w.: 37,000 $\pm$ 4,000 daltons). Data represent the mean $\pm$ SEM () of the PMN·hPa number of a representative of two experiments. The data on diagonal beginning on the right bottom of the table indicate an increase number of PMN·hpf related to the concentration of NCA, consistent with chemokinesis. The data in the 3rd column indicate an increase number of PMN·hpf related to a gradient concentration of NCA, consistent with chemotaxis.

#### Effects of activated EC supernatants on PMN chemiluminescence

As previously known, A23187 was able to induce a high and dose-dependent chemiluminescence in neutrophils. When EC were triggered by A23187, EC supernatants were unable to induce a CL response of neutrophils. This effect was observed whatever the dose of A23187 (fig. 4). In addition, EC supernatants obtained under these experimental conditions could inhibit the CL response of neutrophils stimulated either by A23187 or by fMLP and markedly enhanced the CL response of PMA-stimulated neutrophils (fig. 5).

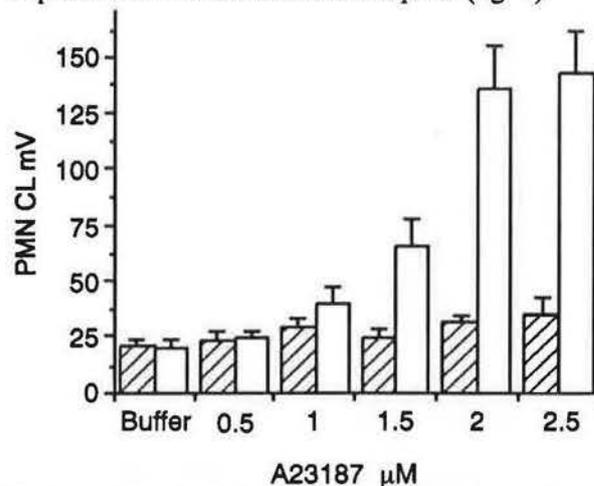


Fig. 4. - Role of activated endothelial cells (EC) supernatants on polymorphonuclear neutrophils chemiluminescence (PMN CL); EC were activated with various doses of A23187 and their supernatants were assessed for PMN CL. The figure represents an average of six experiments. Results are expressed in millivolts (mV).  $\square$  : EC/A23187;  $\square$  : A23187 alone.

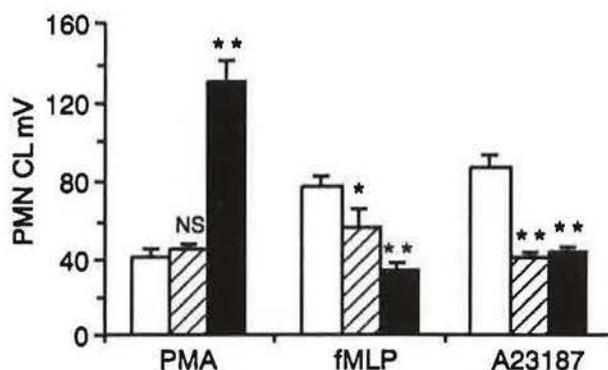


Fig. 5. - Modulation by endothelial cells (EC) supernatants of polymorphonuclear neutrophils chemiluminescence (PMN CL). Either 1.5  $\mu$ M A23187, or 1  $\mu$ M fMLP, or 1 ng·ml<sup>-1</sup> phorbol myristate acetate (PMA) were added to EC supernatants or buffer. Then, these supernatants were assessed for PMN CL. Data are means $\pm$ SEM of 4 experiments. \*:  $p < 0.05$ ; \*\*:  $p < 0.01$ ; ns: not significant by comparison with buffer. Results are expressed in millivolts (mV).  $\square$  : Buffer;  $\square$  : EC;  $\blacksquare$  : EC/A23187.

In order to identify the mechanisms by which the polymorphonuclear neutrophil chemiluminescence (PMN CL) might be regulated, A23187-activated EC supernatants, that had been previously fractionated

by gel filtration chromatography for NCA, were evaluated for their ability to inhibit CL response of neutrophils to 2  $\mu$ M A23187. No peak of inhibitory activity was evidenced, particularly in fractions which exhibited NCA (fig. 3). Thus, it appears that NCA and PMN CL inhibitory activity can be distinguished.

To further investigate the mechanism of PMN CL inhibition, EC were pretreated by indomethacin ( $1 \times 10^{-5}$  M) which completely failed to modulate the inhibitory activity although the measurement of PGI<sub>2</sub> showed a clear-cut inhibition of the cyclooxygenase pathway ( $88.33 \pm 31$  pg·0.1 ml<sup>-1</sup> for A23187-activated EC supernatants;  $4 \pm 2.3$  pg·0.1 ml<sup>-1</sup> for activated EC after pretreatment by indomethacin). NDGA was also evaluated but its own inhibitory effect on PMN CL by a known scavenger effect did not permit interpretation of the results (data not shown).

Because heparin has potent inhibitory effects on PMN CL [21], and that EC were cultured in medium supplemented with heparin, we studied for such a role in our model. The coagulation tests were performed by standard methods, kindly supported by the Department of Haematology (Hopital Calmette, Lille, France). Although the reptilase time was within normal values, the thrombin time of unstimulated EC supernatant was found up to 60 s (normal values: 20 s–25 s) which was completely inhibitable by 10  $\mu$ g·ml<sup>-1</sup> protamine sulphate. The coagulation tests performed with A23187-activated EC supernatants were all within normal values. Moreover, protamine sulphate, used at doses ranging from 2000  $\mu$ g·ml<sup>-1</sup> to 0.2  $\mu$ g·ml<sup>-1</sup>, had not been found to influence EC-derived inhibitory activity (data using 20  $\mu$ g·ml<sup>-1</sup> were shown) (fig. 6).

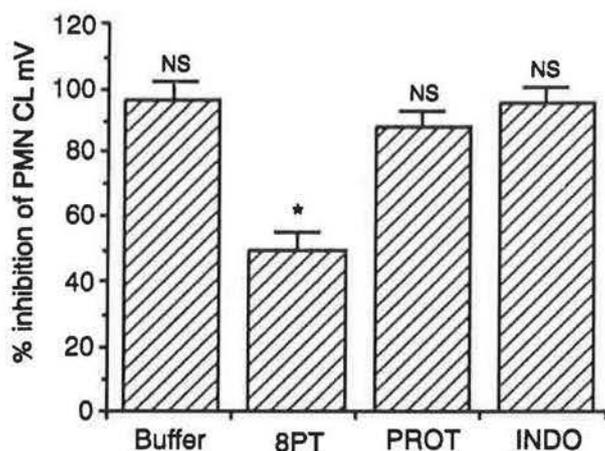


Fig. 6. - Modulation of endothelial cells (EC)-derived inhibitory activity on polymorphonuclear neutrophils chemiluminescence (PMN CL). Either 8-phenyltheophylline (8PT), protamine sulfate (PROT), or indomethacin (INDO) were added to activated EC supernatants and then assessed for PMN CL. Controls showed no activity of these three compounds on A23187-induced PMN CL. Data are means  $\pm$  sem of 3 separate experiments. \*:  $p < 0.05$ ; ns: not significant. Results are expressed in percentage of inhibition of chemiluminescence.

On the other hand, the hypothesis that adenosine may contribute to the inhibitory activity was explored. It has been previously established, under technical conditions quite similar to ours, that EC supernatants contained 1.3  $\mu$ M to 1.4  $\mu$ M adenosine [13]. The addition of 10  $\mu$ M 8-phenyltheophylline (8PT, an adenosine antagonist) with EC supernatants, reduced by 50% the inhibitory activity, suggesting a participation of adenosine in the EC-derived inhibitory activity (fig. 6). In another series of experiments, 2-chloroadenosine (an adenosine agonist) at a concentration which maximally inhibits neutrophil functions (10  $\mu$ M) [22], and dibutyryl-cAMP were compared to EC supernatants in their capacity to inhibit PMN CL induced by either A23187, fMLP, and PMA (fig. 7). Results showed a mimicry between EC supernatants, 2-chloroadenosine, and dibutyryl-cAMP except when PMA was used as a stimulating agent. Moreover, 8PT inhibited 90% of 2-chloroadenosine activity and partially (50%) the EC supernatant activity.

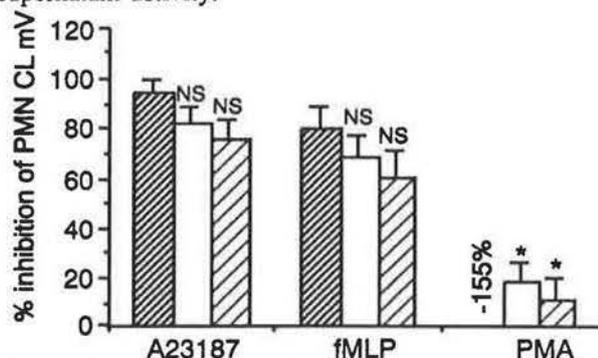


Fig. 7. - Mimicry between 2-chloroadenosine (2-CADO), cyclic adenosine monophosphate (cAMP) and endothelial cells (EC) supernatants. Activated EC supernatants were compared with 2-CADO and cAMP in their capacity to inhibit PMN CL induced by either A23187, fMLP, or phorbol myristate acetate (PMA); Data are means  $\pm$  sem of 3 separate experiments. \*:  $p < 0.01$ ; ns: not significant. Results are expressed in percentage of chemiluminescence. ▨ : EC/A23187; □ : 2-CADO; ▤ : cAMP.

## Discussion

The results support the view that cultured endothelial cells obtained from the human umbilical vein are stimulated by A23187 to produce simultaneously a neutrophil chemotactic factor and an inhibitory activity on PMN chemiluminescence.

Considering that vascular endothelial cells are in close contact with blood inflammatory cells during inflammation, endothelial cells are thought to actively participate in the recruitment of blood cells, especially through the release of chemoattractant mediators. In this way, it has been shown that endothelial cells could release neutrophil recruiting factors either spontaneously [5] or in response to several stimuli such as hypoxia [6], thiourea [7], angiotensin II [8], histamine [9], and cytokines such as TNF $\alpha$  and IL-1 $\beta$  [25]. Endothelial recruiting factors for neutrophils appear to be of a different nature. The protein nature has been suggested based on the gel filtration chromatography and on the

protease sensitivity [5], or on the characterization of some gene products now called IL-8 [25]. On the other hand, the lipidic nature of certain endothelial cell-derived recruiting factors has been suspected when release of the recruiting activity has been prevented by lipoxygenase inhibitors [9]. Based on the reverse-phase high pressure liquid chromatography, such a role for platelet activating factor or for leukotriene B<sub>4</sub> have been excluded [9].

In our study, cultured endothelial cells stimulated by A23187 have produced a chemottractant activity for neutrophils. This NCA was observed after A23187 stimulation, with a dose-dependent response curve with production starting as early as 30 min after ionophore triggering. In addition, this NCA was shown to be independent from arachidonic acid metabolites. Moreover, passage through a gel filtration chromatograph indicated a molecular weight of 37,000 daltons, that might correspond to the spontaneously produced NCF of 35,000 daltons characterized by MERCANDETTI *et al.* [5]. However, the early detection of the NCA beginning at 15 min may indicate a role for some preformed mediators whatever their proteic or lipidic nature. It is unlikely that other cells such as monocytes played a role in this study for two reasons: (a) EC cultures were pure as judged from microscopic examination with fluorescent anti-factor VIII anti-serum; (b) undetected monocytes would not be expected to multiply, but instead be lost after subculture.

The second observation was the detection of inhibitory activity of PMN CL in stimulated EC supernatants. The ability of endothelial cells to inhibit receptor-mediated anion production by polymorphonuclear leukocytes has been suggested by BASFORD *et al.* [26]. They have found that bovine pulmonary artery endothelial cells as well as human umbilical vein endothelial cells could release a soluble inhibitor with an apparent molecular size between 1 and 10 kD. This soluble inhibitor appears to be a polypeptide and is able to inhibit fMLP-stimulated O<sub>2</sub><sup>-</sup> production by neutrophils [26]. In another study, it has been shown that adenosine acted as an endogenous inhibitor of fMLP-stimulated neutrophil injury to EC and prevented both the adhesion of neutrophils and the cytotoxicity that neutrophils caused to EC [13]. Furthermore, the depletion of endogenously released adenosine by adenosine desaminase enhanced neutrophil-mediated injury when either stimulated by fMLP or not [13].

Our results indicate that the release of this inhibitory activity was more marked after A23187-triggering, but also detectable at the baseline and may represent a constitutive property of EC. Several data suggested that the NCA and the inhibitory activity could be distinguished based on the results of gel filtration chromatography. Trying to characterize the nature of this inhibitory activity, a role for prostaglandins and heparin could be excluded. The absence of inhibition of PMA-induced PMN CL suggests that the scavenger pathway is not the major mechanism of inhibition. On the other hand, we have focused our study on the putative role for adenosine, known to be released by EC

and able to inhibit neutrophil-dependent injury [13]. Using an adenosine agonist (2-chloroadenosine), we have reproduced EC-derived inhibitory activity, and focused both the effects of the adenosine agonist and EC supernatant inhibitory activities reduced by 8-phenyltheophylline. However, the small amounts of adenosine in EC supernatants and the complete inhibition by 8PT of optimal dose of adenosine agonist indicated that the inhibitory activity on PMN CL is supported by at least two factors: the first appears to be adenosine and the second is unknown.

Lastly, it is important to point out that PMN CL inhibition differed according to the stimulus used. It has been shown that adenosine, acting *via* A<sub>2</sub> receptors, was able to reduce both superoxide anion production and lysosomal enzyme release from neutrophils stimulated by fMLP or A23187 but not by PMA [23, 24]. The fact that dibutylryl-cAMP mimicks the EC supernatants as well as the adenosine agonist, is in agreement with other reports indicating a cAMP-dependent down-regulation of PMN CL, triggered by adenosine-A<sub>2</sub> receptor coupling [24].

In conclusion, these findings underline a physiologic role of endothelial cells in the influx of neutrophils seen in a positive feedback during PMN influx. The concurrent production by EC of a NCA and an inhibitory activity of neutrophil chemiluminescence, related in part to adenosine, support the view that excited EC could both favour PMN infiltration and prevent oxidative burst, particularly at the early step of PMN transvascular migration. Oxidative burst could then be limited to the inflammatory site. These data offer new insights to PMN-EC interactions during inflammatory processes.

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*Interaction neutrophiles - cellules endothéliales: observation in vitro d'une régulation des fonctions neutrophiliques par les cellules endothéliales.* P. Lassalle, Y. Delneste, P. Gosset, B. Wallaert, A.B. Tonnel, J.P. Dessaint, A. Capron.

RÉSUMÉ: Le but de cette étude est d'étudier les interactions possibles entre les cellules endothéliales humaines de la veine ombilicale (EC) stimulées par l'ionophore A23187 à différentes doses (0.5 à 2.5  $\mu$ M) et le neutrophile polymorphonucléaire (PMN).

Les surnageants de EC s'avèrent posséder une activité chimio-attractante pour les neutrophiles (NCA) ainsi que, parallèlement, un facteur inhibiteur de la chimioluminescence des polymorphonucléaires neutrophiles (PMN CL). Les surnageants obtenus à partir de EC stimulées par A23187 comportent un niveau élevé de NCA ( $73 \pm 5$  PMN·hpf<sup>3</sup> par comparaison à  $21 \pm 4$  PMN·hpf<sup>3</sup> dans les surnageants EC non traités,  $p < 0.01$ ). Ce NCA est indépendant des métabolites de l'acide arachidonique, puisque l'indométhacine et l'acide nordihydroguaiarétique n'arrivent pas à bloquer l'activité chimiotactique. En utilisant la chromatographie sur gel filtration (AcA 54), le NCA se retrouve en un seul pic avec un poids moléculaire apparent de  $37.000 \pm 4.000$  daltons. L'analyse "checkerboard" a indiqué que NCA a à la fois des activités chimiotactiques et chimiocinétiques. En outre, les surnageants de cellules EC stimulées par A23187 et, à un moindre degré, les surnageants de EC non stimulées, inhibent la PMN CL induite par N-formyl-Méthionyl-Leucyl-Phénylalanine (61% d'inhibition,  $p < 0.05$ ), ainsi que par A23187 lui-même (80% d'inhibition,  $p < 0.01$ ), mais non celle induite par le de phorbol-myristate-acétate. L'indométhacine et le sulfate de protamine n'ont pas modulé cette activité inhibitrice. Par contre, l'activité inhibitrice dérivée de EC est inhibée (50%) par un antagoniste de l'adénosine (8-phénylthéophylline), ce qui indique une participation de l'adénosine dans cette activité inhibitrice de PMN CL.

Ces données suggèrent la possibilité que les cellules endothéliales activées pourraient à la fois stimuler la migration des polymorphonucléaires et les protéger eux-mêmes contre les effets potentiellement toxiques de métabolites de l'oxygène produits par les PMN, particulièrement au cours de leur migration transvasculaire.

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