Eur Respir J, 1994, 7, 1585–1592
DOI: 10.1183/09031936.94.07091585

European Respiratory Journal
ISSN 0903 - 1936

Enhancement of reactive oxygen species formation in stable and unstable asthmatic patients

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ABSTRACT: There is increasing evidence to suggest that human blood polymorphonuclear neutrophils (PMNs) and monocytes play an important role in the inflammatory processes of asthma. In asthmatic patients, PMNs and monocytes were shown to be activated more than in healthy subjects.

We investigated the capacity of these two cell populations to generate reactive oxygen species (ROS) in stable and unstable asthmatic patients. The two populations of asthmatic patients were identified by asthma activity, as expressed by clinical events occurring within 2 weeks prior to the study. Oxygen species formation was analysed for isolated purified PMNs and monocytes (Mos) by chemiluminescence (CL) using lucigenin and luminol as luminescent probes. CL was determined on nonstimulated and on phorbol myristate acetate (PMA)-stimulated cells. The stimulatability coefficient (PMA-stimulated/nonstimulated cell ratio) of each cell population was then calculated.

Resting PMNs and Mos generated significantly greater amounts of ROS in stable asthmatic patients, and much more in unstable asthmatic patients, as compared to healthy subjects, both in lucigenin and luminol enhanced CL. Non $O_2^{\cdot -}$ ROS production from PMA-stimulated PMNs and Mos was identical in unstable asthmatic patients and in healthy subjects, whereas a significant decrease was observed in stable asthmatic patients, as assessed by luminol enhanced CL. PMA-stimulated cells showed no difference in $O_2^{\cdot -}$ generation, as assessed by lucigenin enhanced CL. However, the stimulatability coefficient of all asthmatic patients was always significantly lower than that of healthy subjects.

These results suggest that there are differences in priming and stimulation of Ros production from PMNs and Mos between stable and unstable asthmatic patients. Release of oxygen species from these cells may be implicated in the pathophysiology of unstable asthma.

Eur Respir J., 1994, 7, 1585–1592.

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Keywords: Asthma activity
monocytes
oxygen species
polymorphonuclear neutrophils

Received: December 22 1993
Accepted after revision June 23 1994

+This work is dedicated to Dr Marcelle Damon, deceased 1994.

This work was partly supported by a grant from the Comité Départemental de Lutte contre les Maladies Respiratoires, Mont-

Bronchial asthma can be considered as a combination of reversible airway obstruction, increased airway responsiveness and airway inflammation. Various cells which migrate from the bloodstream into the bronchial tree are known to be sources of local inflammation [1].

In a previous study, using chemiluminescence (CL) analysis, we showed that alveolar macrophages from asthmatic patients were activated [2]. As assessed by the release of reactive oxygen species (ROS), this activation was correlated with the severity of asthma defined by the Aas' clinical score [3], which takes into account asthmatic events during the last year. Similarly, blood monocytes (Mos), which are precursors of macrophages, were activated and released higher quantities of ROS in asthmatic patients [4]. Several investigators have also suggested that polymorphonuclear neutrophils (PMNs) from asthmatic patients generate more ROS than those of healthy subjects [5, 6]. This activation is correlated with bronchial hyperreactivity [5]. We thus hypothesized that activation of blood PMNs and Mos could be correlated with the activity of asthma, that is instability or stability, which takes into account asthmatic events over a short period of time (10–15 days).

The final expression of asthma depends on different interactions between the inflammatory cells and on their activation state expressed by the release of numerous mediators. Among them, oxygen radicals and their metabolites, such as superoxide anion ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH°) are important agents causing lung tissue damage in inflammatory processes. These ROS are produced after activation of the nicotinamide adenine dinucleotide phosphate reduced form (NADPH) oxidase system, particularly in phagocytic cells, such as polymorphonuclear and mononuclear cells [7–9]. They can be analysed through their ability to generate luminescence, as amplified by specific probes and measured by a photon counter analyser [10].

The aim of this study was to investigate the capacity of blood leucocytes, PMNs, and Mos, from two different populations of asthmatic patients to generate ROS as compared to cells from healthy subjects. These two populations were classified according to activity of asthma, as described by TURNER WARWICK [11] and ourselves [12], rather than by severity as defined by the Aas' score. Phorbol myristate acetate (PMA) [13] was used for *in vitro* triggering of a protein kinase C activator which stimulates the NADPH oxidase system [14]. This unphysiological stimulus was used to obtained a maximal level of stimulation to make possible comparison between the three groups of subjects.

Methods

Patients

Nine healthy volunteers, aged 22–69 yrs (mean±SEM 43±4 yrs), and 14 patients with asthma, aged 19–66 yrs (44±4 yrs) were studied. Asthma was diagnosed according to the American Thoracic Society Statement [15]. All subjects were nonsmokers.

The severity of asthma was determined according to Aas' clinical score [3] used to grade chronic asthma from very mild forms (score=1) to incapaciting disease requiring permanent medication (score=5). The grading is based on clinical events which took place during the previous year and combines symptoms (number and duration of asthma episodes, total duration of symptoms, presence or absence of symptom-free intervals between attacks) and the requirement for medication. It does not take into account the pulmonary function of the patients. Activity of asthma (stability *versus* instability) was established by a questionnaire as described previously [12]. Briefly, the stability of asthma was defined as the absence of any clinical events or β_2-agonist inhalation during the last 2 weeks. The instability of asthma was defined by the presence of any of the following symptoms: cough, dyspnoea, wheezing, breathlessness, β_2-agonist consumption during the last two weeks. The time of the last asthma attack was defined by the last β_2-agonist inhalation,

Forced expiratory volume in one second (FEV_1) was measured just before venepuncture. Allergy was assessed by clinical history, the presence of at least three positive skin tests and high immunoglobulin E (IgE) levels in the blood. Disodium cromoglycate, nedocromil sodium and inhaled corticosteroids had to have been interrupted for at least 1 month. Stable asthmatic patient were without any treatment. Unstable patients were treated only with β_2-agonists. They did not use theophylline or oral corticosteroids. None of the healthy volunteers were using medication.

Since β_2-agonists can modify the state of blood cell activation in unstable asthmatic patients, three additional healthy subjects were enrolled in the study. A first venepuncture was performed at 8 a.m. The subjects then inhaled β_2-agonist (terbutaline sulphate 1 mg) and a second venepuncture was performed 30 min after inhalation

The study was performed after informed consent and fulfilled the criteria of the Ethics Committee of the University of Montpellier.

Cell preparations

Peripheral blood was recovered by venepuncture using heparin (25 $U \cdot ml^{-1}$) as anticoagulent. Cells were isolated by centrifugation of blood samples over isotonic Percoll solutions (Kabi Phamacia, France) [16] with densities of 1.097 (72%) and 1.086 $g \cdot ml^{-1}$ (63%). A 5 ml volume of 63% Percoll solution was layered over 5 ml of 72% solution in a 15 ml conical tube. Whole blood (5 ml) was layered over the Percoll gradient and then centrifuged at 400×g for 20 min at room temperature, resulting in the formation of one band of mononuclear cells (MNs) and bands of PMNs above the 72% layer. The MN suspension and then the PMN suspension in Percoll were removed and washed once with an equal volume of saline solution. For both populations, contaminating erythrocytes were lysed by incubation for 10 min in a buffer solution of 130 $mmol \cdot l^{-1}$ NH_4Cl, 10 $mmol \cdot l^{-1}$ Tris, and 16 $mmol \cdot l^{-1}$ K_2CO_3, pH 7.4, and recovered by centrifugation. Cells were then washed in saline solution and counted in the initial suspension.

Purity of PMNs (95%) was evaluated by cytocentrifugation and May-Grünwald staining. They were then suspended in medium 199 (Gibco Chemical, Scotland) to obtain a concentration of 5×10^5 PMNs for 800 µl of medium.

MNs were first counted using neutral red staining specific to Mos in proportion to neutral red MNs, 3:1. They (10×10^6 $Mo \cdot ml^{-1}$) were then incubated in Petri dishes with 5 ml RPMI 1640 medium (Gibco) and 20% foetal calf serum (FCS) (Gibco) for 2 h at 37°C in a humid atmosphere of 95% air and 5% CO_2 to separate adherent Mos from suspended lymphocytes. At the end of this incubation, nonadherent cells were removed. Adherent Mos were recovered by gentle scraping with a rubber policeman into RPMI 1640 medium, and then characterized by May-Grünwald staining (purity 95%). Both cell populations (PMNs and Mos) were resuspended in RPMI 1640 medium to obtain a concentration of 5×10^5 PMNs or Mos in 800 µl of medium.

Viability of the two cell populations was determined by the trypan blue exclusion test, and was always greater than 95%.

CL measurements

Reactive oxygen species (ROS)-induced CL was measured using luminol [17, 18] and lucigenin [19, 20] as luminescent probes.

Luminol (Sigma, St Louis, MO, USA) was used to enhance the luminescence induced by the whole set of ROS. Luminol (18 mg) was suspended in a 1% solution of bovine serum albumin (BSA) (Sigma) in 100 ml phosphate-buffered saline (PBS) (pH 7.4) to obtain a

stock solution of 10^{-3} mol·l^{-1} luminol concentration. The final concentration was 10^{-4} mol·l^{-1}.

Lucigenin (Sigma) was used to enhance luminescence induced by superoxide anion ($O_2\cdot^-$) production. Lucigenin (5 mg) was dissolved in 100 ml medium 199 containing 476 mg hydroxyethyl piperazine ethanesulphonic acid (HEPES) (Sigma) and 100 mg gelatin with heating to obtain a stock solution of 10^{-4} mol·l^{-1} lucigenin concentration. The final concentration was 10^{-5} mol·l^{-1}.

PMA (Sigma) was dissolved as stock solution of 1 mg·ml^{-1} in dimethyl sulphoxide (DMSO) (Sigma) maintained at -70°C. Before use, the stock solution was diluted in saline solution to obtain a 10^{-7} mol·l^{-1} final concentration in PMA.

CL was monitored in a model 1251 LKB Wallac luminometer (Wallac Co., Turku, Finland) connected to an Apple computer. All procedures were performed in the dark. Medium (800 µl) containing 5×10^5 purified PMNs or Mos were placed in luminometer vials at 37°C with continuous stirring. Firstly, 100 µl of luminescent probe was added and a basal value was determined, then 100 µl PMA was added and measurements were taken every minute for 20 or 30 min. Spontaneous formation of oxygen species (without PMA) was also analysed simultaneously. The kinetic study of nonstimulated or PMA-stimulated cell responses was performed in duplicate, and the results were analysed as CL peaks (maximal value obtained without any stimulation or after stimulation) for lucigenin and luminol, and expressed as mV per 5×10^5 cells.

Study design and statistical analysis

Samples were recovered at 8 a.m. and studied without delay. PMNs and Mos were isolated from the whole blood. The CL response was analysed on the two cell populations for each subject. The CL peak was defined as the maximum CL obtained after PMA-stimulation. All results were expressed as mean±SEM.

The significance levels between CL peaks in PMA-stimulated and nonstimulated cells from stable and unstable asthmatic patients, and healthy subjects were determined by the nonparametric Mann-Whitney U-test. Differences between stable and unstable asthmatic patients were also analysed in the same way.

The ratio for the CL peak in PMA-stimulated cells/CL peak in nonstimulated cells was also given as a coefficient of cell stimulatability between the spontaneous stimulation of cells and the stimulation of these cells after PMA-stimulation.

Results

Clinical story of subjects

Both groups of patients were identified as described in table 1, based on the activity of asthma (stable *versus* unstable). Seven patients were unstable asthmatic patients, and the last asthma attack was ranked at 12, 18 and 24 h before the analysis. Seven patients were stable asthmatic patients.

There were nine female and five male patients. Six patients presented positive skin tests for common aeroallergens. The Aas' score was 1 in four patients, 2 in four, 3 in three, and 4 in three; and was identical in the two groups of patients. The FEV_1 values were identical in both groups (mean±SEM 85±5 *versus* 82±4% pred). There was no observed difference in gradient efficiency between healthy subjects, stable and unstable asthmatic patients, *i.e.* the number of each cell population recovered was always statistically identical.

Table 1. – Characterization of asthmatic patients

Patient N°	Age yrs	Sex	Time since last attack h	Allergy	FEV_1 % pred	Severity Aas' score
Unstable						
1	19	M	18	+	100	1
2	45	F	24	+	100	1
3	27	F	12	+	80	3
4	36	F	24	+	74	4
5	55	F	18	-	100	2
6	22	F	24	-	75	2
7	60	M	12	-	70	3
Stable						
8	33	M	-	+	100	1
9	52	F	-	+	80	2
10	33	M	-	-	85	1
11	50	F	-	-	100	2
12	62	F	-	-	75	3
13	58	F	-	-	69	4
14	66	M	-	-	70	4

FEV_1: forced expiratory volume in one second; % pred: percentage of predicted value; M: male; F: female; +: positive; -: negative; Aas' score: clinical score used to grade chronic asthma from very mild form (score=1) to incapacitating disease requiring permanent medication (score=5).

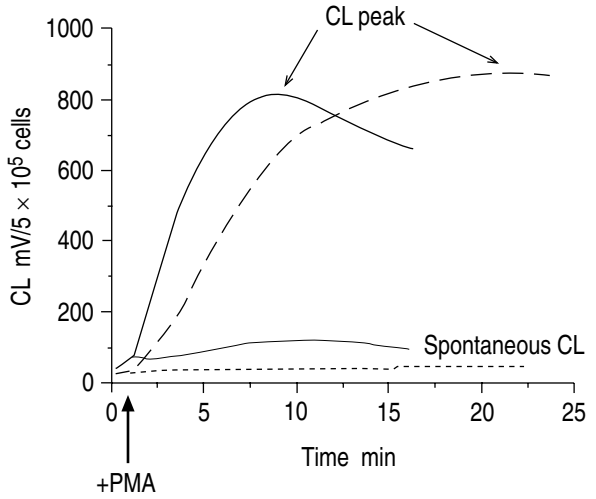

Fig. 1. – Kinetic curve of lucigenin (10^{-5} mol·l^{-1}) enhanced chemiluminescence (CL) on 5×10^5 PMNs or Mos before and after stimulation with PMA (10^{-7} mol·l^{-1}) added after the first measurement made at time 0. Bold line represents the kinetic curve for PMA-stimulated PMNs (continuous line) and stimulated Mos (broken line). Normal line represents the kinetic curve for nonstimulated PMNs (continuous) and Mos (dotted). PMA: phorbol myristate acetate; PMNs: polymorphonuclear neutrophils; Mos: monocytes.

Analysis of CL results

Figures 1 and 2 show the time course variations in CL of PMNs and Mos in lucigenin and luminol, respectively. As indicated in the Methods section, the CL peak was defined as the maximal CL value obtained after PMA-stimulation.

Lucigenin-dependent CL (fig. 3). Nonstimulated PMNs and Mos from asthmatic patients released significantly higher quantities of $O_2^{\cdot -}$ than those from healthy

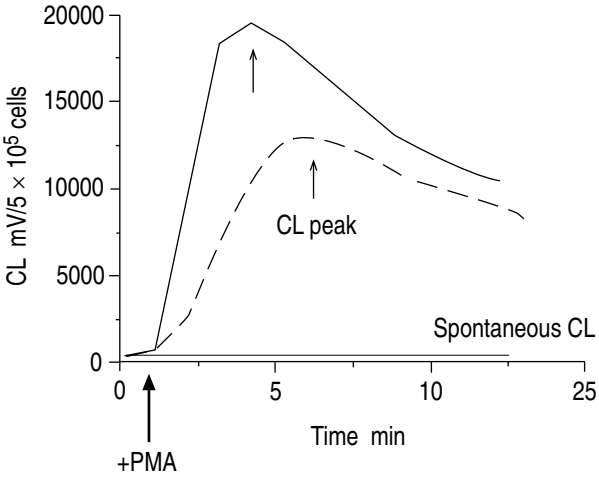

Fig. 2. – Kinetic curve of luminol (10^{-4} mol·l^{-1}) enhanced chemiluminescence (CL) on 5×10^5 PMNs or Mos before and after stimulation with PMA (10^{-7} mol·l^{-1}) added after the first measurement made at time 0. Bold line represents the kinetic curve for PMA-stimulated PMNs (continuous line) and stimulated Mos (broken line). Normal line represents the kinetic curve for nonstimulated PMNs and Mos. For abbreviations see legend to figure 1.

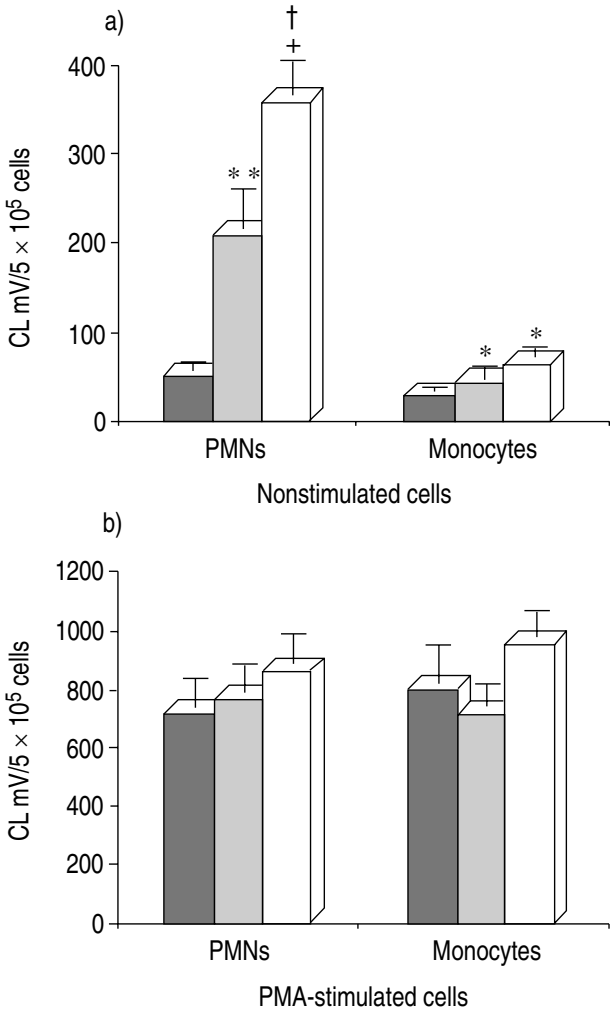

Fig. 3. – Chemiluminescence (CL) peaks of superoxide anion generation using luminometer analysis on PMNs and Mos. Lucigenin (10^{-5} mol·l^{-1}) was used as enhancer for the CL analysis. Cells were purified from whole blood using a Percoll gradient and suspended in medium (5×10^5 cells·ml^{-1}). a) Lucigenin was added for nonstimulated cells, and measurements were carried out every minute until CL peak appeared. b) Lucigenin was added for PMA-stimulated cells, and a first measurement was taken. PMA (10^{-7} mol·l^{-1}) was then added, and the measurements were carried out every min until a CL peak appeared. Results were expressed as mV/5 × 10^5 cells (mean±SEM). *: $p<0.05$; **: $p<0.005$; +: $p<0.001$, for significant differences from healthy subjects. †: $p<0.05$, for significant differences between stable and unstable asthmatic patients. ■: healthy; ▒: stable asthma; □: unstable asthma. For abbreviations see legend to figure 1.

subjects. This was particularly evident in cells from unstable asthmatic patients ($p<0.001$ and $p<0.05$ for PMNs and Mos, respectively), and from stable asthmatic patients ($p<0.005$ and $p<0.05$, respectively). There was a significant difference between stable and unstable asthmatic patients for PMNs ($p<0.05$), with no significant difference for Mos. Moreover, PMNs released significantly higher quantities of ROS than did Mos in the three groups of subjects.

In PMA-stimulated cells, no significant difference was observed between the three populations of subjects, and the results were almost identical for PMNs and Mos.

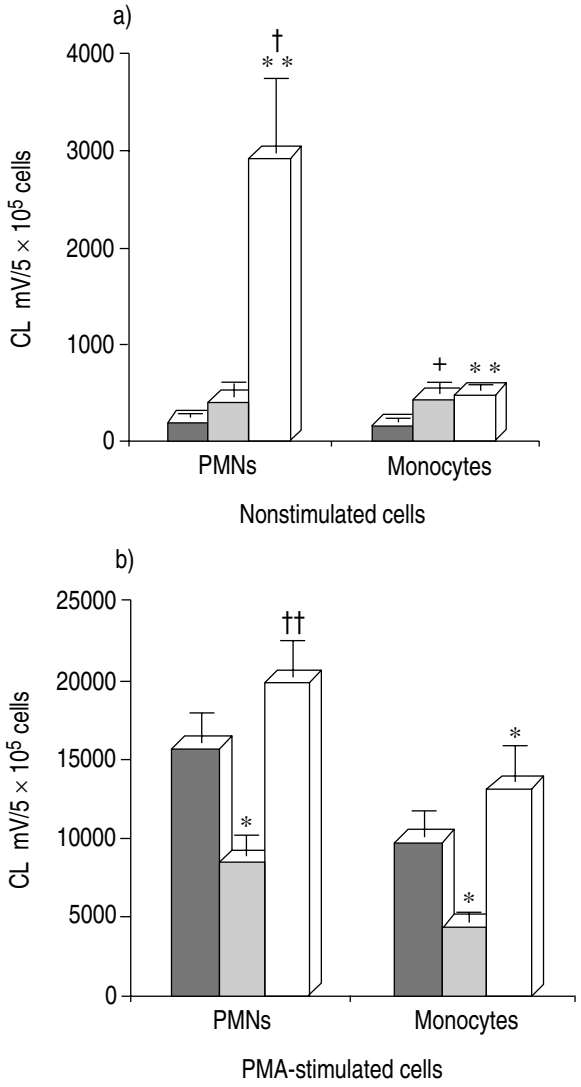

Fig. 4. – Chemiluminescence (CL) peaks of the whole set of reactive oxygen species generation using luminometer analysis on PMNs and Mos. Luminol (10^{-4} mol·l^{-1}) was used as enhancer for the CL analysis. Cells were purified from whole blood using a Percoll gradient and suspended in medium (5×10^5 cells·ml^{-1}). a) Luminol was added for nonstimulated cells, and measurements were carried out every min until CL peak appeared. b) Luminol was added for PMA-stimulated cells, and a first measurement was taken. PMA (10^{-7} mol·l^{-1}) was then added, and the measurements were carried out every minute until a CL peak appeared. The results were expressed as mV5 × 10^5 cells (mean±SEM). *: p<0.05; **: p<0.01; and +: p<0.001, for significant differences from healthy subjects. †: p<0.05; and ††: p<0.01, for significant differences between stable and unstable asthmatic patients. ▩: healthy; ▨: stable asthma; □ : unstable asthma. For abbreviations see legend to figure 1.

Luminol-dependent CL (fig. 4). Nonstimulated PMNs from unstable asthmatic patients released significantly higher quantities of ROS than those from healthy subjects (p<0.01). Nonstimulated Mos from asthmatic patients, either in a stable or unstable state, released higher quantities of ROS than healthy subjects (p<0.01 and p<0.001, respectively). Moreover, the difference between stable and unstable asthmatic patients was significant for PMNs (p<0.05) but nonsignificant for Mos.

When PMNs or Mos were stimulated by PMA, the responses were almost identical in unstable asthmatic patients and healthy subjects, whereas there was a significantly lower CL response in stable asthmatic patients (p<0.05). There were significant differences between stable and unstable asthmatic patients for PMNs (p<0.001), and for Mos (p<0.01).

β_2-agonist inhalation effects

No effect was observed on the efficiency gradient and on the numbers of each cell population before and after inhalation. Thirty minutes after β_2-agonist inhalation, PMA-stimulated PMNs and Mos showed 20–30%

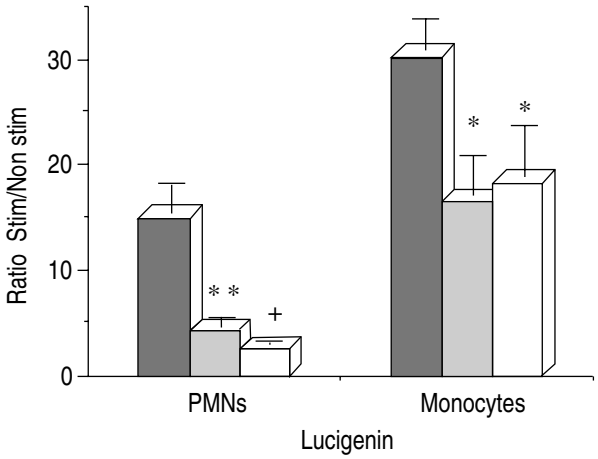

Fig. 5. – Ratio of PMA-stimulated cells *versus* nonstimulated cells for PMNs and Mos, to analyse the coefficient of stimulatability of superoxide anion using lucigenin (10^{-5} mol·l^{-1}) enhanced chemiluminescence (CL). Results are expressed as the ratio of PMA-stimulated/nonstimulated CL peak (mean±SEM). *: p<0.05; **: p<0.005; +: p<0.001, for significant differences from healthy subjects. ▩: healthy; ▨: stable asthma; □: unstable asthma; Stim: stimulated. For abbreviations see legend to figure 1.

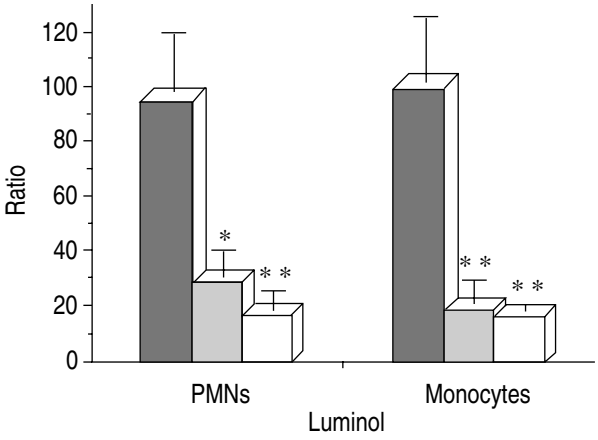

Fig. 6. – Ratio of PMA-stimulated cells *versus* nonstimulated cells for PMNs and Mos, to analyse the coefficient of stimulatability of the whole set of reactive oxygen species using luminol (10^{-4} mol·l^{-1}) enhanced chemiluminescence (CL). Results are expressed as the ratio of PMA-stimulated/nonstimulated CL peak (mean±SEM). *: p<0.05; **: p<0.01, for significant differences from healthy subjects. ▩ : healthy; ▨ : stable asthma; □ : unstable asthma. For abbreviations see legend to figure 1.

decrease in ROS release assessed by lucigenin and luminol enhanced CL, whilst no effect was observed on nonstimulated cells (data not shown).

Analysis of stimulatability coefficient

Lucigenin-dependent CL. As reported in figure 5, the ratio of PMNs and Mos was significantly greater in healthy subjects than in stable asthmatic patients ($p<0.005$ for PMNs and $p<0.05$ for Mos), or unstable asthmatic patients ($p<0.001$ for PMNs and $p<0.05$ for Mos). Moreover, it was higher for Mos than PMNs in all groups of patients.

Luminol-dependent CL. As reported in figure 6, the ratio was significantly greater in PMNs and Mos from healthy subjects than from stable asthmatic patients ($p<0.05$ for PMNs and $p<0.01$ for Mos) or unstable asthmatic patients ($p<0.01$ for both cell types).

In both, luminol and lucigenin, there were no significant differences between stable and unstable asthmatic patients.

Discussion

This study demonstrated that in comparison to healthy subjects, nonstimulated PMNs and Mos from asthmatic patients released higher quantities of $O_2^{\cdot -}$ and the whole set of ROS as assessed by lucigenin and luminol enhanced CL, respectively. This difference was more pronounced in cells from unstable asthmatic patients than from stable asthmatic patients for Mos, and even more so for PMNs.

This increase in ROS release was not correlated with the severity of asthma as defined by the Aas' score [3] and FEV_1. Reactivity of circulating PMNs and Mos differed from that observed by CLUZEL *et al.* [2] on human alveolar macrophages. They showed that alveolar macrophages released higher amounts of ROS, which were correlated with the severity of asthma. In fact, the activation state of alveolar macrophages according to severity cannot only be explained by the migration of blood monocytes. It appears that the high number of eosinophils, observed in bronchoalveolar fluid and bronchial biopsies [21] according to the severity of asthma, could explain this increased activation.

Our results obtained for PMNs in asthma are in agreement with those obtained by KATO *et al.* [22], and NEIJFENS *et al.* [5]. The results of KATO *et al.* [22] suggested that PMNs in asthmatic children, especially those with attacks, generate more ROS than those of healthy subjects, as assessed by a cypridina luciferin analogue enhanced CL. NEIJFENS *et al.* [5] suggested that the increased generation of ROS in children with asthma might also contribute to causing bronchial hyperresponsiveness (BHR).

We also demonstrated that increased generation in unstable asthmatic patients was not the consequence of β_2-agonist inhalation. This is in accordance with previous studies investigating effect of β_2-agonists on the generation of ROS from neutrophils *in vitro* [23], and from eosinophils *in vitro* [24] and *ex-vivo* [25].

Results obtained in stable asthmatic patients with nonstimulated PMNs and Mos showed increased generation of ROS, as assessed by lucigenin and luminol enhanced CL. This observation suggests that PMNs and Mos could be primed *in vivo*, as observed for alveolar macrophages by JOHNSTON and KITAGAWA [26]. However, we cannot say whether this priming was the result of an intrinsic defect in regulation of the cell or the effect of other mediator(s) remaining present after an asthma attack.

After PMA-stimulation, the stimulatability coefficient was higher in PMNs and Mos from healthy subjects than from asthmatic patients, and was identical in both asthmatic patient populations for lucigenin and luminol enhanced CL. Similar results have previously been observed for other mediators, namely that the spontaneous release of mediators by "resting cells" may be very high, without modification of the total release after PMA-stimulation. This has been observed with alveolar macrophages, either for interleukin-1 (IL-1) release by PUJOL *et al.* [27], or for phosphoinositide turnover by DAMON *et al.* [28].

The CL peak of PMA-stimulated PMNs and Mos for ROS release as assessed by luminol enhanced CL in stable asthmatic patients was significantly lower as compared to healthy subjects or unstable asthmatic patients. However, the $O_2^{\cdot -}$ release as assessed by lucigenin enhanced CL was identical in the three groups of subjects. In stable asthmatic patients, PMNs and Mos appeared to be already primed but less stimulatable before asthma attack. These data indicate different release of $O_2^{\cdot -}$ and of other ROS in stable asthmatic patients. This could suggest that in unstable asthmatic patients, non-$O_2^{\cdot -}$ ROS generation could be regulated and/or controlled by a different mechanism.

Resting PMNs released greater amounts of $O_2^{\cdot -}$ as assessed by lucigenin enhanced CL and greater amounts of ROS as assessed by luminol enhanced CL than did Mos. However, after PMA-stimulation, the amounts of $O_2^{\cdot -}$ and ROS were almost identical in the both cell populations. This difference in stimulatability between PMNs and Mos could be the consequence of different enzyme equipment or activity in both cells populations. It is well-known that Mos contains less myeloperoxide (MPO) than PMNs [29], but they contain a peroxidase in cytoplasmic granules which is almost identical to that of the MPO of neutrophils [30]. Could this be related to the different functions of the two cell populations in asthma? Mos are known to be primed [4] and to immigrate into the lungs and form the alveolar macrophage pool [31]. It is possible that PMNs only play a role at the time of antigen challenge to mediate the airway response [32]. The results of HUTCHINSON *et al.* [33] indicate that the effects of PMNs present in the lungs can potentiate the effect on the endothelial compartment, and that circulating PMNs could then be more effective. This would suggest that PMNs have more important

deleterious effects in blood than in the lung tissues. Moreover, KELLENBACH *et al*. [34] and others have also shown a fast PMN recruitment following exercise allergen challenge after bronchoprovocation in asthma.

We would like to propose the hypothesis that PMNs could be implicated in endothelial injury and increased vascular permeability. Indeed, LASSALLE *et al*. [35] showed that activated endothelial cells could enhance PMN migration and protect themselves against potential damaging effects of metabolites produced by PMNs during transvascular migration. PARKER *et al*. [36] showed that the eosinophil peroxide (EPO)-H_2O_2-halide system was a significant contributor to the increased vascular permeability induced by PMA-activated eosinophils, and that EPO-generated hypohalous acids could significantly contribute to endothelial injury in bronchial asthma.

Severity and activity of asthma did not have the same pathophysiological significance. Blood PMNs and Mos seemed to be primed for Ros production in both groups of asthmatic patients, but more pronounced in unstable patients; in stable asthmatic patients, however, these cells were less stimulatable for non $O_2^{\cdot -}$ Ros production. PMNs and Mos appeared to react differently, and additional studies are required to better understand their relative importance in the vascular and cellular components of inflammation observed in bronchial asthma. We have shown that CL measurements can reflect the activity of asthma in individual patients. Further investigations of whole blood samples during a time course study should provide interesting information.

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