

Oxygen radical production by blood eosinophils is reduced during birch pollen season in allergic patients

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Summary

Background The eosinophil granulocyte takes part in allergic inflammatory diseases, like asthma and rhinitis. The aim of this study was to investigate the oxidative metabolism of the blood eosinophils and neutrophils from birch pollen allergic patients, during and after exposure to their allergen.

Methods Thirteen birch pollen allergic patients, with seasonal symptoms of rhinitis, with or without asthma, were followed. The cells were purified using a percoll gradient and the MACS system. The eosinophil purity in all samples was >95%. The oxidative metabolism of both the PMN and the eosinophils was measured by means of a chemiluminescence (CL) assay, with luminol or lucigenin as the amplifier, and using PMA (16 nM) as the activator.

Results In relative terms, the lucigenin CL by the eosinophils was 5–10-fold higher than that of the PMN, $P=0.002$. The eosinophils of the birch pollen allergic patients produced less oxygen radicals during the season, compared to the reference group, measured both with luminol CL and lucigenin CL, $P=0.01$ and $P=0.02$ respectively. Out of season, there was no difference. There was also no difference, during either period, between patients and references in PMN CL. Separating the eosinophils into hypodense and normodense fractions, showed a decreased oxidative metabolism by the hypodense eosinophils.

Conclusion We conclude that the eosinophils of birch pollen allergic patients have reduced production of oxygen radicals when the patients are exposed to their allergen, which could depend on higher amounts of hypodense eosinophils in the blood during season.

Keywords: allergic rhinitis, eosinophils, chemiluminescence, hypodense

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Introduction

The eosinophil granulocyte is an inflammatory cell, participating in the body's natural defence against invading parasites. The eosinophil is found in an increased amount in the peripheral blood and in tissues of patients with allergic inflammatory diseases, for example in asthma and rhinitis [1].

The toxicity of the eosinophil can be a direct action, by means of secretion of different highly basic proteins, for example ECP and EPO, which are deposited in the cytoplasmic granules. It can also be an oxygen-dependent action, via the mechanisms of the respiratory burst and the thereby formed oxygen metabolites [2]. Furthermore, it is shown that both the granule proteins and the oxygen metabolites are needed, and work in synergy, for the killing of *Schistosomula* of *Schistosoma mansoni* [3].

During the respiratory burst, the hexose monophosphate shunt activity increases, creating more NADPH, which is used as the electron donor during

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the following oxidative metabolism. Oxygen uptake and consumption is also increased, and different oxygen metabolites, like H_2O_2 , O_2^- , $\text{OH}^\cdot$, HOCl and $^1\text{O}_2$, are formed. This occurs mostly extracellularly, catalyzed by a membrane-bound enzyme, the NADPH-oxidase [4–5]. The oxidative metabolism seems to be higher in the eosinophil than in the neutrophil granulocyte [6].

Chemiluminescence, which means measuring the emission of light produced by the phagocytes upon activation, is a simple and rapid method for measuring the state of activation of the cell and the different oxygen metabolites produced. Since this generation of light is rather weak, some chemical substrates are used to make it more detectable. These substrates react with the different oxygen metabolites and a molecule of higher energy level is thereby formed, which will emit photons when returning to the ground state [7]. The used chemiluminescent probes react with different oxygen metabolites, giving the chemiluminescence assay some specificity. Eosinophils from patients with allergic rhinitis have shown an enhanced luminol CL, during exposure to the allergen ragweed, when stimulated with opsonized zymosan [8]. In our study, we observed birch pollen allergies and used the soluble stimulus PMA.

Since the eosinophil granulocyte only constitutes such a small amount, about 2–3%, of the peripheral circulating white blood cells in a healthy person, most of the studies on the oxidative metabolism have been performed on eosinophils from patients with hypereosinophilic diseases. It has been shown, however, that there are metabolic differences between the cells from healthy persons and from patients with different diseases with hypereosinophilia [9].

The magnetic cell separating system (MACS) is based on the principle that the eosinophils are separated from the neutrophils due to their lack of CD16 expression on the cell surface. Magnetic beads are coupled to a monoclonal antibody against CD16, which binds to the neutrophils. A column with a ferromagnetic matrix is put inside a magnetic field and as the granulocytes pass through the column, the neutrophils adhere to the column matrix, while the eosinophils travel unaffected through [10–11]. The MACS has made it possible to purify enough eosinophils from one healthy individual for studies of the oxidative metabolism. The aim of this study was to investigate the oxidative metabolism of blood neutrophils and eosinophils from birch pollen allergic patients, during and after the pollen season, and to compare this oxygen radical production with that from a non-allergic control group, during the same periods of time.

Material and methods

Patients

Thirteen patients allergic to birch pollen, six men and seven women, with a mean age of 31 years (range 25–38 years), were studied during the birch pollen season. Nine of these patients were also studied after season. All patients had symptoms of rhinoconjunctivitis, and most also had symptoms of asthma, during the birch pollen season. They were randomized into two different treatment groups: one group received preseasonal immunotherapy with birch pollen extract (Alutard, ALK, Hørsholm, Denmark) and the other group received topical nasal steroid budesonide (Astra, Sweden) 200 µg two times daily, covering 2 weeks preseasonally and the whole birch season. The symptoms of disease during the pollen season were illustrated on a visual analogue scale (VAS) with 1 to 100.

Control group

The control group consisted of healthy and non-allergic blood donors, nine (three men and six women) during and 10 (two men and eight women) after season. The mean age was 34 years, with a range of 24–49 years.

Reagents

Luminol (5-amino-1,2,3,4-tetrahydrophthalazin-1,4 dione) (Merck, Darmstadt, Germany) was dissolved in dimethylsulfoxide (DMSO) and diluted in TRIS(hydroxymethylaminomethane)-NaCl buffer (15 mM TRIS, 150 mM NaCl, pH 7.4) to the concentration 1 g/L. The stock solution was kept dark at 4°C for 1 month. Before use the luminol was diluted to 0.025 g/L in the above TRIS-NaCl buffer. Lucigenin (10,10-dimethyl-9,9-bicaridinium-dinitrate) (Sigma Chemical Company, St Louis, MO, USA) was dissolved in DMSO and diluted in TRIS-NaCl to the concentration 0.5 g/L. The stock solution was kept dark at 4°C for 1 month. Before use the lucigenin was diluted in TRIS-NaCl to 0.1 g/L.

PMA (Phorbol-12-myristate-13-acetate) (Sigma Chemical Company, St Louis, MO, USA) was dissolved in 95% ethanol, to the concentration 1.6×10^{-4} M and kept in –18°C for several months. Before use the PMA was diluted, in two steps, to 16 nM in TRIS-NaCl.

The MACS-system and anti-CD16 beads were from Miltenyi Biotec, Bergisch-Gladbach, Germany.

Percoll (Pharmacia, Uppsala, Sweden) was diluted in two steps, in phosphate buffered saline (PBS), to a 67% (vol:vol) solution before use. Superoxide dismutase (SOD) (Sigma Chemical Co.) was diluted in Gey's

buffer (pH 7.4) to give the final concentration 40 mg/L. Dextran T 500 (Pharmacia, Uppsala, Sweden) was diluted in PBS to a 2% solution.

Preparation of granulocyte suspension and eosinophil isolation

The eosinophils were isolated from peripheral venous blood, 30–40 mL from each donor, with the magnetic cell separation system (MACS). This is described in detail by Hansel *et al.*

Venous heparinized blood was diluted 1:1 in PBS, before it was put on a percoll solution (67%, 1, 119 g/mL). After the following centrifugation (30 min at $1000 \times g$) the mononuclear cells were separated from the PMN. An aliquot of the PMN was diluted in Gey's buffer (pH 7.4) to obtain a final concentration of 1×10^6 /mL and was removed for the chemiluminescence assay. The remaining PMN were incubated for 1 h at 4°C with the anti-CD16 beads before putting the cell suspension on the

MACS column for the final separation. The purity of the eosinophils after the MACS separation was >95% in all samples. The eosinophils were suspended in Gey's buffer to a final concentration of 1×10^6 /mL short before use.

Density separation of eosinophils

Venous blood was diluted 1:1 (vol:vol) with the 2% dextran solution. The red cells were left to aggregate for 30 min at room temperature. The leucocytes were removed and washed twice in Gey's buffer. The few remaining erythrocytes were removed with hypotonic hemolysis. The leucocytes were diluted in 2 mL Gey's buffer, and then put on a percoll gradient, consisting of six different density fractions (60, 65, 70, 75, 80, 90%). The gradient was centrifuged at $600 \times g$ for 35 min at room temperature. Then the different fractions of cells were removed, washed and counted. Fractions 3, 4 and 5 were the only fractions containing enough eosinophils to

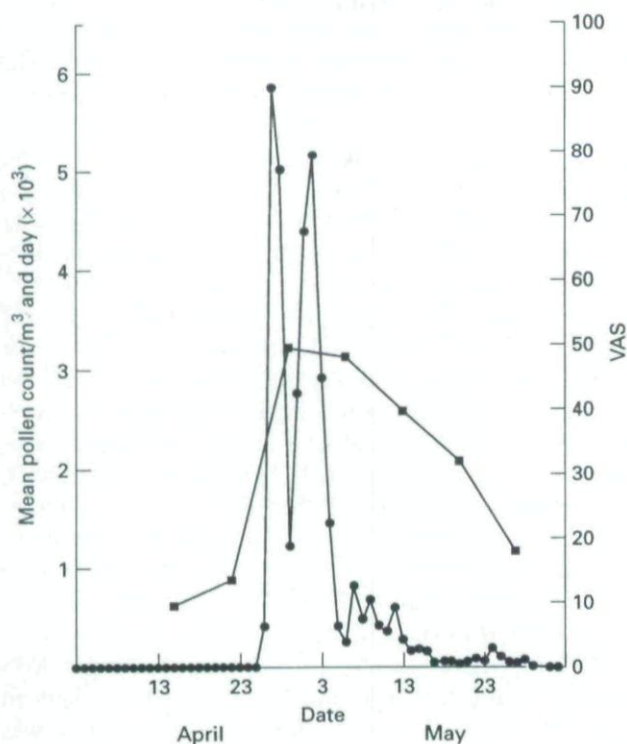


Fig. 1. The total birch pollen count in 1993 was measured before and during the birch pollen season. The pollen grains were measured daily, every second hour, and then a mean value was calculated, and expressed as a mean pollen count per m³ and day (●). Clinical symptoms (■) on a visual analogue scale (VAS), from 1 to 100, showing mean per week.

Table 1. The total white blood cell count, total eosinophil count and per cent eosinophils in the birch pollen allergic patients and the control group

Subjects	WBC (10^9 /L)	EOS (10^6 /L)	% EOS
<i>Controls</i>			
During: <i>n</i> = 9	ND	ND	ND
After: <i>n</i> = 10	6.2 (3.8–8.0)	173 (100–250)	2.8 (1.6–3.8)
<i>Patients</i>			
During: <i>n</i> = 13	5.5 (4.6–7.4)	508 (190–1090)	9.4 (2.6–18.5)
After: <i>n</i> = 9	6.3 (5.3–7.9)	219 (90–400)	3.5 (1.5–7.4)
<i>P</i> *	0.01	0.04	0.01
<i>P</i> **	0.93	0.34	0.39

The total white blood cell count (WBC), total eosinophil count (EOS) and per cent eosinophils of the total peripheral white blood cells (%EOS), in the birch pollen allergic patients and the non-allergic controls, during and after the birch pollen season. Numbers showing mean (range) and ND = not done. *P** value comparing the patients during and after season (Wilcoxon signed rank test). *P*** value showing the comparison between the patients and the references after season (Mann–Whitney *U*-test).

be worthwhile purifying further. These fractions were separated on the MACS column. The purified eosinophils were suspended in Gey's buffer shortly before use, to the final concentration of 0.5×10^6 eosinophils/mL, the rest being mononuclear cells.

Cell counting and quantitation

Cells were stained with Türk's dye and counted under the light microscope. Total blood counts were done with the cell counter Technicon HI (Tournai, Belgium). Differential counts were obtained using a cytocentrifuge preparation (Cytospin, Shandon Southern Instruments,

Sewickley, USA) stained with May Grünwald and Giemsa, and examined under the light microscope.

Chemiluminescence (CL) assay

CL was performed both on mixed granulocytes and on purified eosinophils, the cell concentration always being 1×10^6 /mL in Gey's buffer; 100 μ L cells were added to a measuring tube together with 100 μ L of either luminol or lucigenin, and preincubated at 37°C for 5 min in the measuring chambers of the luminometers (Lumac Bio-counter M2010, Netherlands and Berthold LB 9500 or LB 9505 C, Germany). After that 100 μ L PMA was added to start the reaction. The CL was recorded continuously and the peak curves, for luminol, or the obtained value after 10 min, for lucigenin, were registered and expressed as relative light units (RLU) or counts per min (CPM).

Total pollen count

The total number of pollen was counted as described before [12] and expressed as mean pollen counts per day and per cubic meter.

Statistics

The Wilcoxon signed rank test was used when there were enough data for a paired test; otherwise or due to missing

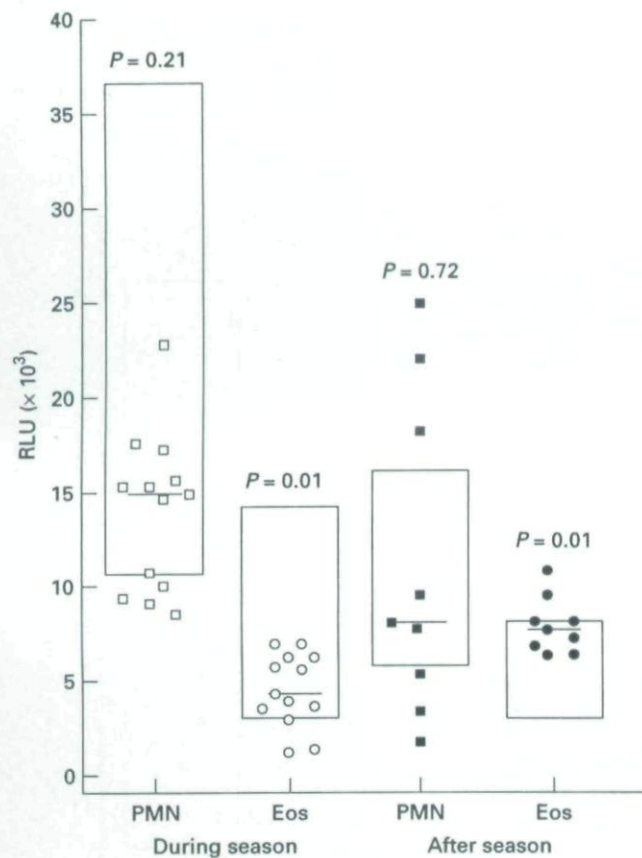


Fig. 2. Luminol chemiluminescence of PMN ($\square$, $\blacksquare$) and purified eosinophils ($\circ$, $\bullet$) from pollen allergics, during and after the birch pollen season. Bars show the medians of the groups. Boxes show the range of the non-allergic control group. The PMN suspension consisted of 89.6% (mean) of neutrophils during the season and 92.8% (mean) of neutrophils after the season. The eosinophil purity was >95% in all samples. The cells (1×10^6 /mL) were prewarmed for 5 min at 37°C, together with luminol or lucigenin, before adding PMA (16 nM). The peak of the curve was registered and expressed as relative light units (RLU).

Table 2. Luminol chemiluminescence of allergics and controls during and after the birch pollen season

	m	P
Eos Con D	8056	0.13*
Eos Con A	6198	
Eos Pat D	4254	0.008**
Eos Pat A	7600	
PMN Con D	15738	0.04*
PMN Con A	11293	
PMN Pat D	14880	0.25**
PMN Pat A	7963	

Luminol chemiluminescence of birch pollen allergic patients and non-allergic controls, during and after the birch pollen season. D = during, A = after pollen season, m = median of the group. P is expressed by means of the Mann-Whitney U-test* or the Wilcoxon signed rank test**.

data, the Mann–Whitney *U*-test was used. *P* values <0.05 were considered significant. All calculations were made on a personal computer by means of the statistical package Instat2, GraphPad, San Diego, CA, USA.

Results

Total pollen counts

The pollen counts peaked between 27 April and 4 May. The blood samples from the patients and the controls during pollen season were obtained between 4 May and 19 May. After the season, when the pollen counts had returned to normal levels, blood samples were obtained from 8 November to 17 November. The symptoms of disease, from the patients during the season, peaked at the same time as the pollen counts (Fig. 1).

Blood eosinophil counts

In the patients, blood eosinophil counts during the season were on average $508 \times 10^6/L$, range $190\text{--}1090 \times 10^6/L$, which was significantly higher ($P = 0.04$) than those after the season, which on average were $219 \times 10^6/L$, range $90\text{--}400 \times 10^6/L$ (Table 1).

Luminol enhanced chemiluminescence of eosinophils and PMN from patients and controls

During the season the eosinophils of the patients had a reduced production of luminol CL as compared to the control population, $P = 0.01$ (Fig. 2). After the season, however, the production was significantly increased compared to the production of the eosinophils by the controls. Eosinophils from the controls had a similar production during and after the season, whereas the production of the patient eosinophils was significantly

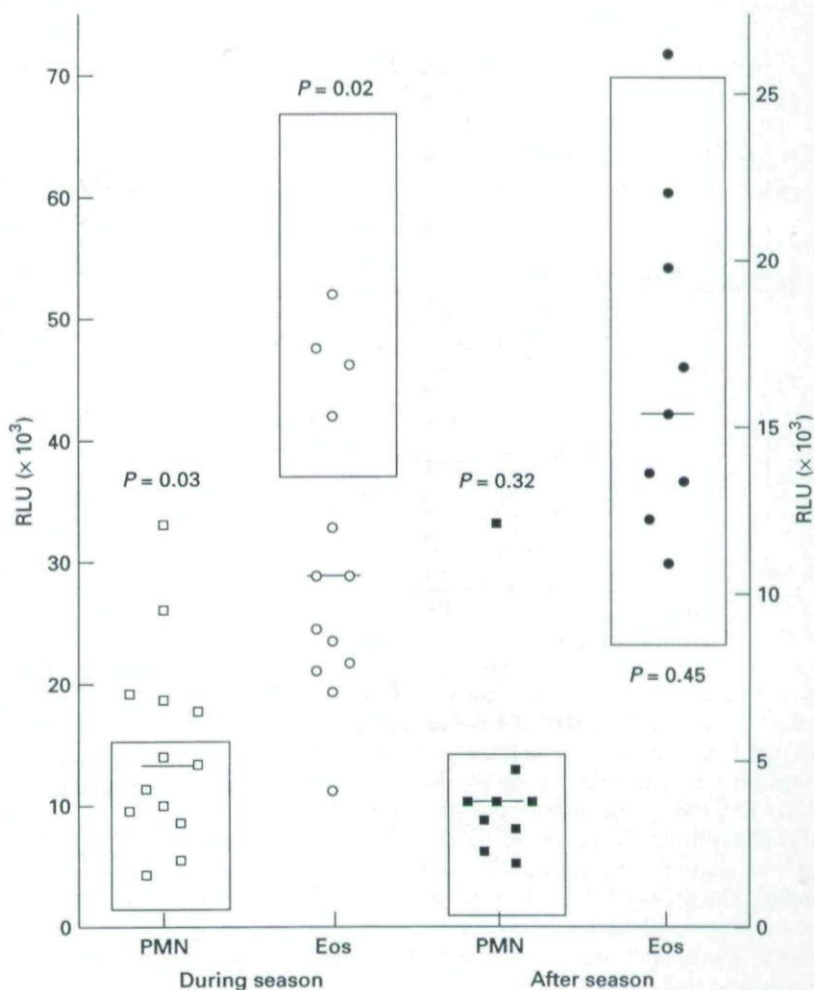


Fig. 3. Lucigenin chemiluminescence of PMN ($\square$, $\blacksquare$) and purified eosinophils ($\circ$, $\bullet$) from birch pollen allergic patients, during and after the birch pollen season. Bars show the medians of the groups. Boxes show the range of the non-allergic control group. Cell suspension and eosinophil purity as in Fig. 2. The obtained value after 10 min was registered and expressed as relative light units (RLU). The statistical differences are expressed by means of the Mann–Whitney *U*-test.

raised after the season compared to the production during the season ($P = 0.008$) (Table 2). The production of luminol CL by the PMN was similar during and after the season for the patients and the controls. There was a tendency towards higher production during the season by both control and patient PMN, which became significant only for the controls ($P = 0.04$).

Lucigenin enhanced chemiluminescence of eosinophils and PMN from patients and controls

During the season the eosinophils of the patients had significantly reduced levels compared to the controls ($P = 0.02$) (Fig. 3). After the season the CL was similar in patients and controls. The PMN from the patients had a larger production of lucigenin CL during the season than the controls ($P = 0.03$), whereas no such difference was observed after the pollen season. Due to a change in methodology no comparisons could be made between the seasons.

Comparison of eosinophils and PMN CL

The purity of the eosinophils was more than 95% in both patients and controls during and after the season. The purified PMNs from the controls were on average made up of 89.6% (range 78.5–96.7%) neutrophils during the season and 92.8% (range 89.6–95.4%) neutrophils after the season, and for the patients were 83.7% (range 66.8–96.0%) neutrophils during the season and 90.6% (range 82.6–96.1%) after the season. The rest of the cells were predominantly eosinophils, with only a very few monocytes. By the way of standardization of the CL-methods, the PMNs showed significantly higher luminol CL than lucigenin CL both during ($P = 0.03$) and after the season ($P = 0.002$) in the control group. In contrast, the eosinophils in this group showed significantly higher lucigenin CL than luminol CL both during ($P = 0.008$) and after the season ($P = 0.002$) (Fig. 4). In the patient group similar relative differences were found in the production of luminol and lucigenin enhanced CL by PMN and eosinophils (Table 3).

Since the relatively higher production of lucigenin CL by eosinophils is a new observation, we tested whether this activity was dependent on superoxide anion production, as has been shown for the neutrophils. Therefore eosinophils were stimulated to CL production in the presence of superoxide dismutase (SOD) at a concentration of 40 mg/L. This experiment showed a 90% reduction in the lucigenin CL with almost no alteration in luminol CL. Thus, these results confirm that the production of lucigenin CL is dependent on superoxide anion formation by the eosinophils.

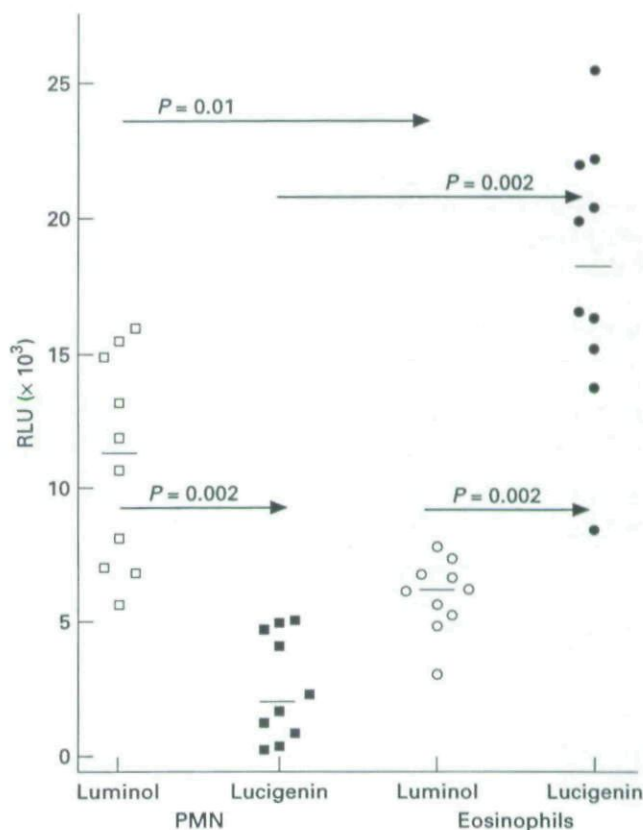


Fig. 4. Luminol and lucigenin chemiluminescence of PMN ($\square$, $\blacksquare$) and purified eosinophils ($\circ$, $\bullet$) from the non-allergic controls after the birch pollen season. The cells were diluted in Gey's buffer to the concentration $1 \times 10^6/\text{mL}$. The peak of the curve, luminol, or the obtained value after 10 min, lucigenin, was registered, and expressed as relative light units (RLU). Bars showing the medians of the groups and the statistical differences are expressed by means of the Wilcoxon signed rank test.

Chemiluminescence of hypodense and normodense eosinophils

Eosinophils were separated from three different donors into different densities by means of percoll separation. Pure eosinophils were obtained at three densities. The CL production of these eosinophil populations was investigated and summarized in Fig. 5. The data show that in all three populations the production of lucigenin CL was 2–3-fold higher by the normodense eosinophils as opposed to the production by those with lower densities. Also, the production of luminol CL was somewhat lower by the hypodense eosinophils. In relative terms, the production of lucigenin CL as compared to luminol CL, was about 3-fold higher by the normodense eosinophils but only 1.5–2-fold by hypodense eosinophils.

Table 3. Chemiluminescence of PMN and purified eosinophils from patients (Pat.) and controls (Con.), during (D) and after (A) the pollen season. m = median, Lum = luminol CL, Luc = lucigenin CL. The statistical differences are expressed by means of the Wilcoxon signed rank test

	Con. D m	Con. D <i>P</i>	Con. A m	Con. A <i>P</i>	Pat. D m	Pat. D <i>P</i>	Pat. A m	Pat. A <i>P</i>
PMN Lum	15738		11293		14880		7963	
PMN Luc	5810	0.03	2040	0.002	13285	1.00	3774	0.10
Eos Lum	8056		6198		4254		7600	
Eos Luc	41815	0.008	18167	0.002	28800	0.0002	15402	0.004
PMN Lum	15738		11293		14880		7963	
Eos Lum	8056	0.008	6198	0.01	4254	0.0002	7600	0.42
PMN Luc	5810		2040		13285		3774	
Eos Luc	41815	0.02	18167	0.002	28800	0.003	15402	0.004

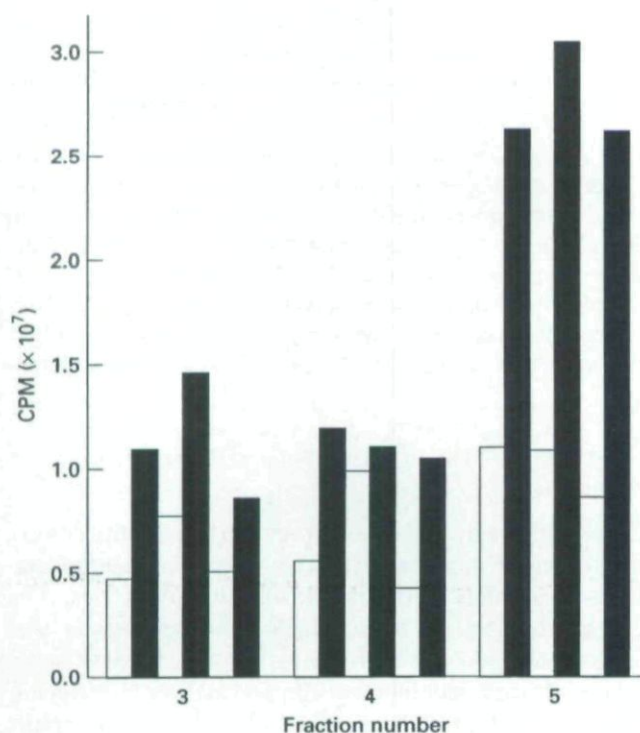


Fig. 5. Luminol (□) and lucigenin (■) chemiluminescence of eosinophils of different densities. Fraction number 5 contains the normodense eosinophils and fraction number 3 the eosinophils with lowest density. The cell concentration was 0.5×10^6 eosinophils/mL in Gey's buffer. The peak of the curves was registered and expressed as counts per min (CPM). Three different experiments with cells from different donors are shown.

Discussion

In this study, the oxygen radical production of eosinophils from birch pollen allergic patients has been studied during and after allergen exposure. A decreased production of oxygen metabolites by the blood eosinophils from the patients, as compared to the healthy controls, was found during the season. No differences in the oxidative metabolism between the groups was seen after the season. Comparing the eosinophil and the neutrophil granulocytes, we found a higher extracellular oxidative metabolism, measured by means of lucigenin enhanced CL, in the eosinophil. Separating the eosinophils due to their density, showed a reduced production of oxygen metabolites by the hypodense eosinophils.

One of the aims was to compare the oxidative metabolism of the eosinophil, with that of another phagocyte, the neutrophil granulocyte. A chemiluminescence assay with two different chemiluminescent probes, luminol or lucigenin, and the chemical stimulus PMA, was used. The luminol CL is believed to measure the production of H_2O_2 from the cells and is peroxidase dependent. This process is dependent on the mobilization of the granule protein within the cell and the process that makes the peroxidase available for the mechanisms of luminol CL [13].

The luminol molecule can pass the cell membrane and therefore the CL can measure both intra- and extracellular events. Lucigenin, the other probe used, is independent of peroxidase and degranulation, and measures O_2^- derived metabolites. Lucigenin, which is a bigger molecule, is believed to measure extracellular events [13–14].

Our results show a decreased luminol CL, but a several-fold higher lucigenin CL of the eosinophil when compared to the neutrophil. This higher oxidative metabolism of the eosinophil has been reported before, using hyper-eosinophilic cells [6] and normal cells [15].

The differences in CL between the eosinophil and the neutrophil, could be due to the fact that the neutrophil is more of a phagocytosing cell than the eosinophil. The neutrophil is engulfing the intruder in a vacuol and destroying it by means of the different granule proteins, e.g. MPO, thus giving an increased luminol CL. The eosinophil on the other side is more a secreting granulocyte, killing parasites extracellularly rather than intracellularly. This is reflected by the higher lucigenin CL. It is also shown that an activated eosinophil has higher hexose monophosphate shunt and NADPH oxidase activity, than the activated neutrophil [6]. The eosinophil also contains more cytochrome b_{558} , another part of the NADPH-oxidase complex, than the neutrophil [16]. The majority of this cytochrome b is shown to be situated in the plasma membrane, and only a small part is presented in the small granules of the eosinophil [17]. Since this enzyme complex is involved in the production of superoxide anion, this could be reflected by the high lucigenin CL from the eosinophil, and the activity difference to the neutrophil.

Another aim of this study was to investigate birch pollen allergic patients during and after the birch pollen season, and to compare their oxidative metabolism to that of healthy non-allergic subjects. The pollen allergic patients were divided into two groups, one that received immunotherapy (IT) before season, and one that was treated with local steroids (LS) during season. When observing the oxidative metabolism of the patients, the IT and the LS treatment groups showed similar results. Therefore the CL results of the patients are being presented as one group only, including all patients. The eosinophils from the pollen allergics had a reduced production of oxygen radicals, both luminol and lucigenin amplified CL, during the birch pollen season, in comparison to the non-allergic control group. This reduction was not seen after the pollen season, when the eosinophils were no longer affected by their allergen. The eosinophil CL from the controls did not differ during or after the birch pollen season, showing that the selected controls were not affected by the unusually high pollen counts that year. The birch pollen count in 1993 had its highest peak at 5838 mean pollen count per m^3 and was several times higher than in 1989, which was considered a normal birch pollen year. These extremely high pollen counts could have caused an *in vivo* influence on the blood eosinophils, that did not reflect the birch pollen influence during other more normal seasons.

Similar reduction of the oxidative metabolism has been reported by Pincus *et al.* [9], studying different types of hypereosinophilia. Heterogeneity of human eosinophils, due to their cell density, has been described [18], indicating the existence of a 'normodense' and a 'hypodense' population of the eosinophils. The normodense eosinophils are believed to change into hypodense eosinophils when activated by different allergens. An increase of hypodense eosinophils has been found in patients with asthma [19] and allergic rhinitis [20], when compared to healthy individuals. The hypodense eosinophils showed a decreased oxidative metabolism compared to the normodense eosinophils [21]. This increased proportion of hypodense eosinophils in patients with allergic diseases, and the reduced production of oxygen radicals by these cells, could be the explanation for the decreased CL observed in our birch pollen allergic patients during the pollen season.

After the season, when no longer exposed to their allergen, the eosinophils of the patients contained more normodense cells than hypodense, giving a higher CL level. On the other side, the clear distinction between the normodense and the hypodense eosinophils in asthmatic individuals has been questioned recently [22]. Also an increased superoxide production and protein kinase C activation in the hypodense eosinophils has been reported in patients with asthma or allergic rhinitis [23–24]. Our experimental data do not contain the normodense:hypodense ratio of these patients needed for that conclusion. Since the eosinophil above all acts as a tissue cell, the few cells remaining in the peripheral blood at allergen exposure, could be the non-activated resting ones, giving an expected lower CL response compared to the activated tissue eosinophils. Our results, however, from the experiments on the density separated eosinophils, will give some guidance in this matter. These results showed that the hypodense eosinophils had a decreased oxidative metabolism, seen both as a decreased luminol and lucigenin amplified CL.

It has been shown by others [8] that ragweed pollen allergic patients have increased luminol CL during their pollen season. These different results, as compared to our findings, could be explained by the usage of a different stimulus. We used PMA, which is a poor peroxidase secretagogue, while Shult *et al.* used zymosan, an effective secretagogue. It has also been shown that eosinophils from birch pollen allergics have a propensity to release EPO when exposed to their allergen (M. Carlson, submitted data). This tendency together with zymosan, would probably give the reported increased production of luminol CL.

The existence of a cytosolic NADPH oxidase deactivator, a thiobis-ester, in the granulocytes, has been

suggested [25]. This thiobis-ester could act as a physiological defence mechanism, protecting the cell and its environment from damaging oxygen radicals when overstimulated by an allergen. As pointed out before, the birch pollen season in 1993 was overwhelming. It is possible that the oxidative metabolism was shut down as a result of this massive influence of pollen on the eosinophils, giving rise to the reduced CL response to PMA.

We conclude that eosinophils of birch pollen allergic patients, when exposed to their allergen, have a reduced capacity to produce oxygen radicals. This reduction could depend on a higher number of hypodense eosinophils in the peripheral blood, since hypodense cells have a reduced oxidative metabolism. We also conclude that the eosinophil granulocyte has higher lucigenin CL than luminol CL, also compared to the neutrophil granulocyte.

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