

Blood eosinophils from asymptomatic allergics have a reduced capacity to produce oxygen-free radicals

Woschnagg C, Rak S, Venge P. Blood eosinophils from asymptomatic allergics have a reduced capacity to produce oxygen-free radicals. Allergy 1998; 53: 1162–1171. © Munksgaard 1998.

Background The eosinophil granulocyte is an inflammatory cell that plays an active part in diseases such as asthma and rhinitis. This study aimed to investigate oxidative metabolism by blood eosinophils taken from allergic rhinitis patients, asthmatics, and nonallergic controls before and during the birch-pollen season.

Methods Twenty patients with allergy to birch pollen and seasonal symptoms of rhinitis, some of whom were also asthmatic, were followed before and during the birch-pollen season in Sweden. The cells were purified using a Percoll gradient and the MACS system. Eosinophil purity in all samples was >95%. Oxidative metabolism was measured by a chemiluminescence (CL) assay, with luminol and lucigenin acting as enhancers, and PMA, serum-treated zymosan (STZ), interleukin (IL)-5, or RANTES as stimuli.

Results The allergic subjects showed reduced luminol CL when activated before the season with PMA ($P=0.040$) or STZ ($P=0.0055$). This was not seen during pollen exposure. STZ-activated lucigenin CL was also reduced before the season ($P=0.0027$). The reduction was most evident in the group with asymptomatic rhinitis. In terms of eosinophil stimulation, IL-5 and RANTES were equally effective in allergic and nonallergic subjects, both before and during the pollen season.

Conclusions Blood eosinophils from asymptomatic allergics may have a lower capacity to produce oxygen-free radicals than eosinophils from nonallergics.

C. Woschnagg¹, S. Rak², P. Venge¹

¹Asthma Research Centre, Department of Clinical Chemistry, University Hospital, Uppsala; ²Department of Lung Medicine, County Hospital, Västerås, Sweden

Key words: allergic rhinitis; asthma; chemiluminescence; eosinophils.

Charlotte Woschnagg
Asthma Research Centre
Department of Clinical Chemistry
University Hospital
S-751 85 Uppsala
Sweden

Accepted for publication 10 July 1998

A raised eosinophil granulocyte count in blood and peripheral tissues is one of the characteristic features of inflammatory diseases such as allergic asthma and allergic rhinitis (1, 2).

Once attracted to the site of inflammation by chemotactic factors, eosinophils become activated and, as a result, secrete several different tissue-toxic mediators. These are either preformed and stored in the cytoplasmic granule (basic proteins such as ECP, EPO, EPX, and MBP) or are freshly synthesized upon activation, like different oxygen radicals, during the process known as oxidative metabolism. Examples of oxygen radicals thus formed are O_2^- , H_2O_2 , OH , and $^1O_2$ (3–6).

These oxygen radicals are formed by the eosinophil, mainly at the cell surface, through attachment and assembly of an enzyme complex,

the NADPH-oxidase, which transports the electron from the donor NADPH, in the cytosol, to the acceptor O_2 on the outside of the cell. NADPH-oxidase consists of cytosol compounds; e.g., $p40^{phox}$, $p47^{phox}$, $p67^{phox}$, and rac , and membrane-bound parts, cytochrome b558. In the resting cell, the various components are not interconnected, but in the activated eosinophil, cytosol proteins translocate to the membrane and attach themselves to cytochrome b558, to form functioning NADPH-oxidase (7, 8).

The eosinophil is attracted to the site of inflammation, drawn in, and subsequently activated under the control and direction of various proteins, known as cytokines and chemokines (9). Among these are RANTES and interleukin (IL)-5.

RANTES is a member of the CC chemokine

family. It is a small, 8–10 kDa, protein, produced mainly by T cells and platelets. RANTES acts as both a chemotactic and activating factor for human eosinophils, affecting the expression of adhesion molecules, secretion of ECP, and eosinophil density (10).

IL-5 is a member of the hemopoietic cytokine family, where it forms a group with IL-3 and GM-CSF through sharing a common receptor chain (11). It is a 40–45-kDa glycoprotein, produced mainly by Th₂ cells, and has been shown to function as a selective activator of human eosinophils, affecting cell proliferation, localization, and activation (12, 13). Both RANTES and IL-5 can induce oxidative metabolism in human eosinophils (14).

In a previous study, oxygen radical production by blood eosinophils taken from patients with an allergy to birch pollen was found to be reduced during exposure to the allergen. Oxygen radical production was measured by means of luminol- or lucigenin-enhanced chemiluminescence (CL), using the phorbol ester PMA as the activator (15). PMA is a very potent, but nonphysiologic, stimulus to NADPH-oxidase production, which works by binding to intracellular protein kinase C (16). For investigation of the mechanisms leading to oxygen radical production in eosinophils from allergic patients, various stimuli were used in this study. These included substances such as serum-treated zymosan, IL-5, and RANTES, which are known to activate the eosinophils through various types of receptor.

This study aimed to investigate oxidative metabolism by blood eosinophils taken from asymptomatic allergic subjects, asthmatic subjects, and nonallergic controls, before and during the birch-pollen season, with different stimuli.

Material and methods

Patients

Twenty patients with birch-pollen allergy were studied before and during the Swedish birch-pollen season. All patients had symptoms of rhinoconjunctivitis during the birch-pollen season, and some also exhibited symptoms of asthma. They were randomized to two different treatment groups, one of which received preseasonal immunotherapy with birch-pollen extract (Alutard, ALK, Hørsholm, Denmark) and the other, the topical nasal steroid budesonide (Astra, Sweden) 200 µg twice daily. Treatment covered 2 weeks before the birch season, and the entire season itself. The rhinitis group consisted of 12 patients, six men and six women, ranging in age from 29 to 44 years with a mean age of 35. In this group, six patients were

treated with immunotherapy and six patients with local steroids.

The asthmatic group contained eight patients, five men and three women, ranging in age from 26 to 44 years, with a mean age of 32. Of these, four patients were treated with immunotherapy and four received local steroids.

During the pollen season, disease symptoms were illustrated on a visual analog scale (VAS) from 1 to 100.

Control group

The control group consisted of 10 healthy, non-allergic blood donors (four men and six women) both before and during the season. The mean age was 34 years before and 33 years during the season, ranging from 27 to 46 and 26 to 46 years, respectively.

Methacholine challenge

A methacholine provocation test was performed, as previously described (17, 18), the methacholine being used in twofold increasing concentrations. The starting concentration for the methacholine solution was 0.03 mg/ml at the first test, after which individual starting doses were used. A 2-ml volume of each concentration of methacholine solution was nebulized in a Pari Inhaler nebulizer (output 0.75 ml/min) and inhaled by tidal breathing for 2 min, at 5-min intervals. FEV₁ was measured before, and also 2 and 4 min after each dose. Once the FEV₁ had fallen by 20%, the provocation was discontinued and a dose-response curve was constructed. The result was expressed as PC₂₀, which is the methacholine concentration required to decrease the FEV₁ by 20%.

Reagents

Luminol (5-amino-1,2,3,4-tetrahydrophthalazin-1,4-dione) (Merck, Darmstadt, Germany) was dissolved in NaOH (0.01 M) and diluted in a TRIS (hydroxymethylaminomethane)-NaCl buffer (15 mM TRIS, 150 mM NaCl, pH 7.4) to give a 1 g/l concentration. The stock solution was kept dark at 4°C for 1 month. Before use, luminol was diluted to 0.025 g/l in the above TRIS-NaCl buffer.

Lucigenin (10,10-dimethyl-9,9-bicaridiniumdinitrate) (Sigma Chemical Company, St Louis, MO, USA) was dissolved in DMSO (dimethylsulfoxide) and diluted in TRIS-NaCl to give a 0.5 g/l concentration. The stock solution was kept dark at 4°C for 1 month. Before use, 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 give a 1.6×10^{-4} M concentration and kept at -18°C for several months. Before use, PMA was diluted, in two steps, to 16 nM in TRIS-NaCl.

Zymosan (ICN Biomedicals, Inc., Aurora, OH, USA) particles were suspended in TRIS-NaCl buffer, to give a concentration of 4 g/l. Opsonization was performed by adding 20% (v/v) pooled normal serum and incubating the mixture for 30 min at 37°C . The suspension was then centrifuged at 2000 g for 5 min, the supernatant discarded, and the pellet resuspended in the TRIS-NaCl buffer, to give the original concentration. The opsonized zymosan particles were stored at 4°C for up to 4 days.

IL-5 (R & D Systems Europe, UK) was diluted in Gey's buffer (pH 7.4) before use, to give the final concentration of 65 ng/ml.

RANTES (R & D Systems Europe, UK) was diluted in Gey's buffer before use, to give the final concentration of 265 ng/ml.

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 give a 67% (v/v) solution before use.

Preparation of purified eosinophils from whole blood

With the magnetic cell separation system (MACS), eosinophils were isolated from peripheral venous blood, 60–80 ml of which was taken from each donor. This is described in detail by Hansel et al. (19).

Heparinized venous blood was diluted 1:1 in PBS, before being put on a Percoll gradient (67%, 1.119 g/ml). After subsequent centrifugation (30 min at 1000 g), the mononuclear cells were removed.

The erythrocytes in the cell pellet were lysed by hypotonic hemolysis for 60 s. The remaining PMN were incubated for 1 h at 4°C with the anti-CD16 beads, before the cell suspension was put on the MACS column for the final separation. Eosinophil purity after MACS separation was $>95\%$ in all samples, with cell viability of $>86.5\%$ in all cases.

Shortly before use, the eosinophils were suspended in Gey's buffer, to give a final concentration of $1 \times 10^6/\text{ml}$.

Cell counting and quantification

Cells were stained with Türk's dye and counted under the light microscope. Cell viability was measured by Trypan blue exclusion. Total blood

cell counts were performed with a Technicon H1 (Tournai, Belgium) cell counter. Differential counts were obtained with a cytocentrifuge preparation (Cytospin, Shandon, Inc, Pittsburgh, PA, USA) stained with May-Grünwald and Giemsa, and then examined under the light microscope.

Chemiluminescence (CL) assay

CL was performed on purified eosinophils, the cell concentration always being $1 \times 10^6/\text{ml}$ in Gey's buffer. A volume of 100 μl cells was added to a measuring tube, together with 100 μl of either luminol or lucigenin, and preincubated at 37°C for 5 min in the measuring chambers of the luminometer (Berthold LB 9505 C, Bad Wildbad, Germany). After that, 100 μl of the stimuli, PMA, STZ, IL-5, or RANTES, was added to start the reaction. CL was recorded continuously, and the curve peaks were registered and expressed as counts per minute (CPM). The within-day variability of the assay was 8.5% (CV).

Total birch-pollen count

The total pollen count was measured by the Palynological Laboratory, Swedish Museum of Natural History, Stockholm. Recording of the pollen was undertaken with the aid of a Burkhard 7-day recording volumetric spore trap, as described previously (20). This trap was placed on the roof of the Eskilstuna Hospital. The pollen count is expressed as the mean number of pollen per m^3 , per 2-h interval over the day.

Statistics

The results are calculated as "results from the individual patients (measured as counts per minute) divided by the median of the results from the control group" and expressed as "percentage of controls". The Wilcoxon signed rank test and the Mann-Whitney U-test were used. *P* values of <0.05 were considered significant. All calculations were performed on a personal computer, using the statistics software, Statistica (Statsoft, USA).

Results

Total birch-pollen counts and VAS

The birch-pollen counts in that year peaked between 4 and 8 May, with some minor peaks between 9 and 28 May. All the blood samples, from the control group and both patient groups, were drawn between 31 January and 9 March, before the start of the season, and then between 19 May and

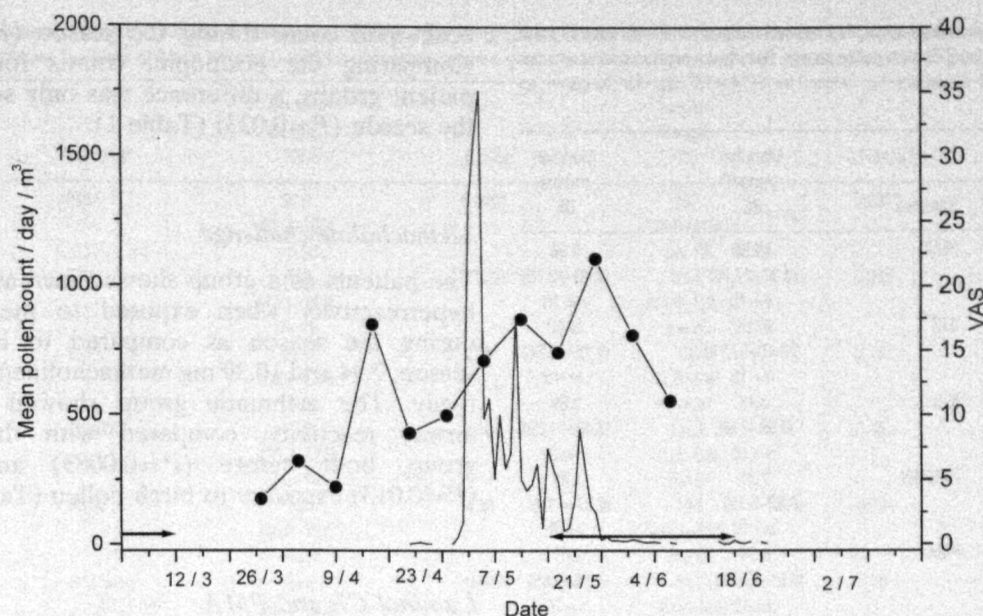


Fig. 1. Total birch-pollen count in 1995 was measured before and during birch-pollen season. Pollen grains in air were measured daily, every 2 h. Mean value was calculated, and expressed as mean pollen count per m^3 and per day (●). Clinical symptoms on visual analog scale (VAS), from 1 to 100, showing mean per week (—).

16 June, during that season. Among the patients exposed to the allergen, disease symptoms peaked during week 21 (22–28 May). There was no difference in VAS between the two treatment

groups, nor were any differences observed in terms of disease symptoms between asthmatic and rhinitis patients. Fig. 1 therefore provides a mean for all the patients who were included.

Table 1. Total eosinophil counts in whole blood from birch-pollen-allergic patients and control group. Wilcoxon test shows comparison before (BS) and during (DS) season within groups. Mann-Whitney U-test shows comparison control and patient groups. Mean (range)

Group	Eosinophils ($10^6/l$)	P value Mann-Whitney
Control BS	0.11	
<i>n</i> =10	(0.05–0.19)	
Control DS	0.12	
<i>n</i> =10	(0.06–0.17)	
Wilcoxon	0.27	
All patients BS	0.17	0.018*
<i>n</i> =19	(0.09–0.37)	
All patients DS	0.23	0.00087*
<i>n</i> =20	(0.12–0.39)	
Wilcoxon	0.0049*	
Rhinitis		
BS	0.15	0.14
<i>n</i> =12	(0.09–0.37)	
DS	0.20	0.0067*
<i>n</i> =12	(0.12–0.37)	
Wilcoxon	0.018*	
Asthma		
BS	0.21	0.0046*
<i>n</i> =7	(0.12–0.35)	
DS	0.27	0.0021*
<i>n</i> =8	(0.12–0.39)	
Wilcoxon	0.12	
Rhinitis vs Asthma		
BS		0.051
DS		0.033*

Total blood eosinophil counts

Compared with the nonallergic subjects, the patients showed an increased number of blood eosinophils both before ($P=0.018$) and during ($P=0.00087$) the birch-pollen season. The average pre-season eosinophil count for the patients, as a single group, was $0.17 \times 10^6/l$ (range 0.09 – $0.37 \times 10^6/l$), which was significantly increased during the season ($P=0.0049$) to $0.23 \times 10^6/l$ (range 0.12 – $0.39 \times 10^6/l$).

Compared with the control group, out of the two patient groups, only the asthmatic subjects had increased numbers of eosinophils ($P=0.0046$ and $P=0.0021$), both before and during the season. However, the rhinitis patients showed an increased

Table 2. PC_{20} before and during pollen season. Figures show mean of groups. Statistical differences are expressed by means of Mann-Whitney U-test and Wilcoxon paired test

Group	PC_{20} BS (methacholine mg/ml)	PC_{20} DS (methacholine mg/ml)	P value
All patients (<i>n</i> =18)	10.39	7.94	0.021*
Rhinitis (<i>n</i> =12)	12.62	10.91	0.068
Asthma (<i>n</i> =6)	4.60	2.50	0.14
	P value	P value	
Rhinitis vs asthma (all)	0.0083*	0.013*	

Table 3. Chemiluminescence by purified eosinophils from nonallergic control group before (BS) and during (DS) birch-pollen season. Results are measured as counts per minute (CPM) and expressed for luminol as $\text{CPM} \times 10^6$ and for lucigenin as $\text{CPM} \times 10^7$.

	Stimulus	Meridian (range) BS	Meridian (range) DS
Luminol	PMA	10.60 (4.37–21.78) $n=10$	9.68 (2.40–13.72) $n=10$
	STZ	37.63 (26.40–57.74) $n=10$	24.62 (4.75–32.56) $n=10$
	IL-5	3.15 (0.86–8.68) $n=10$	3.69 (2.43–11.03) $n=9$
	RANTES	2.31 (0.82–5.12) $n=10$	1.41 (0.87–4.79) $n=9$
Lucigenin	PMA	6.74 (3.51–11.90) $n=10$	2.67 (1.56–9.85) $n=10$
	STZ	4.53 (3.39–5.38) $n=10$	5.64 (1.66–8.21) $n=10$
	IL-5	2.69 (1.39–6.16) $n=10$	1.69 (0.46–3.70) $n=9$
	RANTES	0.94 (0.29–2.29) $n=10$	0.53 (0.17–3.78) $n=9$

eosinophil count during the season ($P=0.0067$). Comparing the eosinophil counts for the two patient groups, a difference was only seen during the season ($P=0.033$) (Table 1).

Methacholine challenge

The patients as a group showed increased airway hyperreactivity when exposed to methacholine during the season as compared to before the season; 7.94 and 10.39 mg methacholine/ml, respectively. The asthmatic group showed increased airway reactivity, compared with the rhinitis group, both before ($P=0.0083$) and during ($P=0.013$) exposure to birch pollen (Table 2).

Luminol CL and PMA

The patients as a group had reduced CL before the season ($P=0.040$), but increased CL during the season ($P=0.031$), as compared with the control group. Of the two groups, the rhinitis subjects had increased CL during the season ($P=0.025$), as compared with the controls. The asthmatic group showed a tendency ($P=0.065$) toward reduced CL, compared with the controls, before the season. The rhinitis group of patients showed increased CL during the season as compared with before the season ($P=0.009$) (Fig. 2 and Tables 3 and 4).

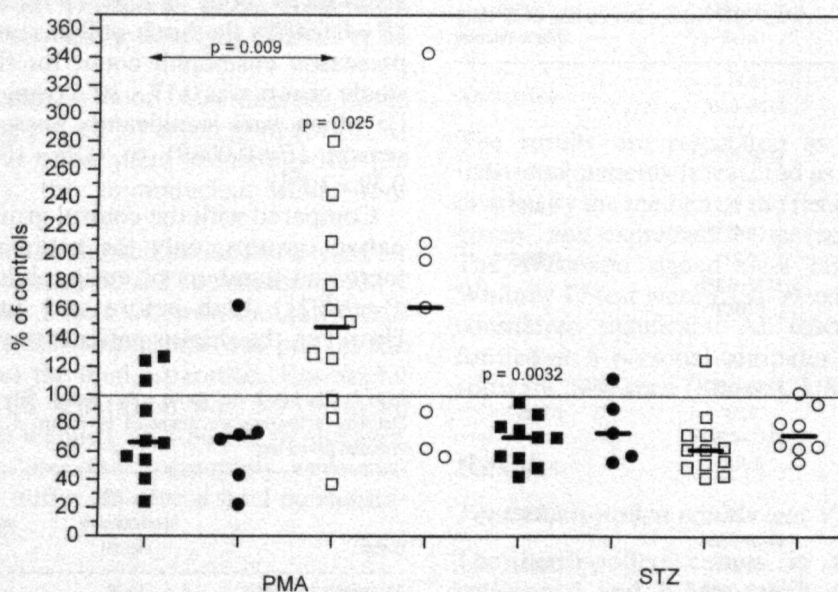


Fig. 2. Luminol chemiluminescence of purified eosinophils from rhinitis patients (■) and asthmatic subjects (●) before (filled) and during (open) birch-pollen season. Either PMA (16 nM) or STZ (4 mg/ml) was used as stimulus. Bars show medians for groups. Eosinophil purity was $>95\%$ in all samples. Cells ($1 \times 10^6/\text{ml}$) were prewarmed at 37° , together with luminol, before stimuli were added. Peak of curve was registered and results expressed as percentage of controls. Statistical differences between groups were expressed by Mann-Whitney U-test, and comparison between seasons within groups was expressed by Wilcoxon signed rank test.

Table 4. Chemiluminescence by purified eosinophils from patients before (BS) and during (DS) birch-pollen season. Results are expressed as percentage of controls. Statistical differences are expressed by means of Mann-Whitney U-test¹ for comparison between groups or Wilcoxon signed rank test² for comparison within groups

	Stimulus	Median (range) BS	P value ¹	Median (range) DS	P value ¹	P value ²
Luminol	PMA	68.0 (22.2–163) <i>n</i> = 16	0.040*	152 (36.8–343) <i>n</i> = 19	0.031*	0.0076*
	STZ	70.4 (42.1–112) <i>n</i> = 15	0.0055*	63.4 (41.4–125) <i>n</i> = 20	0.095	0.61
	IL-5	90.0 (33.3–235) <i>n</i> = 14	0.86	108 (2.2–334) <i>n</i> = 20	0.78	0.55
	RANTES	63.6 (20.3–128) <i>n</i> = 14	0.10	145.5 (18.4–386) <i>n</i> = 20	0.46	0.0052*
Lucigenin	PMA	107 (46.3–257) <i>n</i> = 18	0.34	124 (69.3–362) <i>n</i> = 20	0.19	0.084
	STZ	68.4 (50.1–95.8) <i>n</i> = 15	0.0027*	81.2 (40.8–120) <i>n</i> = 20	0.58	0.061
	IL-5	90.5 (13.0–27.0) <i>n</i> = 14	0.38	123 (76.9–33.0) <i>n</i> = 20	0.14	0.22
	RANTES	138 (8.5–415) <i>n</i> = 14	0.32	184 (28.3–457) <i>n</i> = 20	0.54	0.55

Luminol CL and STZ

The entire group of patients had reduced CL before the season ($P=0.0055$), but not during the season,

compared with the control group. Only the rhinitis group showed a reduced CL response to STZ before the season ($P=0.0032$) (Fig. 2 and Tables 3 and 4).

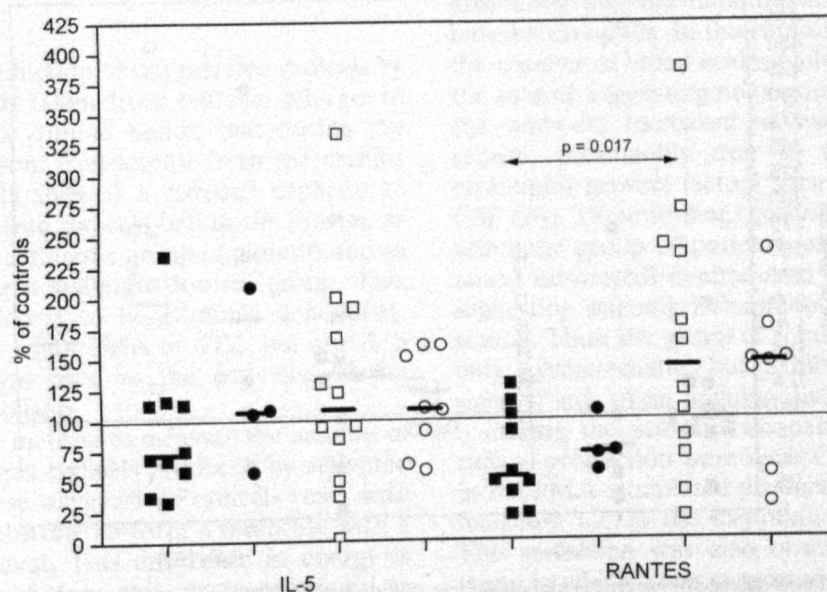


Fig. 3. Luminol chemiluminescence of purified eosinophils from rhinitis patients (■) and asthmatic subjects (●) before (filled) and during (open) birch-pollen season. Either IL-5 (65 ng/ml) or RANTES (265 ng/ml) was used as stimulus. Bars show medians for groups. Eosinophil purity was > 95% in all samples. Cells (1×10^6 /ml) were prewarmed at 37°, together with luminol, before stimuli were added. Peak of curve was registered and results were expressed as percentage of controls. Statistical differences between groups were expressed by Mann-Whitney U-test, and comparison between seasons within groups was expressed by Wilcoxon signed rank test.

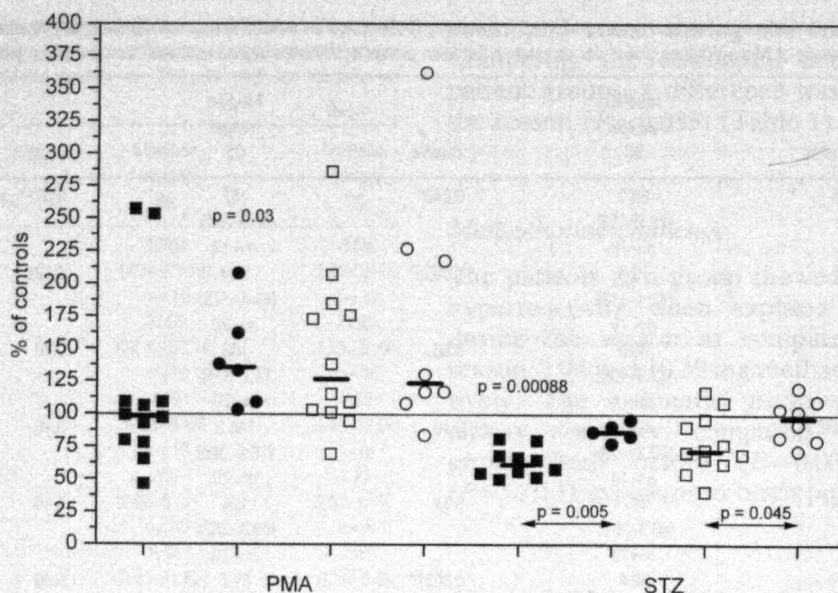


Fig. 4. Lucigenin chemiluminescence of purified eosinophils from rhinitis patients (■) and asthmatic subjects (●) before (filled) and during (open) birch-pollen season. Either PMA (16 nM) or STZ (4 mg/ml) was used as stimulus. Bars show medians for groups. Eosinophil purity was >95% in all samples. Cells (1×10^6 /ml) were prewarmed at 37°C, together with lucigenin, before stimuli were added. Peak of curve was registered, and results were expressed as percentage of controls. Statistical differences between groups were expressed by Mann-Whitney U-test, and comparison between seasons within groups was expressed by Wilcoxon signed rank test.

Luminol CL and IL-5 or RANTES

Both before and during the birch-pollen season, the patient group produced amounts of luminol CL equal to the control group. There were no

differences between the two groups of patients, either before or during the season. However, the group of rhinitis patients showed increased CL, in response to RANTES, during the season as compared to before the season ($P=0.017$) (Fig. 3).

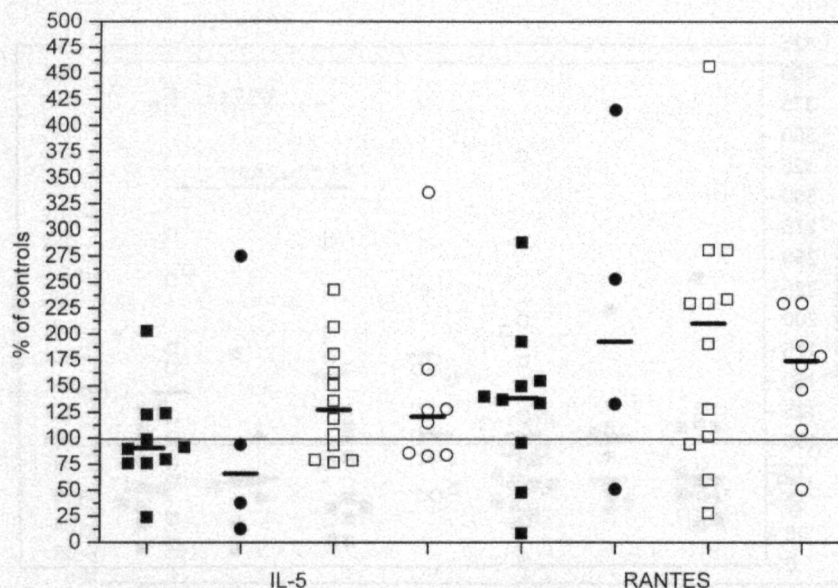


Fig. 5. Lucigenin chemiluminescence of purified eosinophils from rhinitis patients (■) and asthmatic subjects (●) before (filled) and during (open) birch-pollen season. Either IL-5 (65 ng/ml) or RANTES (265 ng/ml) was used as stimulus. Bars show medians for groups. Eosinophil purity was >95% in all samples. Cells (1×10^6 /ml) were prewarmed at 37°C, together with lucigenin, before stimuli were added. Peak of curve was registered, and results were expressed as percentage of controls. Statistical differences between groups were expressed by Mann-Whitney U-test, and comparison between seasons within groups was expressed by Wilcoxon signed rank test.

Lucigenin CL and PMA

There was no difference between the patient group as a whole and the control group, either before or during the season. The asthmatic group showed increased CL, compared with the control group, before the season ($P=0.03$), and a tendency of increased CL compared with the rhinitis group ($P=0.061$). This increase was not seen during the season (Fig. 4 and Tables 3 and 4).

Lucigenin CL and STZ

The whole patient group showed reduced CL ($P=0.0027$) before the season, but the same production during the season, compared with the controls. The reduction in CL was seen in the rhinitis group ($P=0.00088$), but not in the asthmatic group, giving a difference between the two patient groups before the season ($P=0.005$). This difference between the two patient groups was also seen during the pollen season ($P=0.045$) (Fig. 4 and Tables 3 and 4).

Lucigenin CL and IL-5 or RANTES

All the patients, as well as the healthy controls, responded to IL-5 or RANTES, both before and during the birch-pollen season. There was no difference between the two patient groups, either before or during the season (Fig. 5).

Discussion

In this study, production of oxygen-free radicals by blood eosinophils taken from patients allergic to birch pollen was studied before and during the birch-pollen season. Eosinophils from the rhinitis group of patients showed a reduced capacity to produce oxygen-free radicals before the season, as compared to the asthmatic group of patients and as compared to the nonallergic control group. This reduction was found to be stimulus dependent, since it was seen when PMA or STZ, but not IL-5 or RANTES, was used as the activator of the oxidative metabolism.

CL is a simple method to measure the amount of synthesized oxygen radicals produced by activated eosinophils. These oxygen-free radicals react with an enhancing substrate to form a molecule with a higher energy level. This difference in energy is then emitted as photons and can be measured as light by a luminometer (21). The used chemiluminescent substrate reacts with different oxygen radicals, giving the CL assay some specificity. Luminol CL is believed to measure cellular H_2O_2 production. Luminol CL is also peroxidase

dependent, making the process dependent on the mobilization of the granule protein within the cell and the mechanisms that make the peroxidase available for the reaction with luminol (22). The luminol molecule can cross the cell membrane, a fact which means that CL can be used to measure both intra- and extracellular events. Lucigenin, the other probe that is used, is believed to measure O_2^- metabolite derivatives. Lucigenin is not affected by peroxidase or degranulation and, since it is a larger molecule, is believed to measure only extracellular events (22, 23).

In this study, both chemiluminescent probes were used. The major advantages in using CL to measure oxidative metabolism are its high sensitivity and the fact that it requires very few cells for the reaction. This is very important in the context of clinical studies, since the number of purified eosinophils that can be obtained from individual patients is very limited.

The active ongoing disease process was assessed by means of subjective grading of the symptoms on a VAS, the methacholine challenge, and the measurement of PC_{20} . The VAS score showed increased numbers during the season, indicating development of the disease during the birch-pollen season. Likewise, the methacholine PC_{20} decreased during the season, suggesting the development of bronchial inflammation during the season. However, as expected, there was a substantial difference in methacholine PC_{20} between the rhinitic and asthmatic patient groups. An additional way of estimating ongoing inflammation was to measure blood eosinophils. In the rhinitis group of patients, the number of blood eosinophils was normal out of the season, suggesting no ongoing activity, whereas the numbers increased as expected during the season, presumably due to the production of eosinophil growth factors such as IL-5 and GM-CSF (24). These findings contrasted to those of the asthmatic group of patients, since this group had raised eosinophil counts even before the season, suggesting ongoing inflammation even out of the season. Thus, the group of rhinitis patients was not only asymptomatic, but exhibited few objective signs, if any, of an ongoing disease process.

During the season, two patterns of eosinophil radical production were seen. One was a reduction in the PMA-stimulated production as measured by lucigenin CL in the asthmatic group of patients. This reduction was also observed in a previous study, in which it was suggested that it might be due to an increase in the number of hypodense eosinophils in the blood of asthmatic subjects, once they have been exposed to the allergen (15).

The other pattern was an increase in oxygen radical production in the rhinitis group of patients.

We were able to measure this with both lucigenin and luminol CL, in response to either PMA or STZ stimulation.

The increase in STZ-stimulated luminol CL could be explained either by the fact that the C3b receptor may have a greater affinity for STZ, due to IL-5 or GM-CSF priming (25), or by an increased mobilization of eosinophil peroxidase from the eosinophils during season, since eosinophils from birch-pollen-allergic asthmatics have shown an increased propensity to release their granule proteins during an earlier pollen season (M. Carlson, submitted data). Increased CL production by eosinophils from allergic patients during allergen exposure has also been reported by others (26, 27).

The mechanisms leading to radical production by eosinophils from allergics during allergen exposure are not clearly understood. Therefore, different cytokines were used as stimuli, in order to obtain further information. IL-5 and RANTES have been reported to activate oxidative metabolism by human eosinophils (14). In a comparison, IL-5 was reported to be more potent than RANTES in stimulating oxidative metabolism. This confirms our own findings. We found no alterations in the response during the pollen season, nor did we see any differences in the response of the allergic cells to the normal eosinophils. This is in contrast to the results of others, who found that only eosinophils obtained from allergic patients reacted to IL-5 (28). The variations in these results may relate to differences between cellular sources, in the methods used to purify the eosinophils, or in their state of activation.

The finding of a reduced capacity of the eosinophils to produce oxygen-free radicals in the asymptomatic group of allergics out of the season is intriguing and could indicate a constitutional difference between allergics and nonallergics. Whether it constitutes an intrinsic difference in the reactivity of the eosinophil, or is a consequence of the effect on the eosinophils by some cytokine produced by these patients is not known. Indeed, IL-4 has been shown to inhibit eosinophil degranulation and cytotoxicity (29). Thus, it is known that in atopic subjects, T cells exhibit increased production of IL-4 (30). The fact that the asthmatic group of patients showed no reduction in activity out of the season is probably explained by chronic inflammatory activity in these patients.

We conclude that eosinophils from asymptomatic allergics out of season have a reduced capacity to produce oxygen-free radicals, and that this activity is increased and normalized during exposure to the allergen during the pollen season. This reduction could be a genetic abnormality or

the consequence of cytokine exposure, but it needs to be investigated further. The increased activity is unlikely to be due to IL-5 or RANTES, since the eosinophils had an unaltered reactivity to these cytokines, both before and during the birch-pollen season.

Acknowledgments

We thank Dr C. Heinrich of the County Hospital, Västerås, Sweden, for finding the right patients and collecting the samples. This study was supported by grants from the Medical Faculty of Uppsala University, the Uppsala County Association Against Heart and Lung Diseases, and the Bror Hjerpesteds Foundation.

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