

Short communication

Hemin, a heme oxygenase substrate analog, inhibits the cell surface expression of CD11b and CD66b on human neutrophils

Background: Neutrophils are signaled to sites of infection and inflammation by different chemotactic stimuli. In order to reach the airways they have to adhere to, and then migrate through, the endothelium of pulmonary vessels. Carbon monoxide (CO) is a gaseous mediator, endogenously produced in the human airways. Increased CO production has been demonstrated during airway inflammation and CO as well as hemin, a substrate for CO producing enzymes, has been shown to affect neutrophil migration. Our objective was to investigate if the neutrophil cell surface expression of CD11b, CD66b and CD63 was changed during intermittent allergic rhinitis and to establish whether CO could affect the expression of these markers of cellular activation.

Methods: Blood from 10 healthy volunteers was drawn and incubated with different concentrations of hemin. Blood from 12 other healthy volunteers and from 12 patients with intermittent allergic rhinitis was also drawn during grass pollen season. Neutrophils were then isolated from all these three sets, and their expression of CD antigens measured using flow cytometry.

Results: Patients with symptomatic intermittent allergic rhinitis exhibited lower levels of CD11b and CD66b on the neutrophil cell surface. Incubation with hemin decreased the expression of CD11b and CD66b. CD63 was generally weakly expressed and not significantly affected by hemin incubation.

Conclusion: Our results demonstrate that expressions of neutrophil cell surface glycoproteins are changed during the season in patients with intermittent allergic rhinitis and that hemin, a substrate for CO production, may act as an inhibitor of neutrophil activation. This indicates a possible role for CO in the immune defense system.

**J. A. Andersson¹, A. Egesten²,
L. O. Cardell¹**

¹Department of Otorhinolaryngology, Malmö University Hospital, Malmö; ²Department of Laboratory Medicine, Section for Medical Microbiology, Lund University, Malmö University Hospital, Malmö, Sweden

Key words: carbon monoxide; CD11b, CD63, CD66b; flow cytometry; neutrophils introduction.

Lars Olaf Cardell,
Department of Otorhinolaryngology
Malmö University Hospital
Malmö
Sweden

Accepted for publication 5 March 2002

Neutrophils originate in the bone marrow, circulate in the blood for a few days and can be viewed as 'enzymatic storehouses' capable of destroying engulfed micro-organisms and antigen-antibody complexes (1). They are signaled to sites of infection and inflammation by different chemotactic stimuli and, in order to reach the airways, they have to adhere to the endothelium and migrate through to the airway epithelium (2). A neutrophilic glycoprotein central to this adhesion and migration process is the β_2 -integrin CD11b/CD18. Stimulation of neutrophils causes a translocation of CD11b/CD18 from cytoplasmic granules to the cell surface (3). CD66b is another glycoprotein believed to be involved in neutrophil activation and migration, by means of regulating the adhesive activity of CD11/CD18 (4). CD63, also found in intracellular granules, belongs to a family of tetraspanins. It is hard to mobilize and can be used as a marker for mobilization of neutrophil azurophilic granules during cellular activation (5). It has also been implicated as a player in the regulation of neutrophil adhesion (6).

CO is an endogenously produced gas mainly generated by the conversion of heme to biliverdin (7). The rate limiting enzyme for this process, heme oxygenase (HO), exists in two major isoforms: HO-1, an inducible form, and HO-2, a constitutively expressed form (8). The presence of both isoforms has been demonstrated throughout the respiratory tract and the relation between HO and CO production has been established within the airways (9–12). CO can induce bronchodilation (13, 14) and increased levels have been found during allergic rhinitis, asthma and upper respiratory tract infections (15–17). CO has also been reported to affect neutrophil migration (18). Altogether this suggests a role for CO in airway regulation and inflammation. However, the information about CO-induced effects on cells involved in airway inflammation is scarce (10). Therefore, the present study was designed to investigate if hemin, a heme substrate analog and an inducer of HO (19), could influence the expression of different CD antigens on the neutrophil cell surface, thus indicating cell activation.

Material and Methods

Subjects

Three groups of patients were examined: Group A, 10 healthy blood donors, serving as their own controls; Group B, 12 healthy nonallergic controls and Group C, 12 patients with symptomatic intermittent allergic rhinitis. Group B and C were all nonsmokers, age matched and examined during the peak of the grass pollen season. The diagnosis of grass pollen induced allergic rhinitis was based on a positive history of seasonal allergic rhinitis for at least 2 years, and positive skin-prick tests (SPTs) against grass pollen. Patients were excluded if they had a history of perennial rhinitis, had positive SPTs against perennial allergens or had been given local or systemic glucocorticoid treatment before the study. The controls were all symptom-free, had no history of allergic rhinitis or any other atopic disease, and had negative SPTs against a panel of allergens, including grass pollen. Approval for this study was acquired from the Local Ethics Committee of the University of Lund.

Incubation and isolation of neutrophils

From each subject 20 ml of blood was drawn and heparinized. Blood from the subjects in groups B and C was immediately prepared for flow cytometry (see paragraph below). Hemin (Sigma-Aldrich Sweden AB, Stockholm, Sweden) was dissolved in dimethyl sulfoxide (DMSO; Sigma-Aldrich Sweden AB) and further diluted in RPMI-1640 medium (Life Technologies AB, Täby, Sweden) with 1% fetal calf serum (FCS), and the blood from the subjects in group A was incubated at 37°C with either hemin (10^{-13} , 10^{-11} , 10^{-9} , 10^{-7} or 10^{-5} M) or vehicle (DMSO + RPMI + FCS in the same proportions as above). Hemin did not, at concentrations up to 10^{-5} M, alter the viability of the studied cells. Earlier studies have shown that hemin induces HO-1 in various cells, and that an incubation period of 4 h seems to be optimal for the present experiments (20–22). Following 4 h of incubation, 15 ml of a mixture containing two parts Macrodex® (Dextran 70, 60 g/1000 ml 9% NaCl; Pharmalink Basläkemedel AB, Spånga, Sweden) and one part sodium metrizoate 32.8% (Nycomed Pharma AS, Oslo, Norway) was added to the heparinized blood. The solutions were allowed to sediment for 30–40 min at room temperature. The leukocyte-rich supernatant was then carefully layered upon 3 ml Lymphoprep™ (Nycomed Pharma AS) and centrifuged at 450 g for 20 min at room temperature (23–25). The supernatant was discarded and the remaining cells were washed with sterile Hanks' balanced salt solution (HBSS; Life Technologies AB), centrifuged at 340 g for 20 min at room temperature and re-suspended in 1 ml of the same medium. Cells were counted in a Bürker chamber and the cell suspension was diluted with HBSS to a final concentration of 1×10^6 cells/ml. Staining of cells with the May-Grünwald-Giemsa revealed that more than 95% of the cells in the suspension consisted of neutrophils, contaminating cells were mononuclear cells and eosinophils.

Determination of surface activation markers by flow cytometry

For group A the isolated neutrophils, and for group B and C whole blood was put on ice and red blood cells were lysed using formic acid. Thereafter, the cells were incubated with a mixture of a phycoerythrin (PE)-conjugated monoclonal antibody against CD16 (DAKO, Glostrup, Denmark) and a fluorescein isothiocyanate (FITC)-conjugated monoclonal antibody against CD11b, CD63 or CD66b (Immunotech, Marseille, France) or, as a control, CD16 antibody and an isotype-matched FITC-conjugated irrelevant monoclonal antibody (Mab IgG1; Immunotech) at the same concentration as the specific antibodies. Cells were analyzed using an Epics XL-MCL flow cytometry system (Beckman Coulter Inc., Fullerton, CA, USA) and neutrophils were gated using their characteristics in side scatter and forward scatter. Their identity was further confirmed by their

characteristic CD16 signal. The mean fluorescence intensity (MFI) in the FITC-channel was determined for the CD11b, CD63, and CD66b antibodies, respectively, and the control antibody. The MFI for the specific antibodies was subtracted with the MFI for the control antibody, representing background.

Nomenclature

The revised nomenclature for allergy according to recent EAACI recommendations was used (26).

Statistics

Results are expressed as mean $\pm$ SEM, n equals the number of subjects investigated. Statistical comparisons were made by Student's t -test and for multiple comparisons one-way analysis of variance (ANOVA) was performed, followed by Dunnett's post-test. P -values less than 0.05 were considered statistically significant.

Results

The expression of CD11b, CD66b and CD63 on the neutrophil cell surface was analyzed in blood drawn from 12 healthy donors and from 12 patients with symptomatic allergic rhinitis. The expression of CD11b and CD66b was lower among the allergic patients than in the control group (CD11b: 2.24 ± 0.22 MFI in patients and 3.04 ± 0.23 MFI in controls, $P=0.010$, CD66b: 1.10 ± 0.14 MFI in patients and 1.58 ± 0.16 MFI in controls, $P=0.020$, Fig. 1). The expression of CD63 was very weak both among the allergic patients and among the healthy controls (0.068 ± 0.04 MFI in patients and 0.0028 ± 0.011 MFI in controls).

In a separate set of experiments whole blood from healthy donors was incubated with increasing concentrations of hemin. The expression of CD11b was reduced by $22.0 \pm 4.0\%$ when incubated with hemin 10^{-11} M ($P<0.001$, $n=8$) and by $28.2 \pm 3.8\%$ when incubated with hemin 10^{-9} M ($P<0.001$, $n=10$, Fig. 2A). The expression of CD66b was reduced in a similar way (by $23.9 \pm 8.3\%$ with hemin 10^{-11} M, $P<0.05$, $n=8$ and by $22.7 \pm 8.1\%$ with hemin 10^{-9} M, $P<0.05$, $n=10$, Fig. 2B). Incubation with hemin in higher concentration than 10^{-9} M did not induce any further reduction of the CD antigen expression. The cell surface expression of CD63 was also analyzed and found to be very low during control conditions and no change following incubation with hemin could be detected (data not shown).

Discussion

Our results show that the expression of two cell surface markers of neutrophil activation, CD11b and CD66b, on neutrophils isolated from the blood of patients with symptomatic allergic rhinitis is down-regulated. A downregulation is also seen *in vitro* when neutrophils are exposed to hemin, a substrate for the enzyme heme oxygenase which is the limiting factor for CO produc-

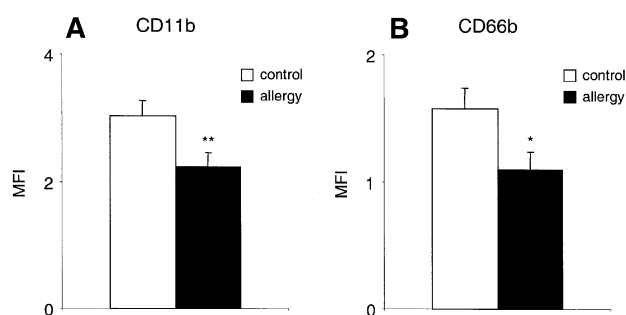


Figure 1. Expression of CD11b and CD66b on neutrophils isolated from allergic patients. Neutrophils from control subjects and allergic patients were isolated and analyzed for their expression of the activation marker CD11b (A) and CD66b (B). Results are expressed as mean fluorescence index $\pm$ SEM. Data derived from 12 patients. ** $P < 0.01$, * $P < 0.05$

tion. Even though the numbers of eosinophils and neutrophils in the blood are reported to increase only in a minority of patients as the result of nasal allergen changes (27), there is little doubt that the attraction of these cells to the nose is of importance in creating the symptoms of rhinitis (28). In addition, neutrophil recruitment to the nose has been suggested to play a major role in nasal secretion via the release of elastase (29, 30). Expression of CD11b/CD18 and CD66b on the neutrophil cell surface is of significance for adhesion and migration (3, 4). Most soluble adhesion molecules in serum are reported to be upregulated during periods of respiratory manifestations of allergic symptoms (31, 32). However, some, such as L-selectin, are in analogy with the present findings for CD11b and CD66b downregulated during the early stage of the season in patients with allergic rhinitis (33). L-selectin initiates neutrophil binding to endothelial cells and is believed to play a key role in the migration of neutrophils to the local site of inflammation (34). A reduction of CD11b has also been described on mononuclear cells in sputum from asthmatic subjects (35) whereas the neutrophil expression of CD11b has been shown to be increased, although this is most likely owing to the migration process *per se* (36). Apart from their involvement in adhesion and recruitment of neutrophils to sites of inflammation, the upregulation of these molecules on the cell surface represents mobilization of different intracellular compartments. CD63 is present in the membrane of azurophil granules and its mobilization to the cell surface correlates well with the release of azurophil granule contents (37, 38). Presence of CD11b/CD18 has been reported in several subsets of granules (specific granules, gelatinase-containing granules, and secretory vesicles) while the presence of CD66b seems to be limited to specific granules. CD63 requires stronger stimuli to be mobilized than CD66b and CD11b/CD18, thus correlating well with the releasability of the content of the respective granules (38). One factor that is known to influence the expression of CD antigens on the

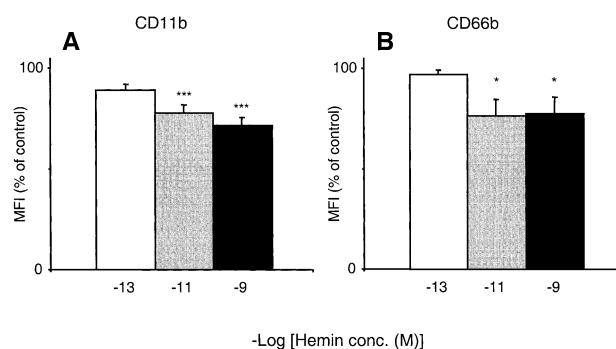


Figure 2. Expression of CD11b and CD66b on hemin treated neutrophils. Whole blood was incubated with varying concentrations of hemin. Neutrophils were subsequently isolated and analyzed for their expression of the activation marker CD11b (A) and CD66b (B). Results are expressed as mean percentage of control $\pm$ SEM. Data derived from 12 patients. * $P < 0.05$, *** $P < 0.001$.

neutrophil surface is nitric oxide (NO). NO can be produced locally in the nose and increased levels have been reported during seasonal allergic rhinitis (39, 40). Studies suggest that NO, derived from an inducible isoform of NO synthase, may act as an endogenous modulator of the inflammatory response by inhibiting adhesion of leukocytes to endothelial cells (41–43). It is also known that exogenous NO facilitates neutrophil chemotaxis *in vitro* (44) and that inhibition of NO synthase decreases neutrophil migration *in vitro* (45, 46). Furthermore, the addition of NO to the inhaled air reduces the cell surface expression of CD11/CD18 on circulating neutrophils, whereas the incubation of isolated neutrophils with L-NMMA, an inhibitor of NO synthase, increases their expression of CD11/CD18 (47, 48). CO is endogenously produced in the upper respiratory tract and increased levels have recently been reported during seasonal allergic rhinitis (49), although the data regarding CO and its potential effects on circulating inflammatory cells is limited. Recent studies have suggested that CO can enhance random neutrophil migration in a cGMP-dependent way (18). Our data demonstrates that hemin at relatively low concentrations inhibits the expression of glycoproteins on the neutrophil cell surface, possibly representing a down-regulation of the neutrophil activity. The mechanism by which CD11b/CD18 and CD66b are downregulated is not clear. Both internalization and proteolytic cleavage are possible mechanisms.

Leukocytes express different sets of cell adhesion molecules. They interact with various combinations of counterligands on the vascular endothelium, respiratory epithelium, matrix proteins and other tissue structures at distinct steps of the inflammatory cascade. Not only are these molecules necessary for recruitment into sites of inflammation, but the pattern and level of expression on a given cell will determine which leukocytes get recruited. The present data, demonstrat-

ing a downregulation of CD11b and CD66b as the result of exposure to hemin and a similar downregulation of neutrophil activity during the season in patients with intermittent allergic rhinitis, further stresses the complexity of this process. Even though a role for CO in the course of neutrophil activation seems likely, the downregulation of CD11b and CD66b in neutrophils from subjects with intermittent allergic rhinitis and in neutrophils exposed to hemin are not necessarily connected. Further research will be needed in order to demonstrate possible links between the increased amount of CO that can be found in the upper airways during seasonal allergic rhinitis and the downregulation of neutrophil activity as a result of hemin exposure reported here. Because neutrophil adhesion is

a critical event occurring locally at the site of inflammation it can not be ruled out that CO, in addition to being a marker of airway inflammation, functions as to attenuate the response in allergic rhinitis.

Acknowledgments

This work was supported by the Swedish Medical Research Council, the Swedish Medical Society, the Swedish Society of Medicine Research, the Swedish Heart Lung Foundation, the Swedish Society of Otorhinolaryngology, AGA AB Medical Research Fund, the Harald Jeansson Foundation, the TH Berg Foundation, The Swedish Foundation for Health Care Sciences and Allergy Research and the Swedish Society against Asthma and Allergy.

References

1. BØRREGAARD N. Current concepts about neutrophil granule physiology. *Curr Opin Hematol* 1996;**3**:11–18.
2. TERAN LM, MONTEFORT S, DOUGLASS J, HOLGATE ST. Neutrophil and eosinophil chemotaxins in asthma. *Q J Med* 1993;**86**:761–769.
3. CARLOS TM, HARLAN JM. Leukocyte-endothelial adhesion molecules. *Blood* 1994;**84**:2068–2101.
4. SKUBITZ KM, CAMPBELL KD, SKUBITZ AP. CD66a, CD66b, CD66c, and CD66d each independently stimulate neutrophils. *J Leukoc Biol* 1996;**60**:106–117.
5. KUIPERS TW, TOOL AT, VAN DER SCHOOT CE, GINSEL LA, ONDERWATER JJ, ROOS D, VERHOEVEN AJ. Membrane surface antigen expression on neutrophils: a reappraisal of the use of surface markers for neutrophil activation. *Blood* 1991;**78**:1105–1111.
6. SKUBITZ KM, CAMPBELL KD, IIDA J, SKUBITZ AP. CD63 associates with tyrosine kinase activity and CD11/CD18, and transmits an activation signal in neutrophils. *J Immunol* 1996;**157**:3617–3626.
7. TENHUNEN R, MARVER HS, SCHMID R. The enzymatic conversion of heme to bilirubin by microsomal heme oxygenase. *Proc Natl Acad Sci USA* 1968;**61**:748–755.
8. MAINES MD, TRAKSHEL GM, KUTTY RK. Characterization of two constitutive forms of rat liver microsomal heme oxygenase. Only one molecular species of the enzyme is inducible. *J Biol Chem* 1986;**261**:411–419.
9. CANNING BJ, FISCHER A. Localization of heme oxygenase-2 immunoreactivity to parasympathetic ganglia of human and guinea-pig airways. *Am J Respir Cell Mol Biol* 1998;**18**:279–285.
10. HORVATH I, DONNELLY LE, KISS A, PAREDI P, KHARITONOV SA, BARNES PJ. Raised levels of exhaled carbon monoxide are associated with an increased expression of heme oxygenase-1 in airway macrophages in asthma: a new marker of oxidative stress. *Thorax* 1998;**53**:668–672.
11. ANDERSSON JA, UDDMAN R, CARDELL LO. Carbon monoxide is endogenously produced in the human nose and paranasal sinuses. *J Allergy Clin Immunol* 2000;**105**:269–273.
12. CARRAWAY MS, GHIO AJ, CARTER JD, PIANTADOSI CA. Expression of heme oxygenase-1 in the lung in chronic hypoxia. *Am J Physiol Lung Cell Mol Physiol* 2000;**278**:L806–L812.
13. CARDELL LO, UEKI IF, STJÄRNE P, AGUSTI C, TAKEYAMA K, LINDEN A, NADEL JA. Bronchodilation in vivo by carbon monoxide, a cyclic GMP related messenger. *Br J Pharmacol* 1998;**124**:1065–1068.
14. CARDELL LO, LOU YP, TAKEYAMA K, UEKI IF, LAUSIER J, NADEL JA. Carbon monoxide, a cyclic GMP-related messenger, involved in hypoxic bronchodilation in vivo. *Pulm Pharmacol Ther* 1998;**11**:309–315.
15. YAMAYA M, SEKIZAWA K, ISHIZUKA S, MONMA M, MIZUTA K, SASAKI H. Increased carbon monoxide in exhaled air of subjects with upper respiratory tract infections. *Am J Respir Crit Care Med* 1998;**158**:311–314.
16. MONMA M, YAMAYA M, SEKIZAWA K, IKEDA K, SUZUKI N, KIKUCHI T, TAKASAKA T, SASAKI H. Increased carbon monoxide in exhaled air of patients with seasonal allergic rhinitis. *Clin Exp Allergy* 1999;**29**:1537–1541.
17. ZAYASU K, SEKIZAWA K, OKINAGA S, YAMAYA M, OHRI T, SASAKI H. Increased carbon monoxide in exhaled air of asthmatic patients. *Am J Respir Crit Care Med* 1997;**156**:1140–1143.
18. VAN UFFELEN BE, DE KOSTER BM, VAN STEVENINCK J, ELFERINK JG. Carbon monoxide enhances human neutrophil migration in a cyclic GMP- dependent way. *Biochem Biophys Res Commun* 1996;**226**:21–26.
19. SHIBAHARA S, YOSHIDA T, KIKUCHI G. Induction of heme oxygenase by hemin in cultured pig alveolar macrophages. *Arch Biochem Biophys* 1978;**188**:243–250.
20. MIRABELLA C, BARONTI R, BERNI LA, GAI P, NDISANG JF, MASINI E, MANNAIONI PF. Hemin and carbon monoxide modulate the immunological response of human basophils. *Int Arch Allergy Immunol* 1999;**118**:259–260.
21. MIRABELLA C, NDISANG JF, BERNI LA, GAI P, MASINI E, MANNAIONI PF. Modulation of the immunological activation of human basophils by carbon monoxide. *Inflamm Res* 1999;**48**:S11–S12.
22. WANG R, WANG Z, WU L. Carbon monoxide-induced vasorelaxation and the underlying mechanisms. *Br J Pharmacol* 1997;**121**:927–934.
23. BÖYUM A. Separation of leukocytes from blood and bone marrow. Introduction. *Scand J Clin Laboratory Invest Suppl* 1968;**97**:7.
24. BÖYUM A. Separation of blood leucocytes, granulocytes and lymphocytes. *Tissue Antigens* 1974;**4**:269–274.
25. BÖYUM A. Isolation of lymphocytes, granulocytes and macrophages. *Scand J Immunol (Suppl.)* 1976;**5**:9–15.

26. JOHANSSON SG, HOURIHANE JO'B, BOUSQUET J, BRUIJNZEEL-KOOMEN C, DREBORG S, HAAHTELA T, KOWALSKI ML, MYGIND N, RING J, VAN CAUWENBERGE P, VAN HAGE-HAMSTEN M, WUTHRICH B. A revised nomenclature for allergy. An EAACI position statement from the EAACI nomenclature task force. *Allergy* 2001;**56**:813–824.
27. PELIKAN Z, PELIKAN-FILIPEK M. Cytologic changes in the nasal secretions during the immediate nasal response. *J Allergy Clin Immunol* 1988;**82**:1103–1112.
28. PIKORIN U, KARLSSON G, ENERBACK L. The cellular response of the human allergic mucosa to natural allergen exposure. *J Allergy Clin Immunol* 1988;**82**:1046–1054.
29. CARDELL LO, AGUSTI C, TAKEYAMA K, STJARNE P, NADEL JA. LTB₄ (4)-induced nasal gland serous cell secretion mediated by neutrophil elastase. *Am J Respir Crit Care Med* 1999;**160**:411–414.
30. CARDELL LO, AGUSTI C, NADEL JA. Nasal secretion in ragweed-sensitized dogs: effect of leukotriene synthesis inhibition. *Acta Otolaryngol* 2000;**120**:757–760.
31. MONTEFORT S, FEATHER IH, WILSON SJ, HASKARD DO, LEE TH, HOLGATE ST, HOWARTH PH. The expression of leukocyte-endothelial adhesion molecules is increased in perennial allergic rhinitis. *Am J Respir Cell Mol Biol* 1992;**7**:393–398.
32. CANONICA GW, CIPRANDI G, PESCE GP, BUSCAGLIA S, PAOLIERI F, BAGNASCO M. ICAM-1 on epithelial cells in allergic subjects: a hallmark of allergic inflammation. *Int Arch Allergy Immunol* 1995;**107**:99–102.
33. KATO M, HATTORI T, MATSUMOTO Y, NAKASHIMA I. Dynamics of soluble adhesion molecule levels in patients with pollinosis. *Arch Otolaryngol Head Neck Surg* 1996;**122**:1398–1400.
34. SCHLEIFFENBAUM B, SPERTINI O, TEDDER TF. Soluble I-selectin is present in human plasma at high levels and retains functional activity. *J Cell Biol* 1992;**119**:229–238.
35. MAESTRELLI P, DE FINA O, BERTIN T, PAPIRIS S, RUGGIERI MP, SAETTA M, MAPP CE, FABBRI LM. Integrin expression on neutrophils and mononuclear cells in blood and induced sputum in stable asthma. *Allergy* 1999;**54**:1303–1308.
36. IN 'T VEEN JC, GROOTENDORST DC, BEL EH, SMITS HH, VAN DER KEUR M, STERK PJ, HIEMSTRA PS. CD11b and I-selectin expression on eosinophils and neutrophils in blood and induced sputum of patients with asthma compared with normal subjects. *Clin Exp Allergy* 1998;**28**:606–615.
37. BORREGAARD N, COWLAND JB. Granules of the human neutrophilic polymorphonuclear leukocyte. *Blood* 1997;**89**:3503–3521.
38. NIESSEN HW, VERHOEVEN AJ. Differential up-regulation of specific and azurophilic granule membrane markers in electroporated neutrophils. *Cell Signal* 1992;**4**:501–509.
39. KHARITONOV SA, RAJAKULASINGAM K, O'CONNOR B, DURHAM SR, BARNES PJ. Nasal nitric oxide is increased in patients with asthma and allergic rhinitis and may be modulated by nasal glucocorticoids. *J Allergy Clin Immunol* 1997;**99**:58–64.
40. ARNAL JF, DIDIER A, RAMI J, C MR, CHARLET JP, SERRANO E, BESOMBES JP. Nasal nitric oxide is increased in allergic rhinitis. *Clin Exp Allergy* 1997;**27**:358–362.
41. DE CATERINA R, LIBBY P, PENG HB, THANNICKAL VJ, RAJAVASHISTH TB, GIMBRONE MA Jr, SHIN WS, LIAO JK. Nitric oxide decreases cytokine-induced endothelial activation. Nitric oxide selectively reduces endothelial expression of adhesion molecules and proinflammatory cytokines. *J Clin Invest* 1995;**96**:60–68.
42. KHAN BV, HARRISON DG, OLBRYCH MT, ALEXANDER RW, MEDFORD RM. Nitric oxide regulates vascular cell adhesion molecule 1 gene expression and redox-sensitive transcriptional events in human vascular endothelial cells. *Proc Natl Acad Sci USA* 1996;**93**:9114–9119.
43. PENG HB, SPIECKER M, LIAO JK. Inducible nitric oxide: an autoregulatory feedback inhibitor of vascular inflammation. *J Immunol* 1998;**161**:1970–1976.
44. BEAUVAIS F, MICHEL L, DUBERTRET L. Exogenous nitric oxide elicits chemotaxis of neutrophils in vitro. *J Cell Physiol* 1995;**165**:610–614.
45. KAPLAN SS, BILLIAR T, CURRAN RD, ZDZIARSKI UE, SIMMONS RL, BASFORD RE. Inhibition of chemotaxis Ng-monomethyl-L-arginine: a role for cyclic GMP. *Blood* 1989;**74**:1885–1887.
46. BELENKY SN, ROBBINS RA, RENNARD SI, GOSSMAN GL, NELSON KJ, RUBINSTEIN I. Inhibitors of nitric oxide synthase attenuate human neutrophil chemotaxis in vitro. *J Laboratory Clin Med* 1993;**122**:388–394.
47. CHELLO M, MASTROROBERTO P, MARCHESI AR, MALTESE G, SANTANGELO E, AMANTEA B. Nitric oxide inhibits neutrophil adhesion during experimental extracorporeal circulation. *Anesthesiology* 1998;**89**:443–448.
48. KUBES P, SUZUKI M, GRANGER DN. Nitric oxide: an endogenous modulator of leukocyte adhesion. *Proc Natl Acad Sci USA* 1991;**88**:4651–4655.
49. ANDERSSON JA, UDDMAN R, CARDELL LO. Increased carbon monoxide levels in the nasal airways of subjects with a history of seasonal allergic rhinitis and in patients with ongoing upper respiratory tract infection. *Clin Exp Allergy* 2001;**31**:in press.