



Investigating lung remodeling in Modul8[®]-treated BALB/c asthmatic animals

H.M. Oberholzer, E. Pretorius*

Department of Anatomy, School of Medicine, Faculty of Health Sciences of the University of Pretoria, Pretoria, South Africa

ARTICLE INFO

Article history:

Received 4 June 2009

Received in revised form 15 July 2009

Accepted 16 July 2009

Keywords:

Asthma
Modul8[®]
Inflammation
Lungs
Eosinophils
Remodeling
BALB/c mice

ABSTRACT

Animal models of bronchial hyperresponsiveness have been successfully used to investigate the pathophysiology of asthma. When mice are sensitized and challenged with an allergen, such as OVA, they experience symptoms and processes similar to that of humans, and are therefore widely used as asthmatic animal models. In the current study the BALB/c murine asthmatic animal model was used to investigate the histological and ultrastructural changes that occur in the lungs of asthmatic animals that received no treatment, compared to two groups of asthmatic animals that were treated with a homeopathic immunomodulator Modul8[®] and hydrocortisone as positive control, respectively. Eosinophil counts in the bronchial lavage of the animals were also analyzed, since it is known that eosinophil counts are increased in the bronchial lavage in asthma. Results indicated that eosinophil counts were elevated in asthmatic animals compared to the controls, but were found to be significantly decreased in the treatment groups. Also, in the asthmatic, untreated animals, histological and ultrastructural changes, typically associated with the inflammatory process were found. Both treatment groups compared well to that of the control animals, indicating that the homeopathic product might be successfully used in the treatment of asthma.

© 2009 Elsevier Ltd. All rights reserved.

1. Introduction

Asthma is associated with a wide range of symptoms and effects, which include chest tightness, wheezing, coughing, increased airway obstruction and airway hyperresponsiveness. The latter part represents the classic physiology of asthma. Airway inflammation is considered to be a central feature of asthma pathogenesis and its clinical manifestations and plays a critical role in airway obstruction and hyperresponsiveness (Wenzel, 1994). Many studies have been done by using bronchial hyperresponsiveness animal models to investigate the pathophysiology of asthma. When mice are sensitized and challenged with an allergen they experience an influx of inflammatory cells into the airways, a process similar to that of humans and are therefore widely used as asthmatic animal models (Evans et al., 2003; Hamelmann et al., 1999; Kumar and Foster, 2002; Leigh et al., 2002; Tomioka et al., 2002). Eosinophilia and mucus cell hyperplasia are some of the most notable histopathological signs of human asthmatics that can also be observed in mouse models of asthma (Uller et al., 2006; Persson et al., 1997).

In the current study the murine asthmatic BALB/c model was used to investigate the histological and ultrastructural changes that occur in the lungs of asthmatic animals by using light and transmission electron microscopy. The lungs of OVA challenged, untreated asthmatic animals were compared to animals treated with a homeopathic immunomodulator, Modul8[®] as well as hydrocortisone, which was used as positive control. White blood cell counts in the bronchial lavage fluid of the animals in the different treatment groups were also analyzed specifically to investigate the eosinophil count, since it was previously found that the number of eosinophils is significantly increased in the asthmatic animals compared to healthy animals (Oberholzer et al., 2008). Eosinophils are also involved in the inflammatory cascade of asthma and migrate into the airways by means of a chemo-attractant (Wenzel, 1994).

Modul8[®] is a complex homeopathic product that consists of a number of different substances namely: *Aconitum napellus* (D20), *Arsenicum album* (D18), *Asafoetida* (D20), *Calcarea carbonica* (D16), *Conium maculatum* (DH17), *Ipecacuanha* (D13), *Phosphorus* (D20), *Rhus toxicodendron* (D17), *silicea* (D20), *sulfur* (D24), *Thuja occidentalis* (D19), alcohol (0.2%, v/v) and purified water (44.050 ml). The composition per 50 ml bottle is expressed as declared potencies (D) which indicates an index of 10-fold dilution. An immunomodulator is an agent that enhances the immunity of an individual (Piemonte and Buchi, 2002). Several studies have shown that products of natural origin can effectively be used in treating diseases and maintaining the resistance to infections of

* Corresponding author at: Department of Anatomy, Faculty of Health Sciences, University of Pretoria, BMW Building, PO Box 2034, University of Pretoria, Pretoria, 0001, South Africa. Tel.: +27 12 319 2533; fax: +27 12 319 2240.

E-mail address: resia.pretorius@up.ac.za (E. Pretorius).

organisms (Pereira et al., 2005) and are also effective against inflammatory conditions. Therefore this product was tested as treatment in the asthmatic mouse model, to investigate its possible effect on the inflammatory process involved in asthma.

2. Materials and methods

2.1. Implementing the asthmatic BALB/c mouse animal model

Six-week-old (female) BALB/c mice (each of average weight 20 g), maintained in the University of Pretoria Biomedical Research Centre and provided OVA-free food (Balanced EpolT mice cubes and pellets, obtained from EPOL – a division of Rainbow Farms PTY LTD, SA) and water *ad libitum*, were used. Polycarbonate type III cages were obtained from Techniplast. The animals were kept in a barrier unit with a temperature range of 20–24 °C and a relative humidity of 40–60% with a 12-h day light and 12-h night time. Six mice were housed per cage and autoclaved pinewood shavings were used as embedding material. White facial tissue paper was also added for enrichment. All experimental protocols complied with the requirements of the University of Pretoria's Animal Use and Care Committee Ethical clearance number: (151/2006) and complies to the WHO/UNESCO standards.

Mice were divided into the following groups: six control mice, six asthmatic mice, six mice exposed to physiologically comparable levels of Modul8® (10%) and six mice exposed to hydrocortisone at a concentration of 100 mg/kg.

Sensitization of mice (on day 0 and 5) involved the intraperitoneal injection of 25 mg ovalbumin (OVA) (Grade V; Sigma-Aldrich) and 2 mg Al(OH)₃ dissolved in 0.5 ml of 0.9% saline solution. All the mice except the control mice were sensitized.

The mice were nebulized twice daily for 1 h on days 13–15 and again on days 30–32 with 1% OVA dissolved in PBS. An inhalation exposure system (IES) (Glas-COL® Inhalation Exposure System, Model 099C A4212, Terre Haute, Indiana) was used for the nebulization. Nebulization involved placing the mice inside a stainless steel wire mesh basket, which is divided into five equal compartments. Each compartment held the six animals from the same experimental group. One complete cycle in the IES included a preheat cycle of 15 min followed by the nebulization with the OVA for 60 min, a cloud decay cycle for 15 min and a decontamination cycle also 15 min.

Treatment involved the administration of Modul8® and hydrocortisone. Hydrocortisone was injected intra-peritoneally, while Modul8® was administered orally. For humans it is generally advised to take 10 drops of Modul8® three times a day. And since the average of 10 drops equals approximately 600 µl, it can be said that the human takes in 600 µl three times per day. This equals an amount of 1800 µl/day (1.8 ml/day). When the average weight of a human adult is also considered (between 60 and 70 kg: average 65 kg): It can therefore be assumed that a normal person that weighs 65 kg will take in 1800 µl = 1800 µl/65 kg. This will imply that the intake is: 28 µl/kg. The average weight of a mouse is around 20 g (0.02 kg). And since it was established that the intake per day is 28 µl/kg, it can be calculated that each mouse should receive 0.56 µl of the Modul8® per day in order have the same values as the human. The first treatment procedures took place on days 15–18, 22–25 and the last set of treatments was on days 36–39.

2.2. Bronchial lavage techniques

After termination, a small skin incision was made in the skin of each mouse in the ventral of the trachea. The trachea was exposed by blunt dissection and a small transversal incision was made below the larynx. Through the sheath of a 21G venous catheter 0.3 ml of saline was injected into the trachea and aspirated with a

syringe. The bronchial lavage fluids collected for the individual groups were pooled, centrifuged for 2 min at 1000 rpm and smears were made. The smears were stained with Rapid Haematological Stain. White blood cells were counted under a 100× magnification and up to a hundred cells were counted per slide.

2.3. Tissue for histology

Lung tissue was collected for histological investigation. Tissue samples were fixed in 4% formaldehyde. The tissue was removed from the fixative and serially dehydrated in 70% and 90% ethanol, followed by three changes of absolute ethanol. The samples were then infiltrated in LR White over three days after which they were polymerized in gelatin capsules at 60 °C for 48 h. 1 µm sections were made with an ultramicrotome and stained with Toluidine Blue. The samples were viewed with a Nikon Optiphot transmitted light microscope (Nikon Instech Co., Kanagawa, Japan).

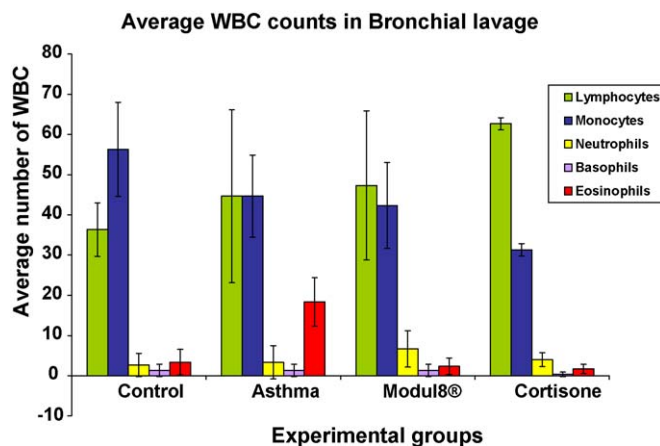
2.4. Tissue for transmission electron microscopy

Lung samples were fixed in 2.5% glutaraldehyde for 1 h, rinsed three times in 0.075 M sodium potassium phosphate buffer (pH 7.4) for 15 min before being placed in secondary fixative, 1% osmium tetroxide solution, for 1 h. Following fixation, the tissues were rinsed again as described above. The tissues were then dehydrated in 30%, 50%, 70%, 90% and three changes of 100% ethanol. The samples were then embedded in resin and ultra-thin sections (80–100 nm), cut with a diamond knife using an ultramicrotome, were contrasted with uranyl acetate for 15 min followed by 10 min of contrasting with lead citrate, after which samples were allowed to dry for a few minutes before examination with the JEOL Transmission Electron microscope (JEM 2100F).

3. Results

Statistical analysis of the recorded white blood cell counts was performed using NCSS. This was done to determine whether any differences existed in white blood cell counts between the different experimental groups. As the data was normally distributed in all cases, one-way ANOVAs were utilized for the between group comparisons for each type of white blood cell and Tukey–Kramer post hoc tests were done.

Graph 1 reveals the average number of white blood cells counted in the bronchial lavage in the different experimental groups. The asthmatic group possessed a significantly greater eosinophil count compared to the control and two treatment



Graph 1. Average number of white blood cells counted in the bronchial lavage in the different experimental groups.

groups (p -value = 0.00132). Statistical analysis of the other white blood cell species revealed that the control group had a significantly higher monocyte count in comparison to the hydrocortisone group (p -value = 0.0389). For lymphocytes, basophils and neutrophils, there were no significant difference between any of the assessed groups.

Fig. 1A (10× magnification) and B (40× magnification) represent a section of the lung of a normal healthy animal where alveoli (Label A) are seen, as well as a few inflammatory cells (Label C). In the higher magnification (Fig. 1B) the black arrow indicates the interalveolar area which is very small due to the lack of many inflammatory cells. Fig. 1C (10× magnification) and D

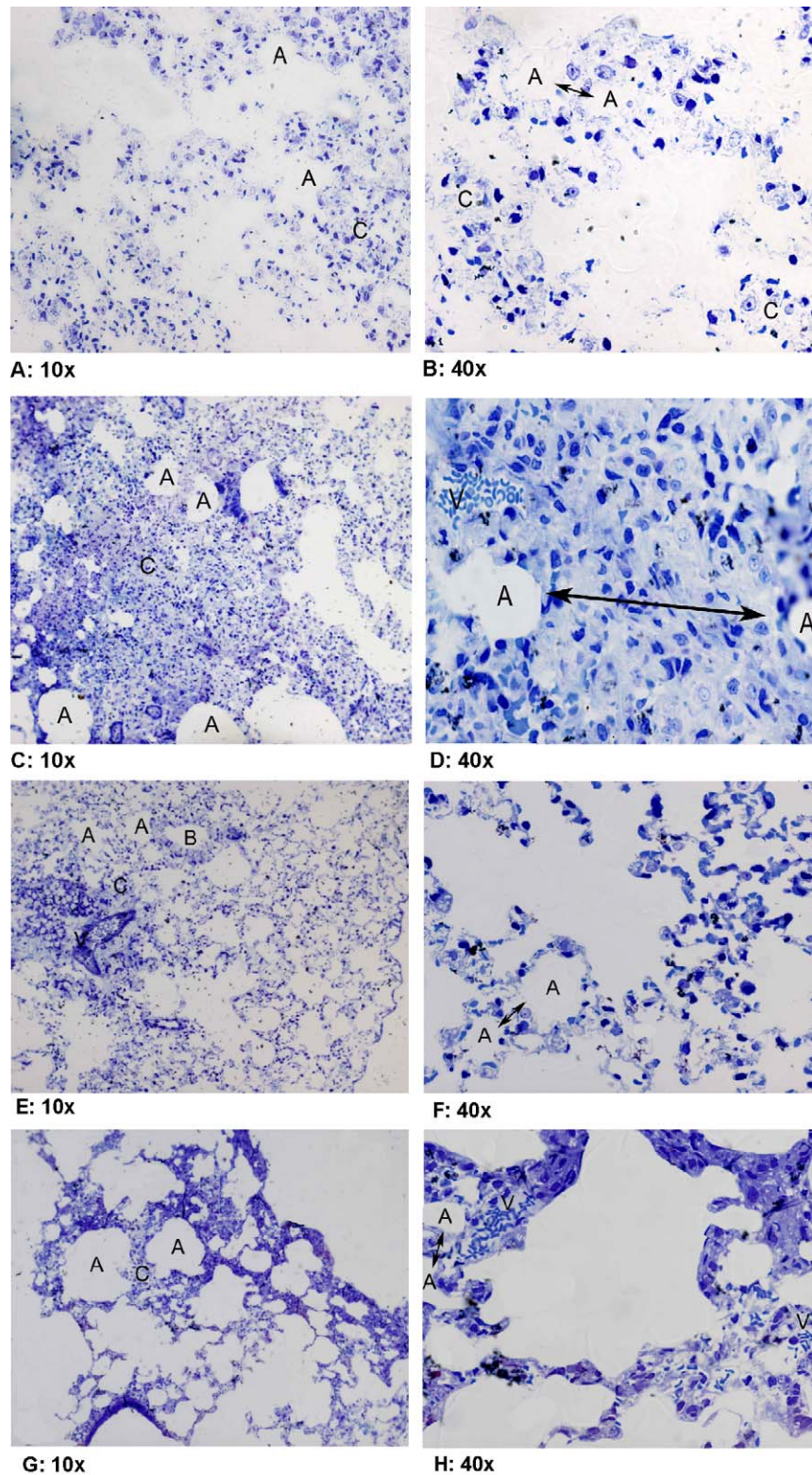


Fig. 1. (A–H) Lung sections of the mice stained with Toluidine Blue. (A and B) Control, (C and D) asthma, (E and F) Modul8[®] and (G and H) hydrocortisone. Label A: Alveoli; Label B: bronchiole; Label C: inflammatory cells; Label V: blood vessel filled with red blood cells.

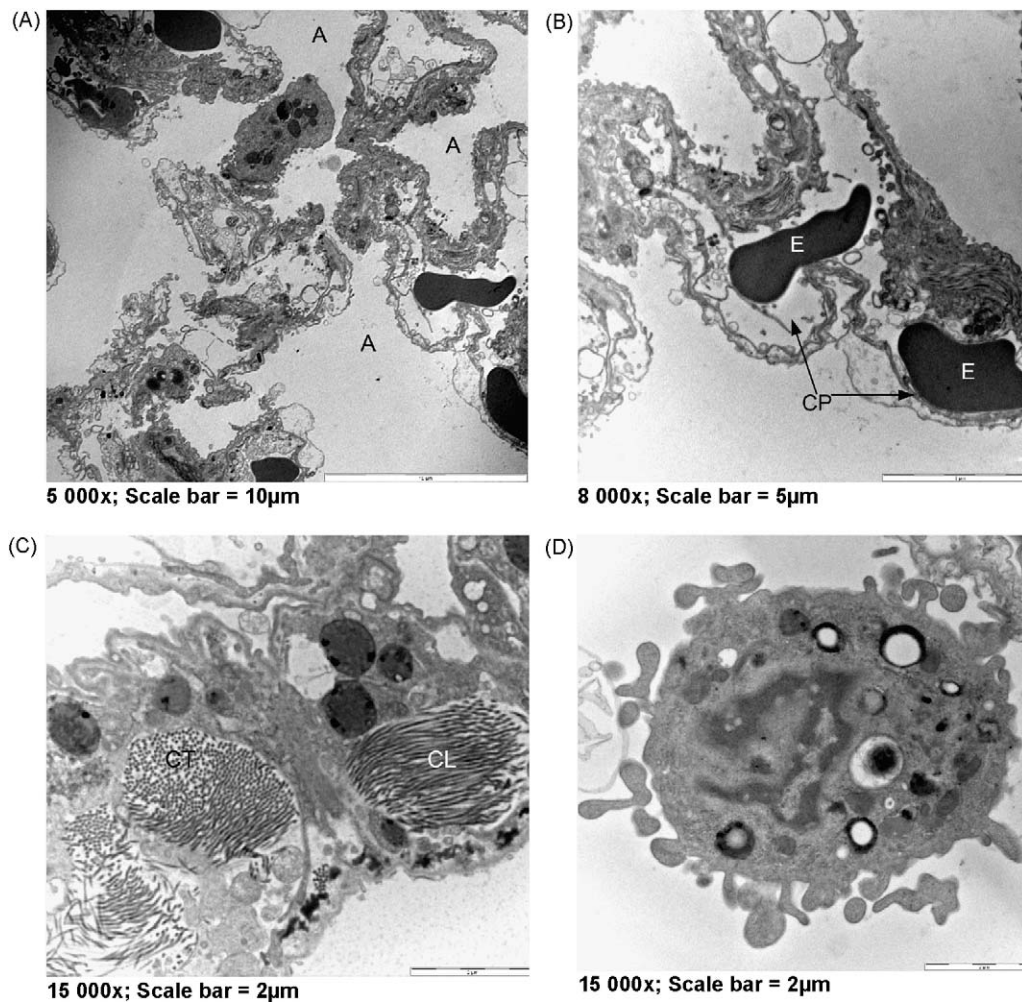


Fig. 2. (A–D) Electron micrographs of the lungs taken from the control group.

(40× magnification) are light micrographs of the lungs of the OVA-challenged asthmatic animals where a marked influx of inflammatory cells (Label C) is observed. In the higher magnification (Fig. 1D) an enlarged, congested capillary is observed. The black arrow indicates the interalveolar area which seemed congested and filled with exudates, containing white blood cells, red blood cells and fibrin – characteristic of the inflammatory condition of the lungs (Ross et al., 2003). This congested appearance is not present in the control animals (Fig. 1A and B). Fig. 1E (10× magnification) and F (40× magnification) show the lung sections of asthmatic animals treated with the homeopathic immunomodulator Modul8®. Alveoli (Label A), a bronchiole (Label B) as well as inflammatory cells (Label C) are visible. The capillary (Label V) appears smaller and less congested as seen in the asthmatic animals (Fig. 1D, Label V). The black arrow in Fig. 1F indicates the interalveolar area which is much smaller than that seen in the asthmatic group (Fig. 1D). Therefore, in this treated group congestion is reduced and much less inflammatory cells are present compared to the asthmatic group. Fig. 1G (10× magnification) and H (40× magnification) represent the micrographs of the hydrocortisone-treated animals, where even less inflammatory cells (Label C) are present. The black arrow (Fig. 1H) indicates the interalveolar area which seems similar to that of the Modul8® treated group and much smaller than that of the asthmatic group; the area has more open spaces and is less congested without the presence of exudate, as are seen in the asthmatic group. This morphology is expected, as

hydrocortisone is an effective anti-inflammatory treatment for asthma.

The results from analyzing the transmission electron microscopy micrographs support those found in analysis of the light microscopy micrographs. Fig. 2A–D are transmission electron micrographs of areas in the lungs of the control group where part A is a representation of the little inflammatory infiltrate present in the interalveolar area whereas in the asthmatic group, as can be seen in Fig. 3A, the interalveolar areas are filled with inflammatory cells and exudate as could also be observed in the light microscopy results. Fig. 2B shows a capillary filled with erythrocytes and Fig. 2C is a representation of the collagen deposition that could be observed which were very little compared to the asthmatic group. Transverse as well as longitudinal collagen fibrils could be seen. Fig. 2D is an electron micrograph of a typical macrophage which could be observed in all the experimental groups.

Fig. 3A–F are transmission electron micrographs of areas in the lungs of the asthmatic group where Fig. 3A shows the marked influx of inflammatory cells into the lung parenchyma and the increase in the area between the alveoli. Fig. 3B shows a congested capillary which could also be observed in the light microscopy results. Fig. 3C shows collagen fibrils in longitudinal section which seems to be more densely packed as could be observed in the other experimental groups where the collagen fibrils could be clearly seen and distinguished. Fig. 3D shows a number of cells that form part of the inflammatory infiltrate in the lungs. A lymphocyte, monocyte, mast cell and fibroblast are seen in this figure. Fig. 3E is a

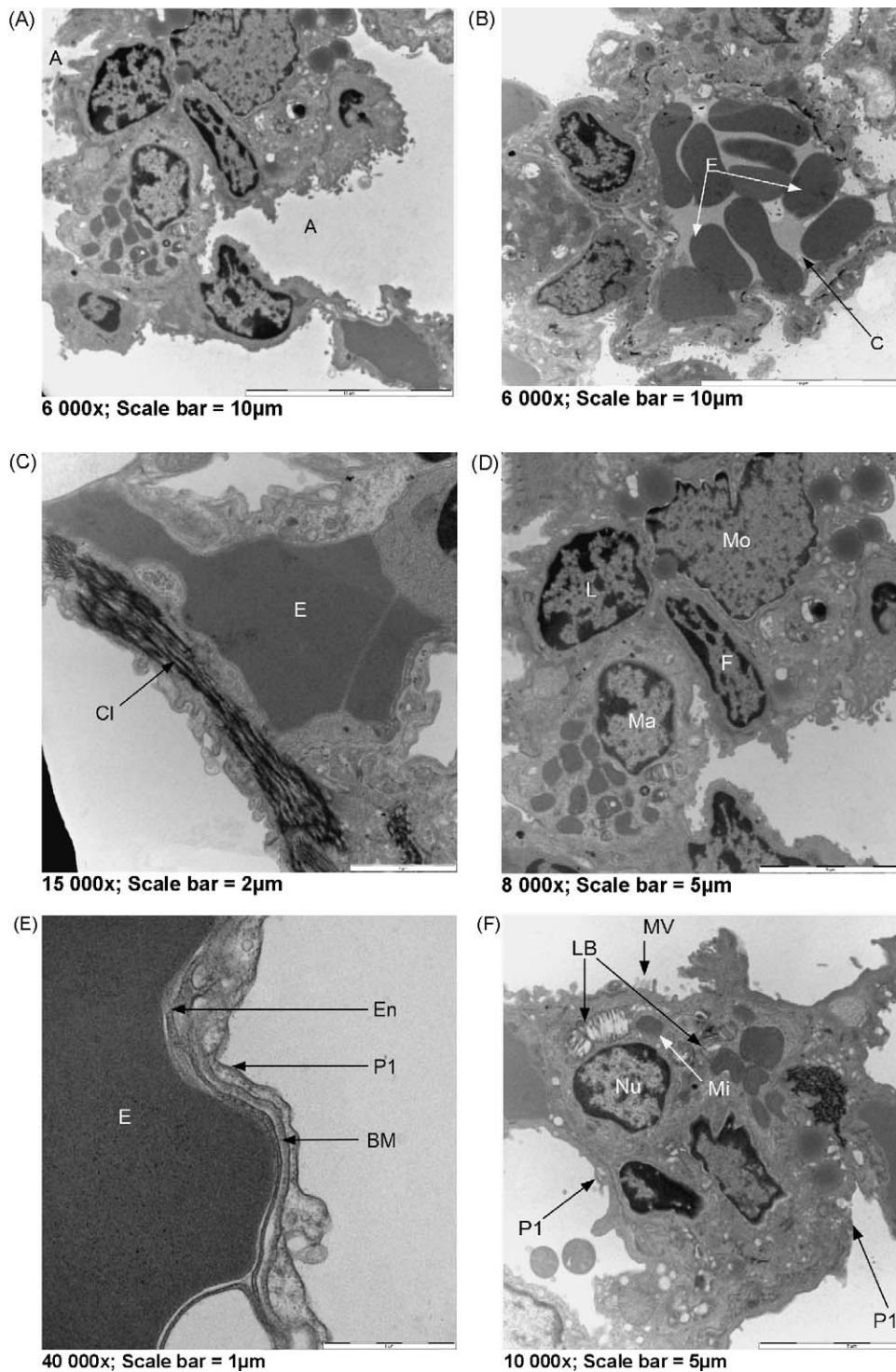


Fig. 3. (A–F) Electron micrographs of the lungs taken from the asthmatic group.

representation of the air–blood barrier showing the components of the diffusion barrier between the blood and alveolar air. A type I pneumocyte, a basement membrane and the thin cytoplasm of the endothelial cells can be seen in this figure. Fig. 3F is a micrograph of a type II pneumocyte. These cells are also known as surfactant cells and are responsible for surfactant production. Type II pneumocytes are recognizable by the presence of lamellar bodies. Most of the cell is surrounded by a basement membrane and only a small portion is in direct contact with the alveolar space where numerous small microvilli could also be observed.

Fig. 4A–F are electron micrograph of the lungs taken from the Modul8[®]-treated group. Fig. 4A is a representation of the alveolar spaces where lots of open spaces can be observed with little inflammatory infiltrate present in the interalveolar areas. Fig. 4B shows a capillary with an erythrocyte and Fig. 4C shows a lymphocyte and two fibroblasts with collagen deposition, which was observed to be not as abundant as could be seen in the asthmatic group. Fig. 4D shows the ultrastructure of a macrophage, revealing cytoplasmic projections or pseudopodia which are involved in movement and phagocytosis. Mitochondria, golgi

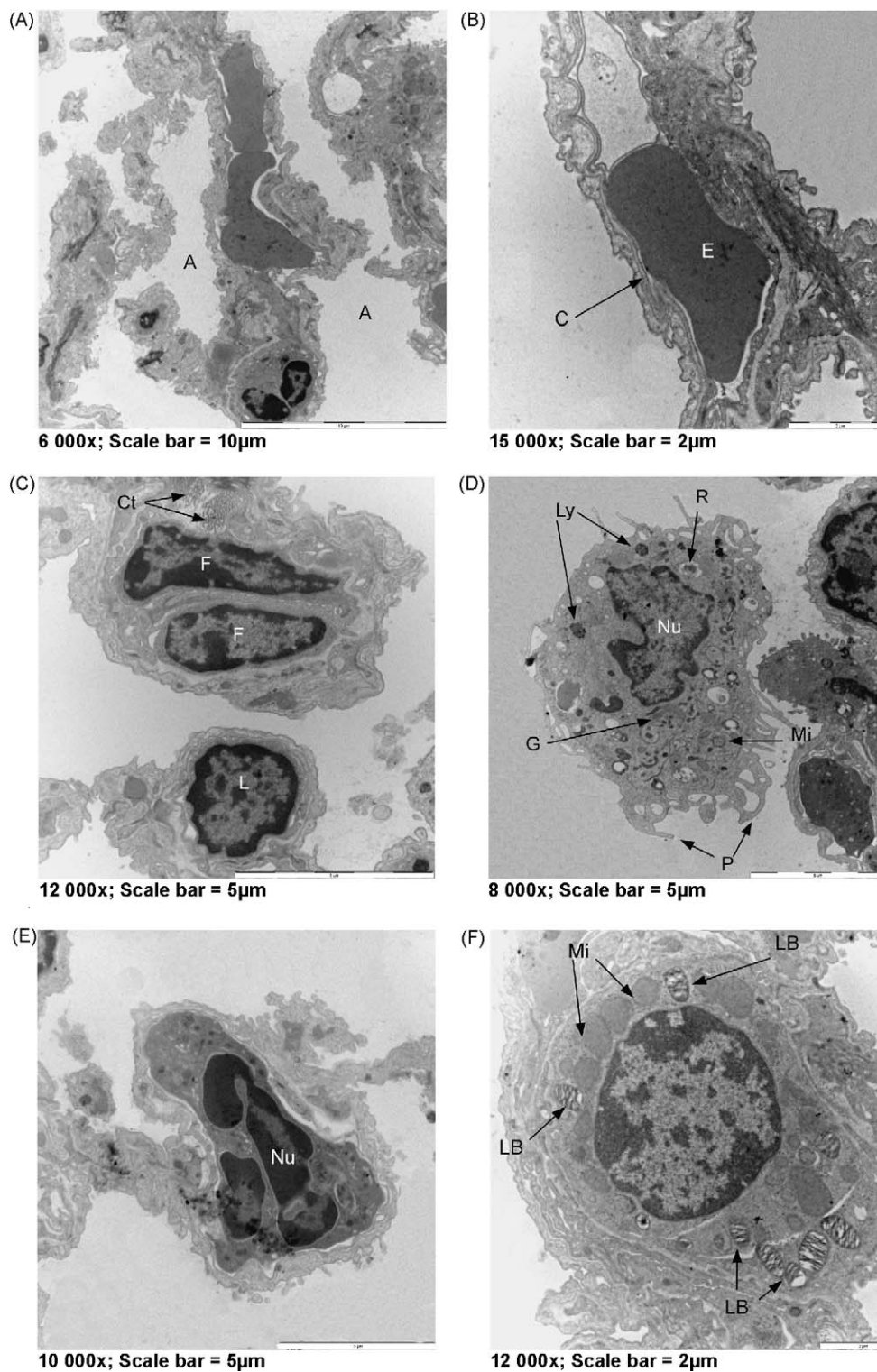


Fig. 4. (A–F) Electron micrographs of the lungs taken from the Modul8[®]-treated group.

apparatus, lysosomes and residual bodies could be identified within the activated macrophage. Fig. 4E is a micrograph of a typical neutrophil with its lobed nucleus which was found in the Modul8[®] group. Fig. 4F is a type II pneumocyte that was found in the Modul8[®] group. Many lamellar bodies could be identified as well as numerous mitochondria in the cytoplasm of this cell.

Micrographs from the hydrocortisone-treated group (Fig. 5A–D) revealed more or less the same ultrastructure as was found in the control and Modul8[®]-treated group. Fig. 5A shows the alveolar

spaces with very little inflammatory infiltrate present in the inter alveolar space. Fig. 5B is a micrograph of a capillary filled with red blood cells and Fig. 5C shows transverse and longitudinal collagen fibrils which appear similar to that found in the control and Modul8[®]-treated group, and were not as densely packed as could be observed in the asthmatic group. Fig. 5D also shows a type II pneumocyte that was found in the hydrocortisone group with numerous lamellar bodies and mitochondria present in the cytoplasm.

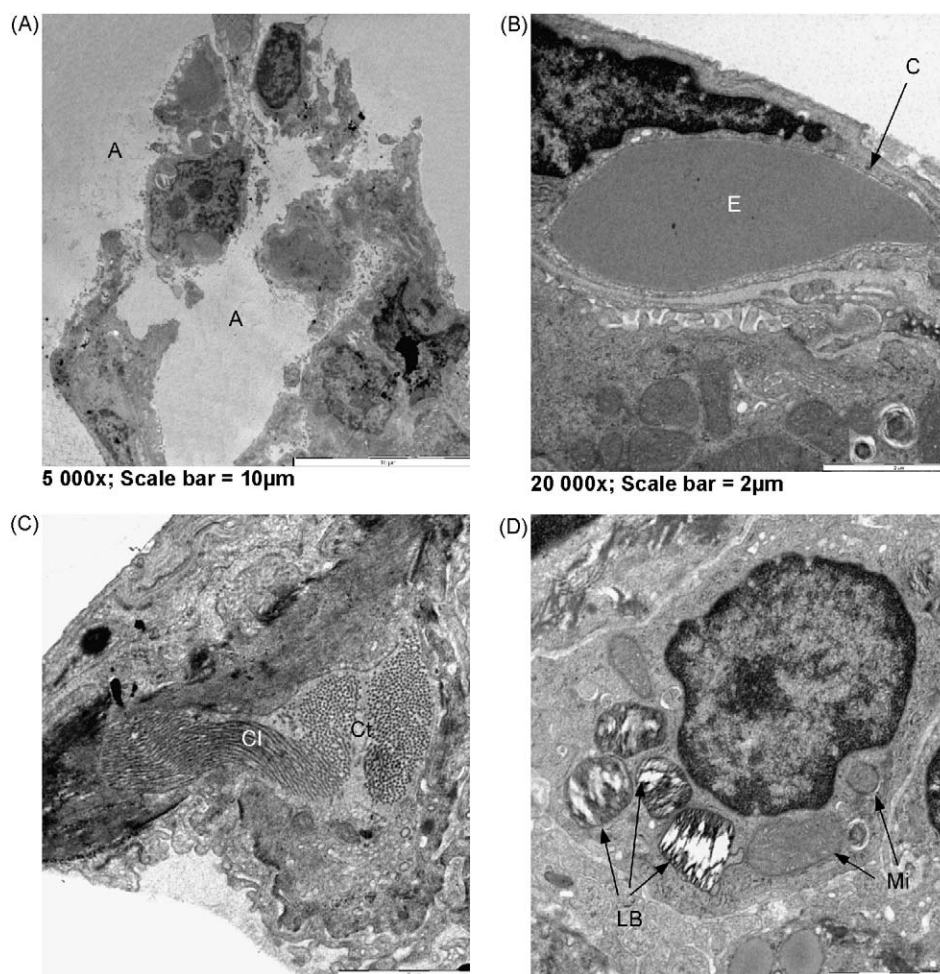


Fig. 5. (A–D) Electron micrographs of the lungs taken from the HC-treated group.

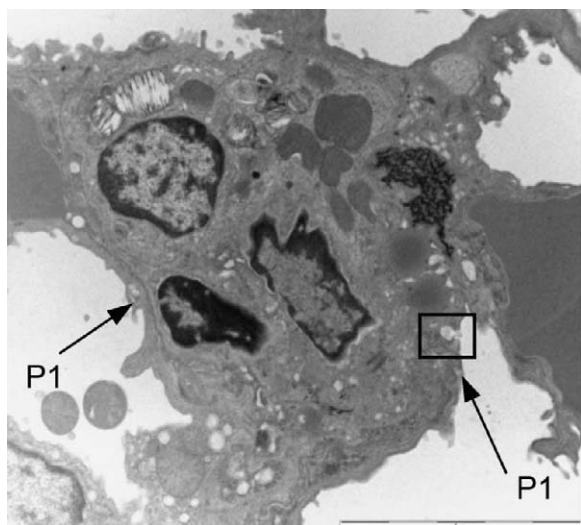


Fig. 6. Presence of possible caveolae in type I pneumocyte (10 000 $\times$; scale bar = 5 μ m). A, alveolus; BM: basement membrane; C, capillary; Cl, collagen fibrils in longitudinal section; Ct, collagen fibrils in transverse section; E, erythrocyte; En, endothelium; F, fibroblast; L, lymphocyte; LB, lamellar bodies; N, neutrophil; Nu, nucleus; M, macrophage; Ma, mast cell; Mi, mitochondria; Mo, monocyte; MV, microvilli; P, pseudopodia; P₁, type I pneumocyte; P₂, type II pneumocyte; R, residual bodies.

Fig. 6 is an electron micrograph taken from the asthmatic group where possible caveolae could be observed in the membrane indicated with the black rectangle. **Fig. 6** is a micrograph of a types I and II pneumocyte and the caveolae could be observed in the type I pneumocyte as indicated with the rectangle.

4. Discussion

Inflammatory conditions such as asthma involve a wide range of cell types and cellular mediators. The inflammatory cascade is a model that was developed to explain the complex process of inflammation. This model suggests that the inflammation associated with the asthmatic response occurs in seven different phases (Wenzel, 1994).

The process starts off with the *sensitization phase*, or the *antigen presenting phase*, which occurs as a result of antigen presentation to a T-lymphocyte. This phase involves a wide range of cells such as dendritic cells, monocytes and B lymphocytes. Upon delivering of the antigen to a T-lymphocyte, the latter responds by changing into an allergic T-Helper 2 or TH-2 cell and emits signals through a cytokine network. The interaction between the cytokines and the B-lymphocytes causes the B-lymphocytes to become plasma cells that produce IgE which binds to mast cells in order to bind the allergen and thereby completing the sensitization phase of the inflammatory cascade (Wenzel, 1994). This phase is followed by the *stimulation phase* where a number of factors play a role in

stimulating an exacerbation of the disease. These factors, such as allergens and environmental agents, act through the triggering of mast cells and IgE together with the triggered mast cells can cause long-term asthmatic inflammation whereas blocking of IgE can limit reaction to allergen and thereby decrease the associated inflammation (Wenzel, 1994).

The next phase in the inflammatory cascade is called the *cell signaling phase* in which the recruitment of inflammatory cells into the airways takes place. In this phase a number of cells, cytokines and markers play a role. These include T-lymphocytes, macrophages and monocytes, IL-4 and IL-13 and TNF- α . The cell signaling phase is followed by the *migration phase* where leukocyte migration into the airways takes place. Eosinophils, neutrophils, lymphocytes and monocytes are some of the cells involved, and this process is possibly mediated by the release of chemo-attractant mediators by the signaling cells.

Activation of the inflammatory cells follow in order to produce the physiological changes associated with asthma. IL-1, IL-5, TNF- α , eotaxin and IL-8 play a role in the activation of the inflammatory cells. Eosinophils are activated in the lungs of asthmatic patients (Wenzel, 1994). An increased number of eosinophils in bronchial lavage fluid of asthmatic animals have also been reported which emphasizes the important role of eosinophils in the inflammatory process associated with asthma (Oberholzer et al., 2008).

One of the characteristics of asthma is the *tissue alteration*. This takes place in the epithelium, basement membrane, smooth muscles and nerves. The changes that occur in the epithelium of asthmatics include abnormal epithelium with the presence of increased mucus-secreting cells. The sub-epithelial basement membrane appears thickened due the action of the eosinophils. The connective tissue associated has an increased collagen deposition. The smooth muscle may appear hyperplastic as well as hypertrophic. The last phase in the inflammatory cascade includes the resolution phase where the hypothesis exists that abnormal or incomplete resolution of the inflammation may play a role in the disease and its severity (Wenzel, 1994).

In 2003, Evans et al. reported that murine models of asthma exhibit a TH2 lymphocyte-driven eosinophilic pulmonary inflammation as well as airway hyperresponsiveness that are very important features of the inflammatory process involved in human asthmatics (Evans et al., 2003). One of the most prominent features of asthma is eosinophil infiltration of the peribronchial regions that is one of the characteristics of airway inflammation (Bousquet et al., 1990; Holgate et al., 1991; Kay, 1991) and eosinophil numbers correlate with the severity of the disease (Corrigan and Kay, 1992).

In asthma, it is known that there is an increase in the total number of white blood cells in the bronchial lavage, but especially the distribution of the cells is changed with an increase in the number of eosinophils as was previously found by Oberholzer et al. (2008). It was also found previously that typically in bronchial lavage of control mice, up to 90% of the cells are monocytes (Larsen et al., 2007). This was also found in the current study as indicated in Graph 1 with the control group possessing a high monocyte count. The presence of eosinophils in the lungs of allergic mice is an indication and confirmation of asthma-like eosinophilic inflammation (Gleich and Kita, 1997; Geba and Askenase, 1997). In the

current study this could also be observed in the lungs of the asthmatic animals (Fig. 1C and D and Fig. 3A) where it appeared congested with very little open spaces between the alveoli. However, the lungs of the treated animals (both Modul8[®] and hydrocortisone) (Fig. 1E and F, Fig. 4A, Fig. 1G and H and Fig. 5A) appeared similar to the control animals (Fig. 1A and B and Fig. 2A) with less severe influx of inflammatory cells and less congested with larger open spaces between the alveoli.

From the findings in the current study it can therefore be concluded that Modul8[®], histologically and ultrastructurally have the ability to stabilize the changes and remodeling caused in the lungs by the inflammatory process involved in asthma. Modul8[®] also significantly decreased the eosinophil counts in the bronchial lavage of the asthmatic animals. It is therefore suggested that Modul8[®] possesses anti-inflammatory properties and might therefore successfully be used in the treatment of asthma.

References

- Bousquet, J.P., Chanez, J.Y., Lacoste, G., Barneon, N., Ghavanian, I., Enander, P., et al., 1990. Eosinophilic inflammation in asthma. *N. Engl. J. Med.* 323, 1033–1039.
- Corrigan, C.J., Kay, A.B., 1992. T cells and eosinophils in the pathogenesis of asthma. *Immunol. Today* 13, 501–507.
- Evans, K.L.J., Bond, R.A., Corry, D.B., Shardonofsky, F.R., 2003. Frequency dependence of respiratory system mechanics during induced constriction in a murine model of asthma. *J. Appl. Physiol.* 94, 245–252.
- Geba, G.H., Askenase, H.W., 1997. Murine models of allergy, asthma and hyperresponsiveness. In: Kay, A.B. (Ed.), *Allergy and Allergic Diseases*. Oxford, Blackwell Science, pp. 1056–1067.
- Gleich, G.J., Kita, H., 1997. Bronchial asthma: lessons from murine models. *Proc. Natl. Acad. Sci.* 94, 2101–2102.
- Hamelmann, E., Takeda, K., Schwarze, J., Vella, A.T., Irvin, C.G., Gelfand, E.W., 1999. Development of eosinophilic airway inflammation and airway hyperresponsiveness requires interleukin-5 but not immunoglobulin E or B lymphocytes. *Am. J. Respir. Cell. Mol. Biol.* 21, 480–489.
- Holgate, S.T., Roche, W.R., Church, M.K., 1991. The role of the eosinophil in asthma. *Am. Rev. Respir. Dis.* 143, 66–70.
- Kumar, R.K., Foster, P.S., 2002. Modeling allergic asthma in mice: pitfalls and opportunities. *Am. J. Respir. Cell. Mol. Biol.* 27, 267–272.
- Larsen, S.T., Hansen, J.S., Hansen, E.W., Clausen, P.A., Nielson, G.D., 2007. Airway inflammation and adjuvant effect after repeated airborne exposures to di-(2-ethylhexyl) phthalate and ovalbumin in BALB/c mice. *Toxicology* 3, 119–129.
- Leigh, R., Ellis, R., Wattie, J., Southam, D.S., De Hoogh, M., Gauldie, J., et al., 2002. Dysfunction and remodeling of the mouse airway persist after resolution of acute allergen-induced airway inflammation. *Am. J. Respir. Cell. Mol. Biol.* 27, 526–535.
- Kay, A.B., 1991. Eosinophils and asthma. *N. Engl. J. Med.* 324, 1514–1515.
- Oberholzer, H.M., Pretorius, E., Smit, E., Ekpo, E., Humphries, P., Auer, R.E., Bester, M.J., 2008. Investigating the effect of *Withania somnifera*, selenium and hydrocortisone on blood count and bronchial lavage of experimental asthmatic BALB/c mice. *Scand. J. Lab. Anim. Sci.* 35 (4), 239–248.
- Pereira, W.K.V., Lonardoni, M.V.C., Grespan, R., Caparroz-Assef, S.M., Cuman, R.K.N., Bersani-Amado, C.A., 2005. Immunomodulatory effect of Canova medication on experimental *Leishmania amazonensis* infection. *J. Infect.* 51, 157–168.
- Persson, C.G., Erjefalt, J.S., Korsgren, M., Sundler, F., 1997. The mouse trap. *Trends Pharmacol. Sci.* 18, 465–467.
- Piemonte, M., Buchi, D., 2002. Analysis of IL-2, IFN- γ and TNF- α production, $\alpha 5\beta 1$ integrins and actin filaments distribution in peritoneal mouse macrophages treated with homeopathic medicament. *J. Submicrosc. Cytol. Pathol.* 34, 255–263.
- Ross, M.H., Kaye, G.I., Pawlina, W., 2003. *Histology: A Text and Atlas*. Lippincott Williams & Wilkins, Philadelphia, p. 588.
- Tomioka, S., Bates, J.H., Irvin, C.G., 2002. Airway and tissue mechanics in a murine model of asthma: alveolar capsule vs. forced oscillations. *J. Appl. Physiol.* 93, 263–270.
- Uller, L., Lloyd, C.M., Rydell-Tormanen, K., Persson, C.G., Erjefalt, J.S., 2006. Effects of steroid treatment on lung CC chemokines, apoptosis and transepithelial cell clearance during development and resolution of allergic airway inflammation. *Clin. Exp. Allergy* 36, 111–121.
- Wenzel, S.E., 1994. Asthma as an inflammatory disease. *Ann. Allergy* 72, 261–271.