ERJ Express. Published on March 28, 2007 as doi: 10.1183/09031936.00011606

KGF improves alterations of lung permeability and bronchial epithelium in allergic rats

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Running Head: KGF improves lung permeability in asthma

Key Words: asthma, inflammation, growth factor, lung permeability, rat.

Word count: 3581

Abstract

Chronic allergic asthma is associated with marked inflammatory reaction, microvascular leakage and epithelium injury. As previously shown in a rat model of chronic asthma, these alterations increased lung permeability and distal airway fluid clearance. Keratinocyte growth factor (KGF) has been shown to induce epithelial cell proliferation and to protect from acute lung injuries. Therefore, we evaluated the potential role of KGF treatment on lung permeability and airway inflammation in rats with chronic asthma.

KGF (1mg/kg) was administered intravenously, before the last ovalbumin (OVA) challenge in sensitized rats. Permeability was assessed by the leak of radiolabeled albumin from the alveolar and the systemic compartments. Histopathologic analysis was also performed.

Treatment with KGF decreased the leak of both markers and the level of extravascular lung water in sensitized rats challenged with OVA. KGF treatment also reduced the inflammatory cell number in bronchoalveolar lavage but not in bronchial mucosa. KGF markedly limited the allergen-induced alterations in epithelium integrity and expression of the intercellular junction proteins: β-catenin and ZO-1.

In conclusion, KGF administration markedly limits lung permeability and airway inflammation, an effect associated with a decrease in epithelium alterations during chronic allergic asthma. These data open new prospects in the therapeutic strategy of asthma.

Introduction

Microvascular leakage represents a classical feature in pathogenesis of asthma [1]. Many authors previously showed that an active exsudation involving both the epithelial layer and lamina propria of the bronchial wall associated with hyperplasia of smooth muscle is responsible for the airway obstruction [2-4]. The mechanisms responsible for this exsudation are probably multifactorial but certainly involve the increase in epithelium and endothelium permeability [5]. We recently studied lung permeability and lung fluid movement in chronic bronchial allergic inflammation and showed that the increased permeability is associated to a TNF-α dependent upregulation of distal airway fluid clearance [6]. The modulation of this response could represent an attractive research pathway in asthma treatment. Indeed, alveolar epithelial repair is critical in acute lung injury and the acceleration of this process at an early stage could represent a major therapeutic target.

Keratinocyte Growth Factor (KGF) is a heparin binding growth factor [7] which has been shown to have a protective effect in acute lung injury [8, 9]. KGF stimulates both *in vitro* and *in vivo* alveolar type II cells proliferation and differentiation [10, 11]; its activity is first thought to be limited to epithelial cells [12]. This hypothesis was challenged by Gillis et al who showed that KGF induces neovascularization in the rat cornea and protect the endothelium from hydrogen peroxide toxicity [13]. The protective effect of KGF is multifactorial : we showed, in the α-naphthylthiourea-induced lung injury, a potentiation of alveolar fluid clearance through Na, K-ATPase stimulation [14]. In *P. aeruginosa* pneumonia, KGF restored lung liquid clearance related to an improvement of the endothelial barrier [15]. An anti-apoptotic activity of KGF is evidenced in hepatocytes after TNF-α injury [16] and finally, in oxidant lung injury, KGF protects alveolar epithelial cells through enhanced DNA repair and prevents the disruption of tight junctions [17, 18]. KGF also influences the inflammatory response: in acid aspiration lung injury, KGF pretreatment decreases MIP-2α

concentrations and neutrophil recruitment [19]; another group showed that KGF could decrease transcripts for many interferon-induced genes [20]. *In vitro*, KGF downregulates ICAM-1 and VCAM-1 expression on bronchial epithelial cells, leading to a decreased granulocyte adherence to these cells [21].

We therefore decided to evaluate the effect of KGF in the late stages of ovalbumin (OVA)-induced chronic asthma in brown-Norway rats. KGF was injected intravenously, three days in a row prior to the last ovalbumin challenge (out of 6). We evaluated the effect of this treatment on lung permeability, inflammatory response and on the epithelium alteration associated with the allergic reaction). We also measured the impact of KGF treatment on the inflammatory response and on the bronchial and alveolar repair 7 days after the last challenge.

Methods

1. Animals

Specific pathogen-free male Brown-Norway rats (280-300g, Centre d'élevage Dépré, St Doulchard, France) were housed in the Lille University Animal Care Facility and allowed food and water *ad lib*. All experiments were performed with approval of the Lille Institutional Animal Care and Use Committee. A total number of 44 rats were used for all the experiments.

2. Keratinocyte Growth Factor injection

For the intravenous (IV) injection, animals were anesthetized with sevoflurane (Servorane™, Abbott, UK), and the injection was performed in the dorsale penile vein, once a day three days in a row starting 3 days prior to the experimental study. Either KGF (recombinant human KGF 1mg/kg in 1 ml PBS, Amgen, Thousand Oaks, CA) or an equal volume of isotonic saline was injected. The animal was then allowed to recover. Effective dose was established in models of lung injury [14,15].

3. Immunization and exposure to ovalbumin

Rats were immunized to OVA on the first and the fifteenth days, by intraperitoneal injection with 10μg OVA associated with 1.5×10^{10} heat-killed bordetella pertussis organisms and 1 mg Al_2O_3, (Vaxicoq; Institut Mérieux, Lyon, France)) [22]. Control animals were injected on day 1 and 15 with 0.5 ml of sterile isotonic saline.

Animals were exposed for 20 min to aerosolised OVA (1%weight for volume), or sterile saline, in an inhalation chamber [22]. The aerosol was generated into the chamber with an ultrasonic nebuliser (Pariboy, Starnberg, Germany). Rats were exposed to a total of 6 nebulisations with OVA solution or sterile saline for controls, for the first time on day 21 (6 days after the last OVA intraperitoneal injection) and on days 25, 29, 33, 37 and 41.

4. Histopathology

Lung and bronchial samples from each group were analysed. The sample was fixed in formaldehyde and embedded in paraffin. Paraffin sections were stained with Haematoxylin eosin (HE) and by May-Grünwald Giemsa (MGG) (Sigma-Aldrich) for analysis of inflammatory cells. Cells were counted (eosinophils, neutrophils and mononuclear cells) by two independent observers and expressed by high power field (HPF: x100). Four fields, preferentially selected on peribronchial, peribronchiolar and perivascular areas, were evaluated for each section. The epithelial integrity was also determined using computer assisted-morphometry (IM500, Leica Microsystems, Heerbrugg, CH) as the percentage of bronchial epithelium length with desquamated (lack of epithelial cell onto the basement membrane) or damaged (shrinkage, thinning down, detachment) epithelium.

Immunohistochemistry analysis for β-catenin and zonula occludens (ZO)-1, two adaptor proteins required for the formation of adherens and tight junctions, respectively, was performed on paraffin-embedded lung sections. First, sections were deparaffinised and treated for Ag retrieval by incubation at 90°C for 20 min in citrate buffer. Then, the sections were incubated with rabbit IgG, anti-β-catenin (Santa-Cruz Biotech., San Diego, Ca) and anti-ZO-1 (Zymed, Invitrogen, Paisley, UK) at the concentration of 2 µg/ml in PBS with 2% fetal calf serum. After washings, the binding was detected by incubation with biotinylated anti-rabbit IgG goat IgG (Sigma-Aldrich) and then with the ABC-alkaline phosphatase kit (Vector, Burlingame, CA). Enzymatic activity was revealed in red with Fast Red and sections were counter-stained with haematoxylin (Sigma-Aldrich).

5. In vivo measurement of the alveolar permeability

Surgical preparation and ventilation

As previously described [15], Brown Norway male rats were anesthetized with pentobarbital (Sanofi, Libourne, France). A catheter (PE-50) was inserted into the left carotid artery to monitor systemic arterial pressure and obtain blood samples. Pancuronium bromide (0.3 $mg.kg^{-1}.h^{-1}$ iv) was given to achieve neuromuscular blockade. An endotracheal tube (PE-220) was inserted through a tracheostomy. The rats were ventilated with a constant volume pump (Harvard Apparatus, South Natick, MA) with an inspired O_2 fraction of 1.0, peak airway pressures of 8-12 cmH_2O, and a positive end expiratory pressure of 2 cmH_2O.

Preparation of instillate

The test solution that was used for alveolar instillation was prepared as follows : briefly, a 5% bovine albumin solution was prepared using Ringer lactate [6, 15, 23]. We added 1µCi of ^{125}I-labeled human serum albumin (^{125}I-HSA; CIS biointernational, Gif sur Yvette, France) to the 5% albumin solution. Also, anhydrous Evan's blue dye (0.5 mg) was added to confirm the location of the instillate at the end of the study. A sample of the instilled solution was saved for total protein measurement, radioactivity counts, and water to dry weight ratio measurements.

General protocol

For all experiments, the following general protocol was used. After the surgical preparation, heart rate and blood pressure were allowed to stabilize for 1 hour. The rat was then placed in left lateral decubitus position (to facilitate liquid deposition into the left lung).

To calculate the flux of plasma protein into the lung interstitium, a vascular tracer, 1µCi of ^{131}I-labeled human albumin, was injected into the bloodstream [6, 23]. ^{131}I-HSA was prepared in our institution according to a standardized technique [24]. To calculate the flux of protein from the airspaces into the circulating plasma, 3ml/kg of the 5% bovine albumin solution with

1μCi of ^{125}I-albumin were instilled 30 min later into the left lung over a 10-min period, using a 1-ml syringe and polypropylene tube (0.5 mm ID) [25].

One hour after the beginning of the alveolar instillation, the abdomen was opened, and the rat was bled. Urine was sampled for radioactivity counts. The lungs were removed through a sternotomy, and fluid from the distal airspaces was obtained by passing a propylene tube (0.5 mm ID) into a wedged position in the left lower lobe. The total protein concentration and the radioactivity of the liquid sampled were measured. Right and left lungs were homogenized separately for water to dry weight ratio measurements and radioactivity counts.

Hemodynamics, pulmonary gas exchange, and protein concentration

Systemic arterial pressure and airway pressures were measured continuously. Arterial blood gases were measured at one hour intervals. The arterial PO_2 was used to quantify the oxygenation deficit [6, 23, 26]. Samples from instilled protein solution, from final distal airspace fluid, and from initial and final blood were collected to measure total protein concentration with an automated analyzer (Hitachi 917, Japan).

Albumin flux across endothelial and epithelial barriers

Two different methods were used to measure the flux of albumin across the lung endothelial and epithelial barriers. The first method measures residual ^{125}I-albumin (the alveolar protein tracer) in the lungs and accumulation of ^{125}I-albumin in plasma. The second method measures ^{131}I-albumin (the vascular protein tracer) in the extravascular spaces of the lungs as previously described [6].

6. Bronchoalveolar lavage (BAL)

Lungs from each experimental group were lavaged with a total of 30 ml of PBS with 3mM EDTA in 5 ml aliquots. Total and differential cell count was determined after MGG staining by counting 300 cells/sample and results were expressed for each cell type as the percentage of total cell number.

7. Experimental Protocols

The following experimental groups were studied for analysis of permeability and pathological analysis:

- Group (C): rats immunized and challenged by sterile saline, analysed at Day 43 (n =9).
- Group (Se): rats immunized with OVA and sham-challenged, analysed at Day 43 (n=9).
- Group (OVA): rats immunized and challenged with OVA, analysed at Day 43 (n=9).
- Group (KOVA): rats immunized and challenged with OVA, analysed at Day 43, KGF was administered intravenously: 1mg at day 38, 39, and 40 (n=9).

Two additional experimental groups were devoted only to histological analysis:

- Group (ROVA): rats immunized and challenged with OVA, analysed at Day 50 (n=4).
- Group (KROVA): similar to the KOVA, but analysed at Day 50 (n=4).

In vivo studies to evaluate permeability of the alveolo-capillary barrier to proteins were performed 24 hours after the last nebulization on each of the first four groups. Pathological analysis and BAL were obtained with the same timing.

8. Statistical Analysis

Results are presented as mean ± SEM. Data were analyzed using the Kruskal-Wallis test and the Mann Whitney test where appropriate. p values less than 0.05 were regarded as statistically significant.

Results

1. OVA sensitization is associated with an increase in epithelial and endothelial permeability

The OVA challenge in sensitized animals (OVA group) is associated with an increase of radio-labelled albumin flux both from the alveolar or the systemic compartments. In fact, we observed an increase of the leak of the alveolar protein tracer, ^{125}I-Alb, into the systemic circulation in the OVA group compared to both the (Se) and the control groups (Fig 1). Similarly, the evaluation of endothelial permeability using the leak of the vascular tracer, ^{131}I-Alb, into the alveoli was consistent with those results (Fig 1). The interstitial compartment was significantly increased in the OVA group compared with the sensitized animals, as reflected by the increase of total interstitial plasma equivalent and lung wet to dry weight ratio (Table 1). These results are comparable to our previous data [6].

2. KGF pre-treatment improves alterations of permeability observed after OVA sensitization and challenge test.

Administration of KGF is associated with a decrease of the leak of both markers, ^{125}I-Alb and ^{131}I-Alb in respectively the systemic and alveolar compartment. The interstitial plasma equivalent and the lung wet to dry weight ratio were also significantly lower after KGF treatment compared to the OVA group (100 and 65 % inhibition, respectively) (Table 1).

3. KGF pre-treatment inhibits lymphocyte and neutrophil recruitment in the BAL.

Compared to control animals, OVA challenge leads to the development of a peribronchial inflammation (Fig 2A-B) and a leucocyte cell influx in the BAL (Fig 3). This cell infiltrate was mainly composed of lymphocytes and granulocytes in the BAL from OVA rats (Fig 3). In addition, the number of eosinophils and mononuclear cells was significantly higher in OVA rats compared to control or to sensitized rats ($p< 0.05$; table 2). OVA challenge induced

epithelial lesions as illustrated by their shedding in the BAL. In the KOVA group, KGF administration significantly decreased both lymphocyte and neutrophil counts in the BAL compared to OVA (Fig 3), whereas there was no significant effect on peribronchial infiltrates (Fig 2C-D and table 2). Surprisingly, KGF did not affect the OVA-induced recruitment of eosinophils in the BAL and bronchial mucosa. Moreover, histopathologic analysis performed 7 days after the last challenge showed that a bronchial inflammatory infiltrate mainly composed of mononuclear cells persisted at a similar level in both rats from the ROVA and KROVA groups; although the difference was not statistically different with the control rats. In the BAL, no significant inflammatory cell infiltrate was detected in both ROVA and KROVA groups.

4. KGF limits epithelium alterations

Since KGF targets mainly epithelial cells, we focused on the effect of this treatment on epithelial lesions associated with asthma. OA challenge in sensitized animals induced epithelial damages in the bronchial epithelium as shown in fig 2A-B (24 h after the last inhalation). KGF treatment limited these epithelial alterations and restored the epithelial integrity (Fig 2D-E). Indeed, only 4.3% the epithelial surface was altered in the KOVA group compared with 43.4 % in the OVA group ($p<0.05$). Moreover, a marked decrease of perivascular oedema was observed after KGF administration (Fig2G-H).

In addition, 7 days after the last challenge, there was a restoration of bronchial epithelium in KROVA group (7 % damaged epithelial) (Fig 2F) whereas restrained epithelial alterations persisted in the ROVA group (24 % damaged epithelium).

5. Expression of protein belonging to the intercellular junctions.

The expression of intercellular junction proteins, β-catenin and ZO-1 was evaluated by immunohistochemistry. Challenges with OVA in sensitized rats decreased the level of expression for both proteins in epithelial cells in comparison with control rats (Figure 4). KGF treatment strongly increased the level of β-catenin and ZO-1 expression in both the epithelial cells and the vascular walls as compared with OVA rats. Some cells within the bronchial mucosa are also positive for both proteins in control and KOVA rats. The expression of ZO-1 and β-catenin in KROVA was similar to that observed in KOVA group whereas in ROVA, the intensity of staining is intermediate between OVA and KOVA (data not shown). No staining was detected with the control IgG (Fig 4).

Discussion

Since microvascular leakage and epithelial alteration represent an important feature in the pathogenesis of severe asthma, we evaluated the effect of the treatment with a factor able to facilitate airway mucosa repair like KGF. In the present study, we demonstrated that KGF improves OA-induced increase in alveolar permeability in our model of allergic bronchial inflammation in Brown Norway rats. Moreover, the decrease in epithelial and alveolar permeability is associated with an improvement in epithelial alterations and a decrease in neutrophil and lymphocyte influx in the BAL.

Epithelial damage is a characteristic feature of asthma [1]. The epithelium is a barrier but also generate mediators that play a key role in the inflammatory response and remodelling in asthma. Many growth factors such as Hepatocyte Growth Factor (HGF), Granulocyte Macrophage Colony-Stimulation Factor (GM-CSF), Transforming Growth Factor (TGF)-β, the Epithelial Growth Factor (EGF) are implicated in this process. EGF stimulates epithelial cell proliferation and migration. KGF, also called FGF-7, belongs to the family of the fibroblast growth factor and predominantly stimulates the proliferation of epithelial cells [7, 11, 12, 27, 28]. In the lung, KGF has been demonstrated to be a potent mitogen of alveolar epithelial type II cells, but also bronchial epithelial cells *in vitro* and *in vivo* [10, 11, 29]. The protective effect of KGF is demonstrated in different situations, including the prevention of cell loss but also the epithelium repair after injury [14-17].

In asthma, little is known about the role of KGF. We demonstrated in chronic asthma that the treatment by KGF before the last ovalbumin nebulisation was associated with a decrease of the leak of epithelial and endothelial markers. The effect of KGF on the permeability of airway epithelial cell has already been investigated by Waters et al [30]. Indeed, KGF reduces the basal albumin flux and prevents the increase in permeability induced by H_2O_2 on airway epithelial cell monolayers. This effect is probably due to changes in

paracellular permeability, but not to cell proliferation since the reduction in epithelial permeability was rapidly observed (1h after KGF addition). One mechanism could be due to the KGF-induced stabilisation of the tight junctions in this model [18]. In our study, KGF was administered 3 days consecutively before the last challenge, a period which should be sufficient to restore bronchial epithelium in rats. KGF was injected after the fifth challenge with OVA, when important epithelial lesions were observed in the bronchial mucosa of sensitized rats. As shown in fig 2, treatment with KGF markedly limits the alteration of the epithelial barrier with an improvement of the permeability increase. However, with this method, we cannot distinguish whether this increase of permeability is related to large or small airways. Both sites are probably involved since KGF treatment clearly induced small and large bronchial epithelial repair. Michelson et al. also showed *in vitro* and *in vivo* that KGF induced bronchial epithelial cell proliferation in rats, with a dose-dependent increase in DNA synthesis [31]. Since our effect was observed after 3 days treatment, cell proliferation was probably involved in epithelial regeneration in our model. Moreover, both prevention of tight junction disruption and bronchial cell proliferation may act together to limit the increase in lung epithelial permeability induced by allergen. Indeed, we observed that KGF prevents the OVA-induced decreased expression of both ZO-1 and β-catenin, two adaptors playing a key role in the formation of tight and adherens junctions, respectively. We also identified their expressions in cell infiltrate within the bronchial mucosa; this might be due to their transient expressions by some inflammatory cells as previously reported [32,33]. One other function has been assigned to β–catenin i.e. that of being a co-activator of transcription factors Lef/Tcf, a process implicated in cell proliferation and in production of metalloproteases [34]. ZO-1 might also recruit signalling proteins that participate in the regulation of cell proliferation and differentiation [35]. Additional experiments are required to determine if KGF acts at the transcriptional or at the translational level, and to determine if this KGF-

induced upregulation is associated with a reformation of adherens and tight junctions. Moreover, epithelium restitution was achieved at day 7 after the last challenge although an inflammatory cell infiltrate persisted within the mucosa. So, these data showed that KGF is able to repair epithelial damages and to limit the effect of OVA challenge in our experimental model.

We did not evaluate the anatomic level of liquid clearance. Most data obtained with the same procedure (labelled tracers) for evaluating the epithelial and alveolar permeability are issued from the ARDS and pneumonia experimental models. The pneumocytes II seem to be the main cells involved in fluid movement [14,15,23]. Nevertheless, other data obtained in asthmatics showed that cells issued from bronchiolar mucosa are also potentially involved [1,3]. In our rat model, injection of 1ml in one lung did not allow to localize the cells involved in liquid clearance.

Endothelial permeability increase is also limited by KGF treatment in asthmatic rats. There are little data concerning the implication of KGF in endothelial cell proliferation. *In vivo,* KGF induces neovascularization in the rat cornea [13]. Moreover, it blocks the increase in permeability induced by hydrogen peroxide and vascular endothelial growth factor/vascular permeability factor (VEGF/VPF) in monolayers of microvascular but not aortic endothelial cell [13]. KGF is also capable to induce angiogenesis and to stabilize endothelial barriers in microvascular tissue. In an *ex vivo* model of hydrostatic pulmonary oedema, KGF attenuates the injury via mechanisms that prevent increased in alveolar-capillary permeability showing its implication in endothelial permeability [36]. In the model of allergic rats, these data suggest that the action of KGF may implicate a direct effect on endothelial permeability. Since administration of KGF limits lung permeability increase and epithelial alterations associated with repeated OVA challenge in sensitized rats, we can hypothesize that this treatment affects airway inflammation. Whereas the infiltrate within the bronchial mucosa is

not modified, neutrophil and lymphocyte counts decrease in the BAL after KGF administration in rats challenged with OVA. Indeed, KGF has the capacity to limit the production of some chemokines [19]. Just et al demonstrated, *in vitro*, that KGF down regulates the expression of adhesion molecule on epithelial cells, this effect being associated with a decrease in neutrophil adherence [21]. In addition, the increased expression of both ZO-1 and β-catenin may also limit the leucocyte transmigration through the potential reformation of intercellular junctions within the bronchial epithelium.

In conclusion, our data show that administration of KGF limits lung permeability increase in our rat model of chronic allergic asthma and limit OVA-induced epithelial damages. However, this treatment has a restrained effect on the inflammatory infiltrate within the lung. These observations suggest that such a therapeutic strategy designed to restore the epithelial and the endothelial barrier integrity may have a great potential to limit the consequences on lung functions of increased permeability in severe asthma. Further studies are necessary to define whether the effects of KGF might reduce at long term the inflammatory reaction and/or the allergen-specific immune response.

Acknowledgements : The authors would like to thank Dr Johan Kips for the help and support he provided in the elaboration of this work. We are also very grateful to Pr Xavier Marchandise, Pascal Briche, and Nathalie Zenani for their daily encouragements.

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Table 1. Effect of KGF pretreatment on wet to dry lung weight ratio, and intertistial plasma equivalent.

Group	W/D weight ratio	Interstitial Plasma Equivalent (μL)
C	3.31±0.28	0.003±0.02
Se	3.43±0.22	0.06±0.02
OVA	4.34±0.29*	0.103±0.05*
KOVA	3.75±0.31	0.03±0.018

* Statistically different from the other groups, $p<0.05$

Table 2 : Modulation by KGF pretratment of leucocyte infiltrate in bronchial mucosa from allergic rats. KGF was administered before the last exposure to OVA except in control rats. Leucocyte infiltrate was measured 48h (OVA and KOVA) or 7 days after the last challenge (ROVA and KOVA) and expressed as mean +/- SD of 10^{-2} cell number / mm2.

	Mononuclear cells	*Neutrophils*	*Eosinophils*
Control	33.2±17.1	6±3.32	1.8±2.68
Sensitized	38.83±8.93	7.17±3.2	3.5±2.6
OVA	70.83±10.8*	7.75±4.85	13.8±7.73*
KOVA	67.33±17.2*	8.5±4.64	9.67±4.23*
ROVA	57.43±18.2	3.57±1.81	2.29±3.9
KROVA	56.29±22.7	4.43±3.05	1.57±1.62

*: $p < 0.05$ compared with the rats from the control or the sensitized group.

Figure Legends

Figure 1. Measurement of the epithelial permeability assessed with the leak of the alveolar tracer (^{125}I-Alb) in the systemic compartment (Blood 125I-alb) expressed as a percentage of the total instilled radioactivity. Measurement of the endothelial permeability expressed as the ratio of the systemic tracer (^{131}I-Alb) recovered in the BAL, to the systemic count (Asp/Plasma). *: $p < 0.05$ compared with both Sens and Ctr groups; #: $p < 0.05$ compared with OVA..

Figure 1

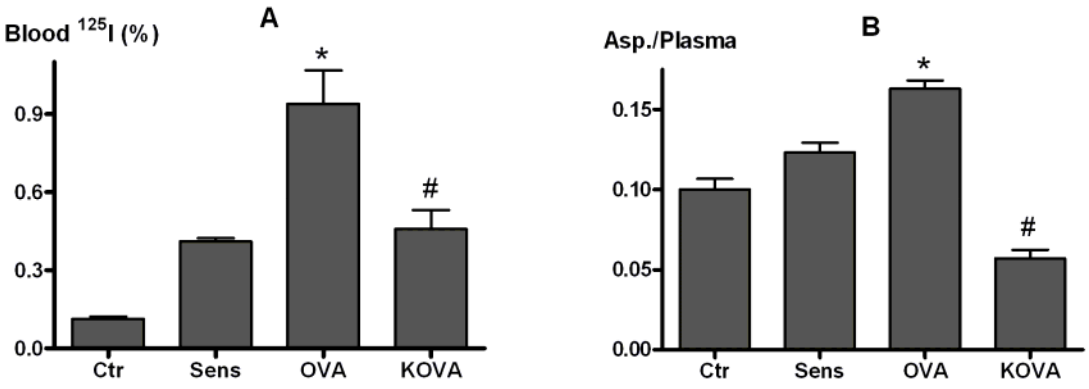


Figure 2. Effect of KGF treatment on the peribronchial inflammatory infiltrate and on bronchial epithelium alteration in rats sensitized and challenged with ovalbumin. Paraffin-embedded sections from OVA (A and B), Sens (C), KOVA (D and E) and KROVA (F) rats were stained with hematoxylin-Eosin. KGF treatment did not markedly modify the inflammatory infiltrate (black arrows) but inhibited the epithelial lesion observed in OVA rats (arrowheads) and allowed to restore nearly undamaged epithelium in KOVA rats (part D and E) and 7 days after the last challenge (part F) (magnification: x 300). KGF also decreases

perivascular oedema (see the stars, part G: group OVA versus part H: Group KOVA; magnification: x 500).

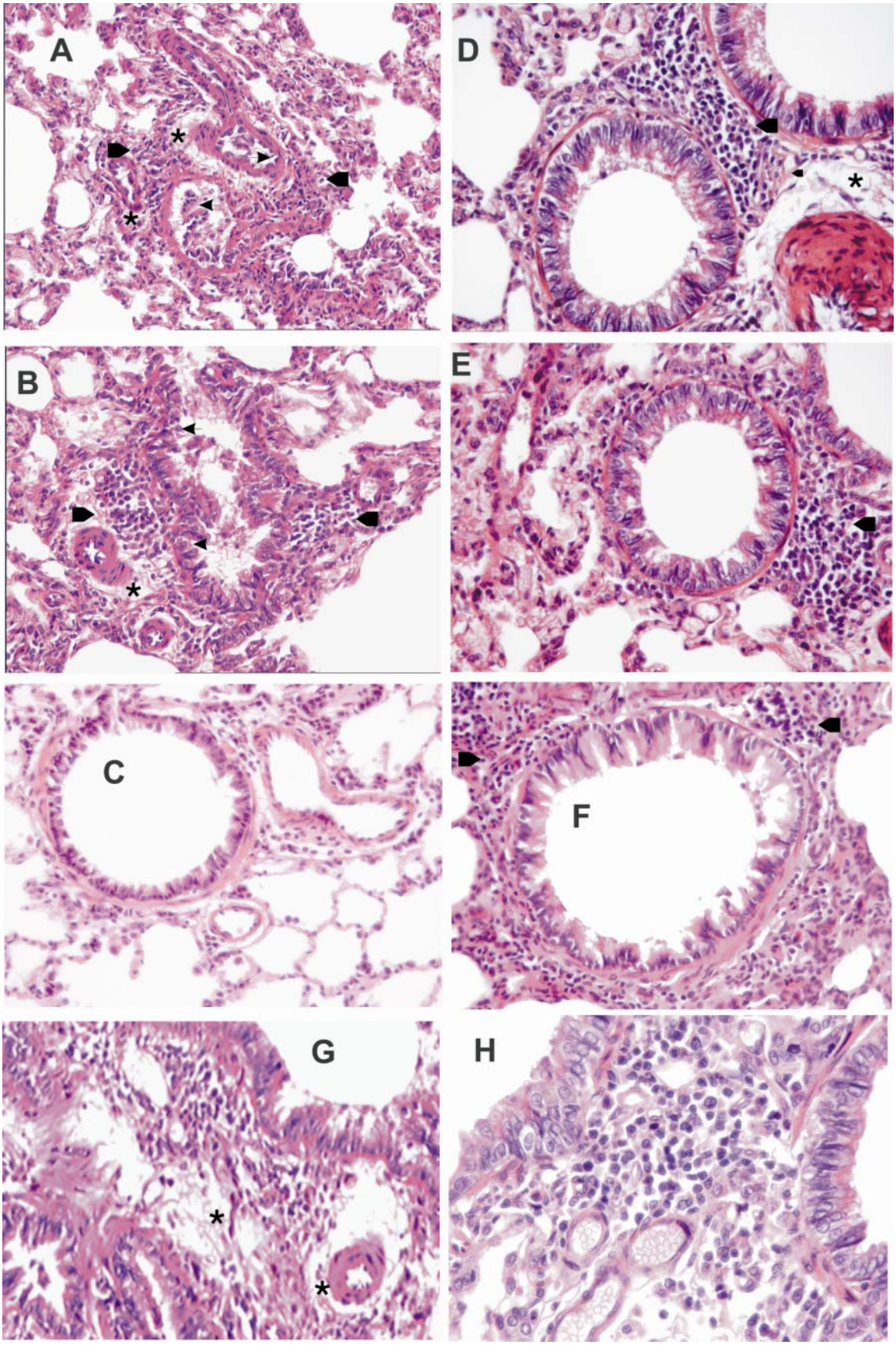

A
D
B
E
C
F
G
H

Figure 3. Differential cell count analysis in the BAL from rats sensitized and challenged with saline serum (Sens), challenged with Ova (OVA), and treated with KGF (KOVA) collected 24h after the last challenge. BAL procedure was also performed 7d after the last challenge in rats treated with KGF (KROVA) or not (ROVA). Treatment with KGF significantly reduced the OVA-induced influx of lymphocytes, and neutrophils and tended to increase the number of BEC. Results were expressed in percentage (upper part of the figure) or in absolute counts (lower part). *: $p < 0.05$ compared with both Sens and Ctr groups; #: $p < 0.05$ compared with OVA.

Figure 3

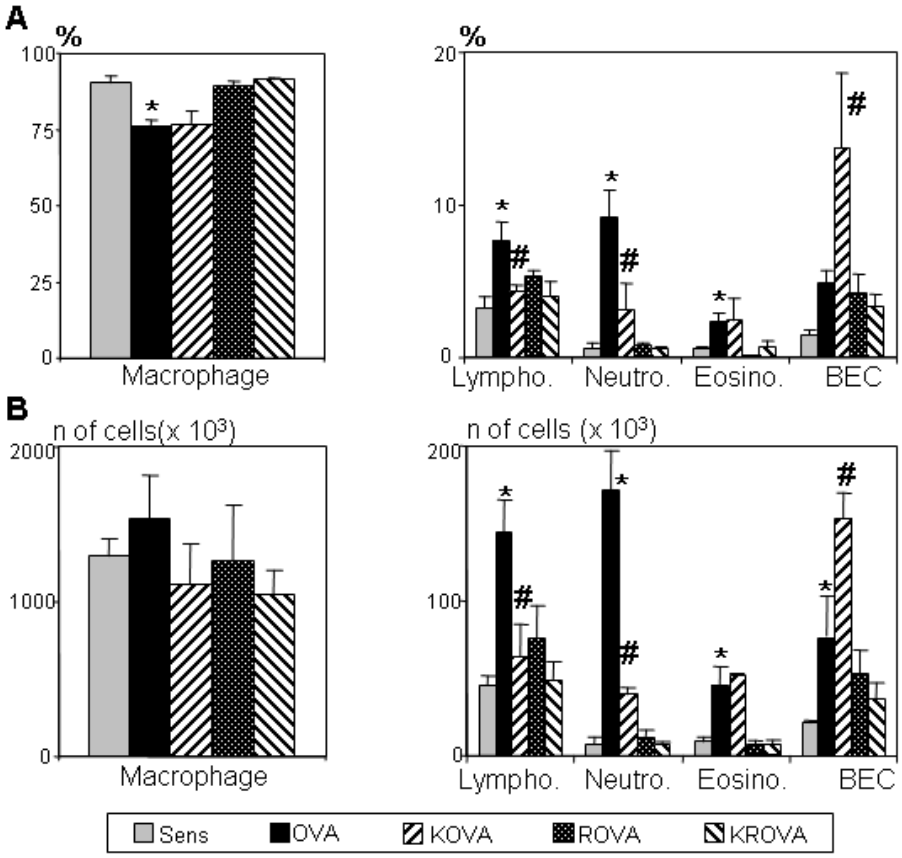
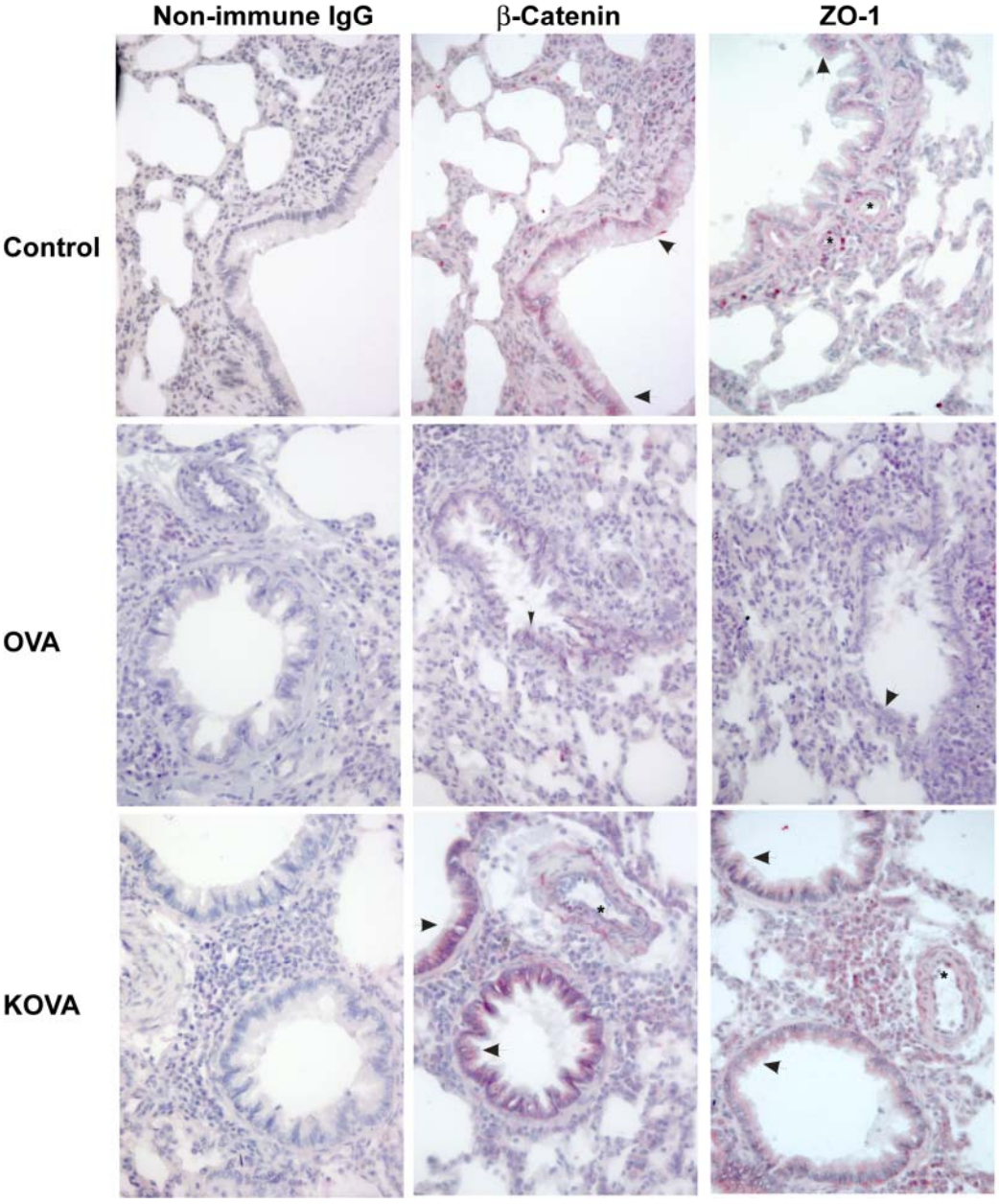


Figure 4: Evaluation of the expression of the intercellular junction proteins, β-catenin and ZO-1 by immunohistochemistry. Lung sections from rats belonging to the groups of controls,

OVA and KOVA were analyzed. Positive staining was detected in red and the sections were counterstained with haematoxylin. Both β-catenin and ZO-1 expression in epithelial cells (black arrow) and vascular wall (star) was expressed in control and KOVA group whereas there is a faint staining in OVA rats. Positive cells were also present within the inflammatory infiltrate. No staining was detected with the non-immune rabbit IgG (magnification: x 300).


Non-immune IgG
β-Catenin
ZO-1
Control
OVA
KOVA