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Antibiotics counteract the worsening of airway remodelling induced by infections in asthma

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ABSTRACT

Asthma is associated with structural remodelling processes, including basement membrane thickening, increased vascularity and smooth muscle alterations. It is known that respiratory infections are associated with asthma exacerbation; infections can worsen asthma symptoms and influence susceptibility to asthma onset. How infections affect asthma is not fully elucidated. It is possible that the immune response, due to recurrent infections, leads to the pathogen's eradication but also increases bronchial inflammation, which induces airway remodelling in asthmatic subjects. We evaluated how infection affects lung remodelling and inflammatory responses and assessed the impact of antibiotic treatment in a murine model of asthma. Ovalbumin-sensitised BALB/c mice were divided into control, mild and chronic asthmatics. A subset of animals in each group was infected with *Streptococcus pneumoniae* and was treated with antibiotics. The results show an increase in key lung remodelling factors in mice with chronic asthma, particularly those infected with *S. pneumoniae*. Notably, antibiotic therapy attenuated these effects. These findings demonstrate for the first time that prompt antibiotic therapy may be useful to reduce lung remodelling progression in infected asthmatic subjects.

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1. Introduction

The World Health Organization (WHO) estimates that asthma affects up to 33% of children and 10% of adults in Westernised countries, and 180 000 people die from asthma every year. The disease is characterised by chronic and eosinophilic inflammatory airway responses; the immune response increases bronchial hyper-responsivity causing dyspnoea, wheezing and coughing.

As with many chronic inflammatory disorders, airway inflammation causes tissue injury that results in structural changes, including airway fibrosis, goblet cell hyperplasia, increased smooth muscle mass, extracellular matrix alterations and increased vascularity [1].

The increase in basement membrane thickness could be a consequence of epithelial damage, which involves continuous tissue repair via growth factor production that induces fibroblasts to proliferate and deposit collagen fibres [2]. A hallmark of asthma is marked smooth muscle cell proliferation, which results in an

increased muscular component. Woodruff et al. [3] demonstrated that the percentage of smooth muscle content in the bronchial wall of asthmatics is increased compared with non-asthmatics, with a 50–83% rise in the volume fraction of smooth muscle.

Some growth factors, such as fibroblast growth factor (FGF), are correlated with angiogenesis in bronchial asthma [4]. The resultant increase in vessel number contributes to wall thickening, which induces airway narrowing.

The causes of asthma include environmental, immunological and host genetic factors. Epidemiological studies have demonstrated a strong association between respiratory infections and asthma pathogenesis, but how the presence of micro-organisms impacts the airways of asthmatics has not been elucidated. In 1991, Hahn et al. [5] provided the first evidence of a direct association between chronic *Chlamydomydia pneumoniae* infection and asthma. Chu et al. [6] demonstrated that *Mycoplasma pneumoniae* infection induced fibrosis in a murine model of airway inflammation.

A key feature of micro-organism-mediated asthma promotion is the ability to induce lung inflammation and injury, which adversely impacts airway physiology. Micro-organisms may damage and compromise lung integrity by infecting airway epithelial cells and causing cell death [7] and shedding [8]. Micro-organisms

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can also affect epithelial permeability, leading to increased airway inflammation and creating opportunities for increased infection [9], allergen uptake [10] or environmental pollutant exposure.

It is likely that repeated acute and/or chronic infections play a role in disease worsening through increased inflammation that causes airway remodelling. Here we analysed a number of markers associated with bronchial remodelling in a murine model of allergic airway inflammation to evaluate the influence of acute and recurrent respiratory infections on remodelling to test the hypothesis that infections worsen remodelling and that antibiotic therapy may attenuate these changes.

2. Materials and methods

2.1. Animals

The study protocol was reviewed and approved by the Committee on Ethics in Animal Experiments of the Italian National Institutes of Health. Sixty male BALB/c mice (8 weeks of age) were obtained from Charles River Laboratory (Calco, Italy). Mice were fed a normal mouse diet ad libitum, were housed under biosafety level 2 conditions and were cared for according to the standard and specific procedures outlined by the Italian National Institutes of Health.

Experiments were performed under barrier conditions, and sentinel mice were monitored by a quality assurance programme. Animals were divided into three groups (control, mild and chronic asthma) and subgroups:

- non-infected control and infected control mice, sacrificed 12, 24 or 48 h after infection;
- mild asthmatic and infected mild asthmatic mice, sacrificed 12, 24 or 48 h after infection; and
- chronic asthmatic and infected chronic asthmatic mice, sacrificed 48 h after infection, with and without antibiotic therapy.

At the end of the respective treatments, mice were sacrificed under anaesthesia with urethane (1.6 g/kg, intraperitoneal; Sigma–Aldrich, St Louis, MO) and the lungs were removed.

2.2. Asthma model

A murine model of allergic bronchial asthma characterised by airway inflammation and hyper-responsiveness was performed as described by Chiba et al. [11] with some changes. Briefly, mice were sensitised by intraperitoneal injection of 8 µg of ovalbumin (OVA) (Sigma–Aldrich, Milan, Italy) in 2 mg of alum (Imject Alum; Pierce Biotechnology, Rockford, IL) on Days 0 and 5. Sensitised mice were challenged with aerosolised OVA–saline solution (5 mg/mL) for 30 min on Days 12, 16 and 20. The control group was aerosolised with saline only. The aerosol was generated with an ultrasonic nebuliser (Universal Plus; Flaem, San Martino della Battaglia, Brescia, Italy) and was introduced to a Plexiglas chamber box (100 mm × 200 mm; 100 mm height) where mice were placed.

Control and mild asthmatic mice received a further cycle of saline and OVA aerosol, respectively. Following aerosol exposure, a group of mice was infected and killed under anaesthesia with urethane (1.6 g/kg, intraperitoneal; Sigma–Aldrich, St. Louis, MO) and the lungs were removed.

Lungs were immediately dissected and were separated into individual lobes. One lobe was fixed in formalin (4%), routinely processed and embedded in paraffin for histological analysis and the other was frozen in isopentane and stored at –80 °C for real-time PCR.

Chronic asthmatic animals were aerosolised as described for acute asthma, but treatment was performed for an additional 5 weeks, once a week. A group of animals was infected once a week (immediately after OVA cycle) and was subcutaneously treated with antibiotics.

2.3. Experimental infection

Streptococcus pneumoniae ATCC 49619 (ATCC, Rockville, MD) was inoculated in mice three times to increase virulence. The bacterial strain was then identified by standard criteria and by susceptibility to optochin (Oxoid Ltd., Milan, Italy). Bacteria were stored at –70 °C in brain–heart infusion (BHI) broth (Oxoid S.p.A, Rodano, Milan, Italy) with 10% glycerol (Sigma–Aldrich, Milan, Italy). The inoculum of *S. pneumoniae* for infection was obtained by overnight incubation in BHI broth and was suspended to a final inoculum of 10⁸ CFU/mL. Mice were anaesthetised by ether inhalation to induce hyperventilation, and each animal was inoculated intranasally with 30 µL of 10⁸ CFU/mL. The inoculum was checked by plating 10-fold dilutions on trypticase soy agar with 5% defibrinated sheep blood (Oxoid S.p.A).

2.4. Antibiotic treatment

A portion of the infected and chronic asthmatic mice were subcutaneously treated with 50 mg/kg/mouse of azithromycin (Zitromax®; Pfizer, Latina, Italy) or ceftriaxone (Rocephin®; Roche, Milan, Italy) once a day for 2 consecutive days, started 12 h after bacterial inoculation. Mice were killed 24 h after the last treatment and lungs were excised as previously described.

2.5. RNA isolation and reverse transcription

Total RNA was purified from the lung using TRIzol® reagent (Life Technologies, Carlsbad, CA). After purification, RNA was treated with Ambion® DNA-free DNase (Life Technologies, Monza, Italy), and cDNA was synthesised with a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions.

2.6. Real-time PCR

cDNA was subjected to real-time PCR using an ABI PRISM® 7000 system (Applied Biosystems). *TaqMan* PCR was performed using *TaqMan* Universal PCR Master Mix. *TaqMan* probe/primers specific for β-actin (Mm01205647.g1), transforming growth factor beta (*TGFβ*) (Mm01176413.m1), alpha smooth muscle actin (*αSMA*) (Mm00725412.s1), fibroblast growth factor 2 (*FGF2*) (Mm00433287.m1) and tumour necrosis factor alpha (*TNFα*) (Mm00443258.m1) were *TaqMan* Assays-on-Demand™ Gene Expression Products. All reagents were from Applied Biosystems. All PCR assays were performed in duplicate and the data were pooled before using the $\Delta\Delta C_T$ method for relative quantification.

2.7. Histological analysis

Lung sections (4 µm) were stained with haematoxylin–eosin (H&E) (Bio Optica, Milan, Italy) to measure bronchial wall thickness and with Alcian blue–Periodic acid Schiff (AB–PAS) (Bio Optica) for assessment of goblet cell numbers. Images of lung tissue sections were captured using a digital camera (AxioCam ICc1 R4; Zeiss, Oberkochen, Germany). A minimum of ten bronchi measuring 150–350 µm in luminal diameter were analysed per mouse using AxioVision v.4.8 software (Zeiss), which was calibrated with a reference micrometre slide. The thickness of the bronchial wall was measured by tracing around the basement membrane and the

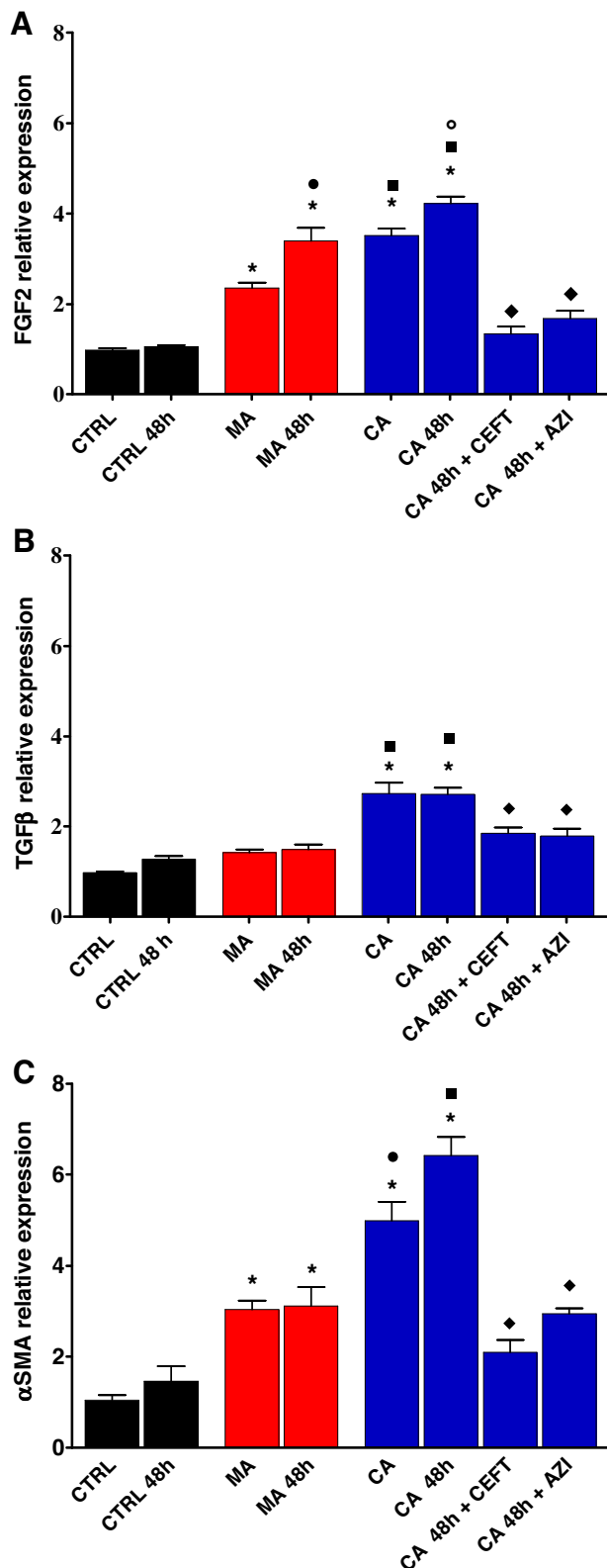


Fig. 1. (A) Fibroblast growth factor 2 (*FGF2*), (B) transforming growth factor beta (*TGFβ*) and (C) alpha smooth muscle actin (*αSMA*) mRNA expression in lungs determined by real-time PCR analysis. CTRL, control mice; CTRL 48 h, infected mice sacrificed 48 h after *Streptococcus pneumoniae* inoculation; MA, mild asthmatic mice; MA 48 h, mild asthmatic mice, infected and sacrificed 48 h after inoculation; CA, chronic asthmatic mice; CA 48 h, chronic asthmatic mice, infected and sacrificed 48 h after inoculation; CA 48 h+CEFT and CA 48 h+AZI, chronic asthmatic mice, infected, treated with ceftriaxone or azithromycin, respectively, and sacrificed 48 h after inoculation. Data represent the mean $\pm$ standard deviation. (A) * $P < 0.001$ vs. CTRL and CTRL 48 h; $\blacksquare P < 0.001$ vs. MA; $\blacklozenge P < 0.001$ vs. CA and CA 48 h; $\bullet P < 0.01$ vs. MA; and $\circ P < 0.05$ vs. CA. (B) * $P < 0.001$ vs. CTRL and CTRL 48 h; $\blacklozenge P < 0.001$ vs. MA and MA 48 h; $\blacklozenge P < 0.001$ vs. CA and CA 48 h. (C) * $P < 0.001$ vs. CTRL and CTRL 48 h; $\blacksquare P < 0.001$ vs. CA; $\blacklozenge P < 0.001$ vs. CA and CA 48 h; $\bullet P < 0.01$ vs. MA.

luminal surface of epithelial cells and calculating the mean distance between the lines using AxioVision v.4.8 software. For goblet cell determination, the basement membrane length was measured by tracing in AxioVision v.4.8 software and goblet cells were counted in AB-PAS-stained sections and expressed as the percentage of cells per 100 μm of basement membrane.

2.8. Immunohistochemical staining and quantification

The applied antibodies were subjected to in-house validation by the manufacturer for immunochemistry analysis on paraffin-embedded material. The antibodies used in the study were anti-FGF2 (Abcam, Cambridge, UK), anti-TGFβ and anti-αSMA (Thermo Fisher Scientific, Waltham, MA). Sections were deparaffinised with xylene and rehydrated with ethanol. Antigen retrieval was performed by placing the specimens in 0.25 mM ethylene diamine tetra-acetic acid (EDTA) solution (pH 8.0) at 95 °C for 45 min. Slides were incubated in 3% H_2O_2 in methanol for 10 min to block endogenous peroxidases. Primary antibodies were incubated overnight at 4 °C and then with ImmPRESS™ reagents (VECTOR Laboratories, Burlingame, CA) for 30 min. Sections were incubated in DAB (3,3'-diaminobenzidine) Peroxidase Substrate Kit (VECTOR Laboratories) and counterstained.

Using ImageJ software, immunostained sections were evaluated and the percentage of positive area of each bronchus was assessed. A minimum of ten bronchi per mouse were analysed by two separate double-blinded operators.

2.9. Statistical analysis

Data were analysed using GraphPad Prism statistical software (GraphPad Software Inc., San Diego, CA) using two-way analysis of variance (ANOVA) followed by post hoc comparison using Bonferroni *t*-test. *P*-values of < 0.05 were considered significant.

3. Results

3.1. Recurrent respiratory infections enhance the lung remodelling pathway, which is attenuated by antibiotic treatment

To evaluate lung remodelling in the presence of infections, mRNA levels of key factors involved in this process were assessed. No significant variations in *FGF2*, *TGFβ* or *αSMA* gene expression were observed in non-asthmatic or mild asthmatic mice sacrificed 12 h or 24 h after infection (data not shown). *FGF2* levels progressively increased in mild and chronic asthmatics ($P < 0.001$) (Fig. 1A). Infection caused a further increase in gene expression levels in asthmatic mice, particularly in those with chronic asthma ($P < 0.001$). Antibiotic treatment significantly reduced *FGF2* levels to those observed in control mice ($P < 0.001$) (Fig. 1A). Mild and chronic asthmatic mice sacrificed 48 h after infection showed increased *αSMA* expression compared with asthmatic mice ($P < 0.001$) (Fig. 1C), but *TGFβ* levels were not significantly higher compared with asthmatic mice (Fig. 1B). Antibiotics significantly reduced both *TGFβ* and *αSMA* expression ($P < 0.001$) (Fig. 1B and C). This trend was confirmed with immunochemistry of FGF2, TGFβ and αSMA protein (Fig. 2).

MA; $\blacklozenge P < 0.001$ vs. CA and CA 48 h; $\bullet P < 0.01$ vs. MA; and $\circ P < 0.05$ vs. CA. (B) * $P < 0.001$ vs. CTRL and CTRL 48 h; $\blacklozenge P < 0.001$ vs. MA and MA 48 h; $\blacklozenge P < 0.001$ vs. CA and CA 48 h. (C) * $P < 0.001$ vs. CTRL and CTRL 48 h; $\blacksquare P < 0.001$ vs. CA; $\blacklozenge P < 0.001$ vs. CA and CA 48 h; $\bullet P < 0.01$ vs. MA.

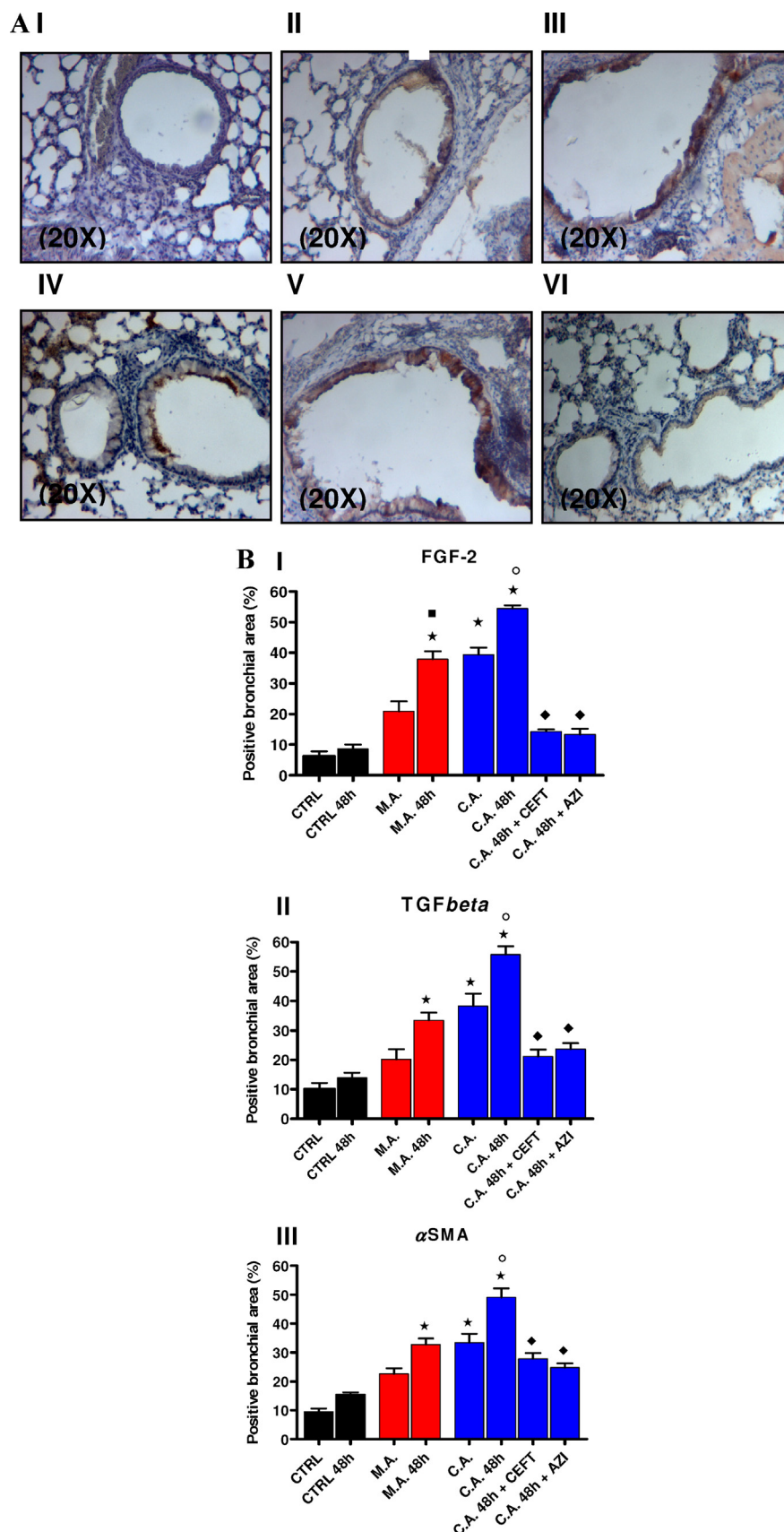


Fig. 2. (A) Representative photomicrographs of immunochemistry-stained lung sections (20 $\times$) for fibroblast growth factor 2 (FGF2) protein: (I) control mice; (II) mild asthmatic mice; (III) mild asthmatic mice, infected and sacrificed 48 h after *Streptococcus pneumoniae* inoculation; (IV) chronic asthmatic mice; (V) chronic asthmatic mice, infected and sacrificed 48 h after inoculation; and (VI) chronic asthmatic mice, infected, treated with azithromycin and sacrificed 48 h after inoculation. (B) Percentage of bronchial areas with positive stain for (I) FGF2, (II) transforming growth factor beta (TGF β) and (III) alpha smooth muscle actin (α SMA) protein. CTRL,

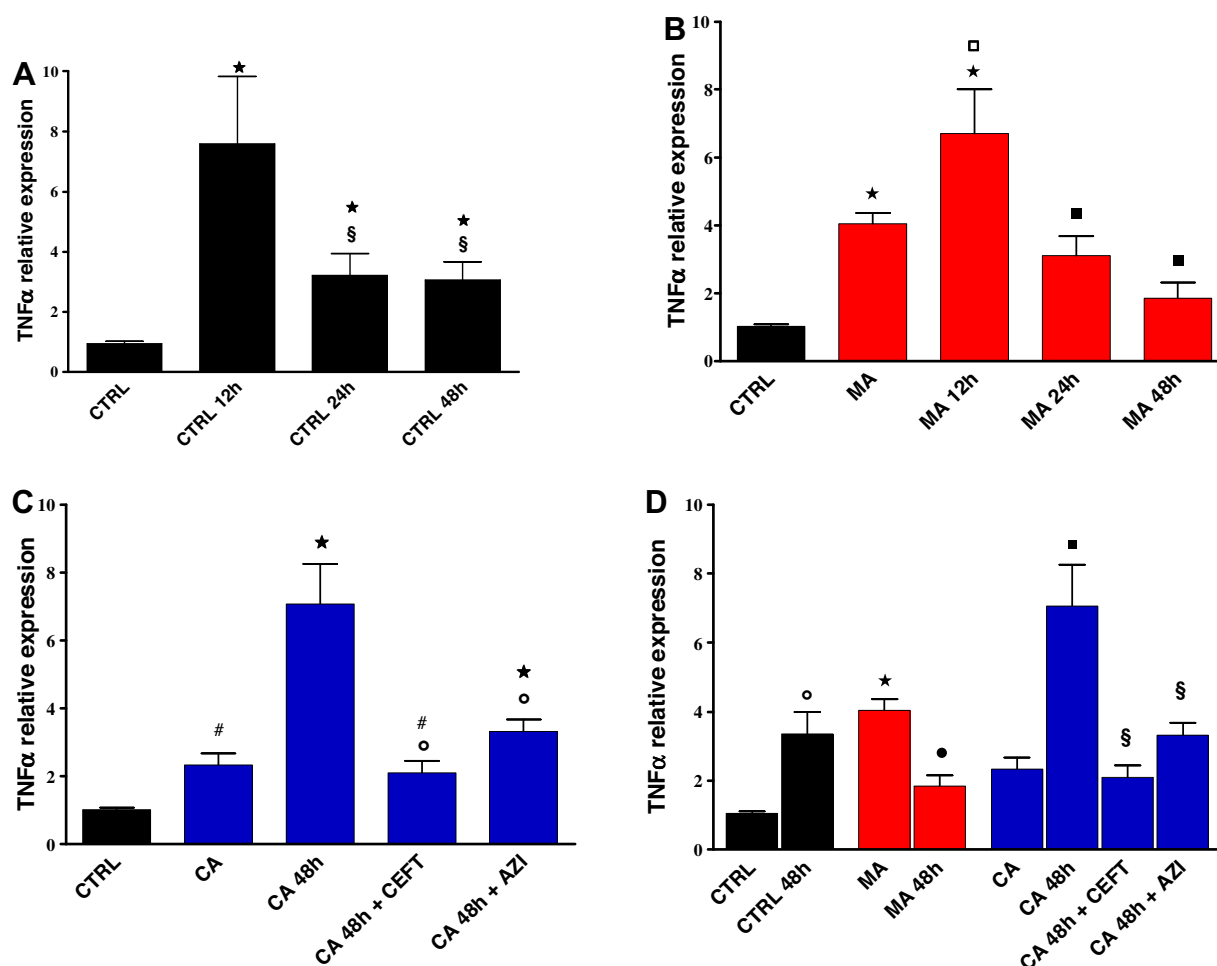


Fig. 3. Tumour necrosis factor alpha (*TNFα*) mRNA expression in lungs determined by real-time PCR analysis: (A) control mice (CTRL) and mice infected and sacrificed 12, 24 and 48 h after *Streptococcus pneumoniae* inoculation (CTRL 12 h, CTRL 24 h and CTRL 48 h, respectively); (B) control mice (CTRL), mild asthmatic mice (MA) and mild asthmatic mice, infected and sacrificed 12, 24 and 48 h after inoculation (MA 12 h, MA 24 h and MA 48 h, respectively); (C) control mice (CTRL), chronic asthmatic mice (CA), chronic asthmatic mice, infected and sacrificed 48 h after inoculation (CA 48 h) and chronic asthmatic mice, infected, treated with ceftriaxone or azithromycin and sacrificed 48 h after inoculation (CA 48 h + CEFT and CA 48 h + AZI, respectively); (D) overview of all groups. Data represent the mean $\pm$ standard deviation. (A) $\star P < 0.001$ vs. CTRL; $\S P < 0.05$ vs. CTRL 12 h; (B) $\star P < 0.001$ vs. CTRL; $\blacksquare P < 0.001$ vs. MA 12 h; $\square P < 0.01$ vs. MA; (C) $\star P < 0.001$ vs. CTRL; $\circ P < 0.001$ vs. CA 48 h; $\# P < 0.05$ vs. CTRL; and (D) $\star P < 0.001$ vs. CTRL; $\blacksquare P < 0.001$ vs. CA; $\S P < 0.001$ vs. CA 48 h; $\circ P < 0.05$ vs. CTRL; $\bullet P < 0.05$ vs. MA.

3.2. Lung infections induce *TNFα* expression in asthmatic mice, which is reduced by antibiotic therapy

A significant increase in *TNFα* gene expression was observed 12 h after control mice were inoculated with *S. pneumoniae* ($P < 0.01$) (Fig. 3A). At 24 h and 48 h, *TNFα* expression decreased to non-infected control levels. In mild asthma, *TNFα* significantly increased at 12 h after infection ($P < 0.05$) (Fig. 3B); conversely, no variations were observed at 24 h and 48 h post inoculum. In contrast to data obtained both in control and mild asthmatic animals, in chronic asthmatic mice *TNFα* significantly increased 48 h after intranasal inoculation of *S. pneumoniae* ($P < 0.001$) and returned to control values following antibiotic treatment (Fig. 3D).

3.3. Recurrent respiratory infections contribute to increase bronchial thickness and goblet cell hyperplasia

Histological and morphometric analysis of lung sections stained with H&E revealed that infected mild asthmatic mice had no

significant variations in bronchial thickness compared with mild asthmatic mice. However, the bronchial wall diameter significantly increased in infected chronic asthmatic mice compared with non-infected mice ($P < 0.001$) (Fig. 4A and B). The percentage of AB-PAS-positive goblet cells in stained airway sections was quantified: infected mild asthmatic mice showed no significant variations in goblet cell numbers compared with mild asthmatic mice, whereas infected chronic asthmatic mice had significantly more goblet cells than uninfected mice ($P < 0.001$) (Fig. 5A and B). Antibiotic treatment appears to prevent lung remodelling, reducing both wall thickness ($P < 0.001$) and goblet cell hyperplasia ($P < 0.001$). Infected mild asthmatic mice showed no significant variations in bronchial thickness compared with mild asthmatic mice.

4. Discussion

Respiratory infections induce acute inflammation that exacerbates the chronic inflammatory state already present in asthmatic

control mice; CTRL 48 h, infected mice sacrificed 48 h after *Streptococcus pneumoniae* inoculation; MA, mild asthmatic mice; MA 48 h, mild asthmatic mice, infected and sacrificed 48 h after inoculation; CA, chronic asthmatic mice; CA 48 h, chronic asthmatic mice, infected and sacrificed 48 h after inoculation; CA 48 h + CEFT and CA 48 h + AZI, chronic asthmatic mice, infected, treated with ceftriaxone or azithromycin, respectively, and sacrificed 48 h after inoculation. Data represent the mean $\pm$ standard deviation. $\star P < 0.001$ vs. CTRL and CTRL 48 h; $\circ P < 0.001$ vs. CA; $\blacksquare P < 0.001$ vs. MA; $\diamond P < 0.001$ vs. CA and CA 48 h.

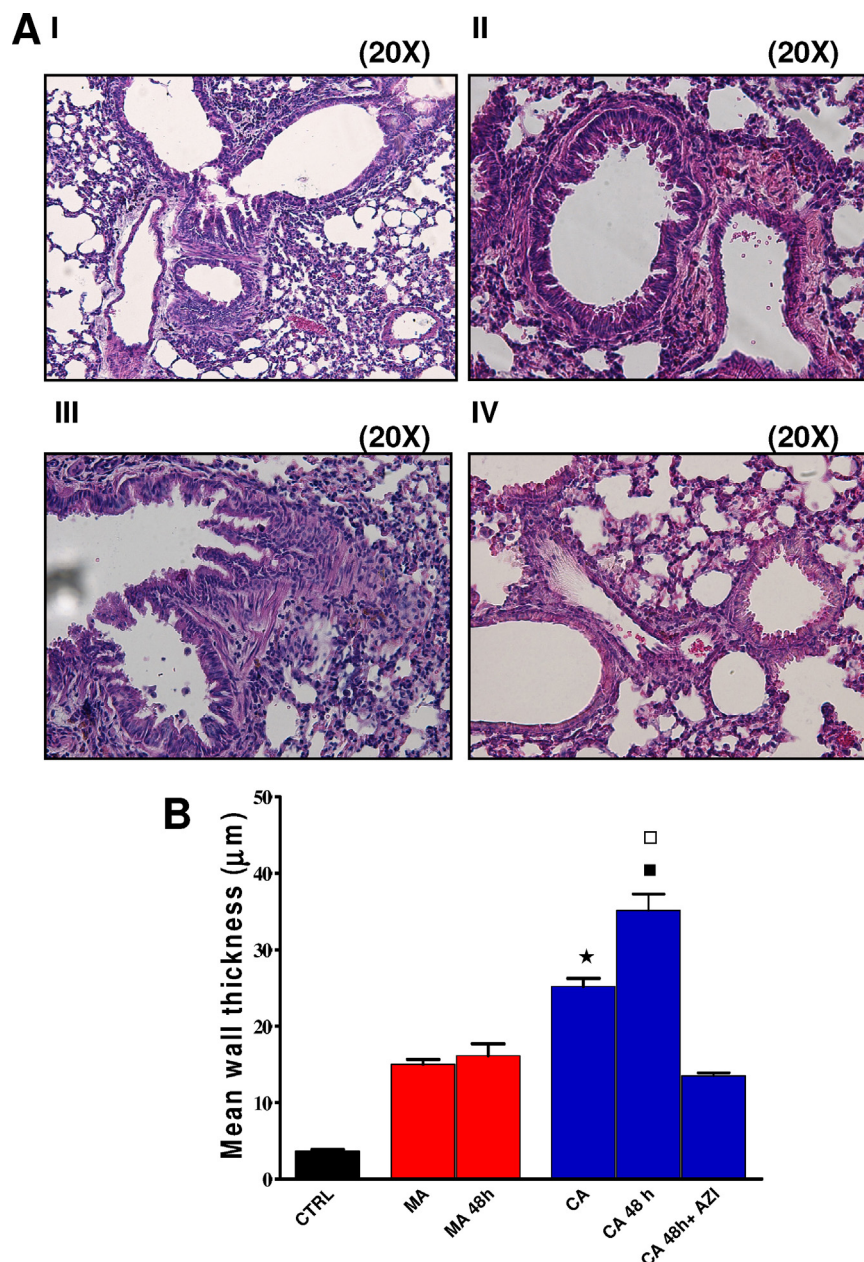


Fig. 4. (A) Representative photomicrographs of haematoxylin–eosin (H&E)-stained sections of mice airways (20×): (I) control mice; (II) chronic asthmatic mice; (III) chronic asthmatic mice, infected and sacrificed 48 h after *Streptococcus pneumoniae* inoculation; and (IV) chronic asthmatic mice, infected, treated with azithromycin and sacrificed 48 h after inoculation. (B) Morphometric analysis of epithelial thickness in the lungs. Airway epithelial thickness of H&E-stained lung tissue sections was traced and assessed using AxioVision v.4.8 software (Zeiss, Oberkochen, Germany). Data represent the mean epithelial thickness values obtained from mice of each group. CTRL, control mice; MA, mild asthmatic mice; MA 48 h, mild asthmatic mice, infected and sacrificed 48 h after *S. pneumoniae* inoculation; CA, chronic asthmatic mice; CA 48 h, chronic asthmatic mice, infected and sacrificed 48 h after inoculation; CA 48 h + AZI, chronic asthmatic mice, infected, treated with azithromycin and sacrificed 48 h after inoculation. Data represent the mean ± standard deviation. * $P < 0.001$ vs. CTRL; $\star P < 0.001$ vs. CA; $\square P < 0.001$ vs. CA 48 h + AZI.

patients. This could accelerate the cellular and molecular processes involved in lung remodelling, particularly if the infections are recurrent.

In this study, the role of acute and recurrent respiratory infections in lung remodelling both in mild and chronic mouse models of asthma was evaluated by assessing key factors involved in remodelling.

Mice do not spontaneously develop asthma, therefore there is a need to reproduce mouse models of allergic response to inhaled allergens in order to investigate immunological and inflammatory responses in asthma. A large variety of allergen challenge models in mice have been reproduced, characterised by different sensitisation times (intraperitoneally), followed by challenges with

allergen administrations by intratracheal/intranasal instillation or via aerosols, usually over a period of several days [12,13]. OVA derived from chicken eggs is the allergen most frequently used to induce a strong inflammatory response in the lungs of rodents. In particular, acute exposure to OVA induces hyper-responsiveness, airway inflammation, Th₂ cytokine production and hyperplasia of goblet cells [14]; however, in this model no other asthma features are observed, such as a chronic inflammatory state and bronchial remodelling. For this reason, many researchers have investigated the effect of chronic exposure to OVA. McMillan and Lloyd [15] demonstrated that repeated allergen challenge of sensitised mice results in persistent remodelling of the airways, with chronic inflammation and eosinophilia, hypertrophy of smooth muscle and

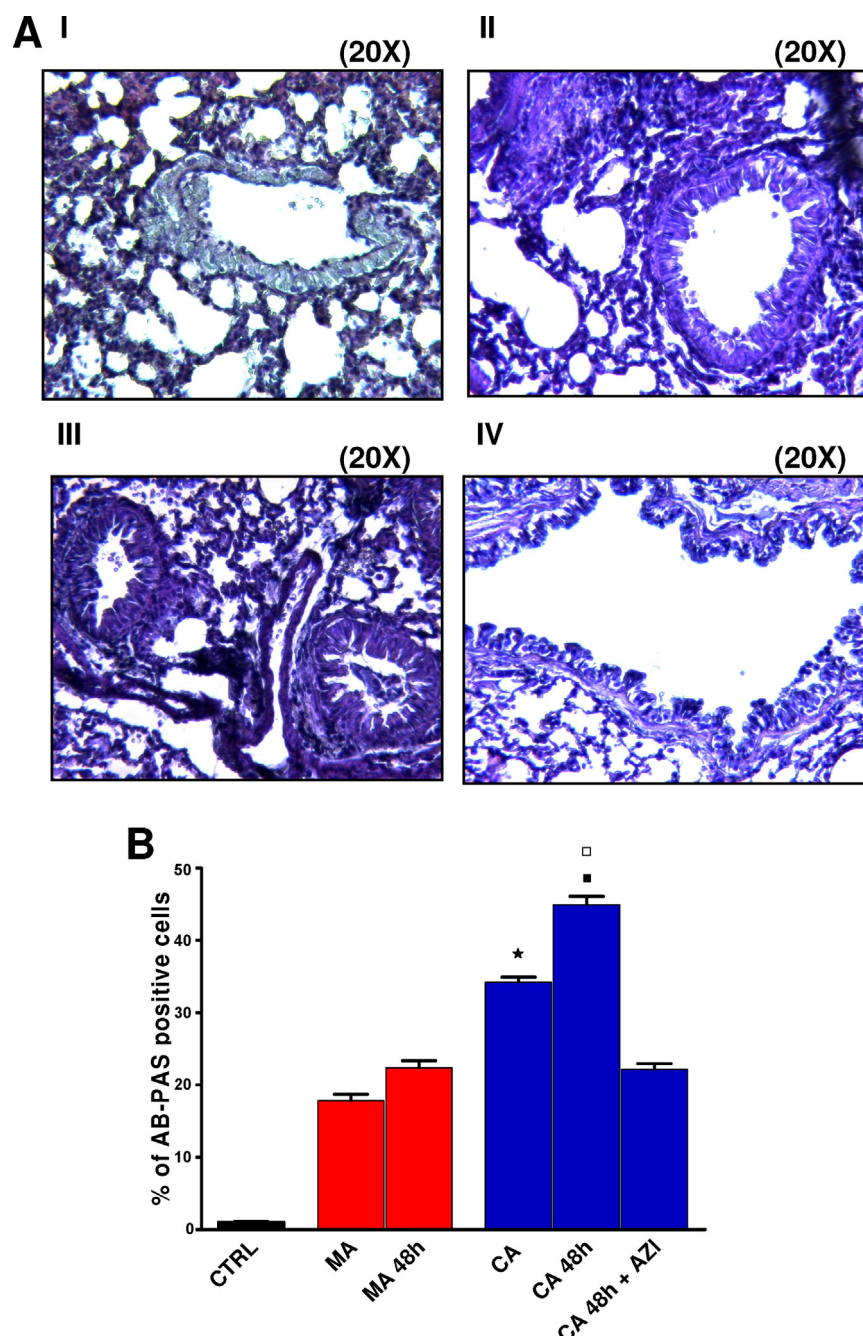


Fig. 5. (A) Representative photomicrographs of Alcian blue-Periodic acid Schiff (AB-PAS)-stained sections of mice airways (20 $\times$): (I) control mice; (II) chronic asthmatic mice; (III) chronic asthmatic mice, infected and sacrificed 48 h after *Streptococcus pneumoniae* inoculation; and (IV) chronic asthmatic mice, infected, treated with azithromycin and sacrificed 48 h after inoculation. (B) Percentage of AB-PAS-positive goblet cells in mouse airways. Sections of mouse lung tissue were stained with AB-PAS and the percentage of AB-PAS-positive goblet cells was determined. CTRL, control mice; MA, mild asthmatic mice; MA 48h, mild asthmatic mice, infected and sacrificed 48 h after inoculation; CA, chronic asthmatic mice; CA 48h, chronic asthmatic mice, infected and sacrificed 48 h after inoculation; CA 48h + AZI, chronic asthmatic mice, infected, treated with azithromycin and sacrificed 48 h after inoculation. Data represent the mean $\pm$ standard deviation. $\star P < 0.001$ vs. CTRL; $\blacksquare P < 0.001$ vs. CA; $\square P < 0.001$ vs. CA 48h + AZI.

hyperplasia of goblet cells; moreover, these structural alterations persist even without further allergen challenge. In the current work, mice were exposed to an acute and a repeated OVA treatment in order to induce a phenotype of mild and chronic asthma, respectively.

The remodelling process is orchestrated by a pathway that involves FGF2, TGF β and α SMA. FGF2, a potent fibroblast mitogen, is involved in tissue morphogenesis, repair processes, inflammation, angiogenesis and tumour growth. In the airways, FGF2 is predominantly expressed by epithelial cells and in the extracellular matrix of the epithelial basement membrane [16,17]. Moreover,

inflammatory cells, such as eosinophils, macrophages and T-cells, are also potent sources of FGF2.

In pathological conditions such as asthma, FGF2 is likely released after epithelial damage. Lung tissue is the primary target for several inflammatory insults entering the airways; following such challenges, cell membrane integrity of the epithelium could be compromised, with mobilisation and activation of inflammatory cells into the airways, which leads to the release of cytoplasmic FGF2 [18]. Activation of FGF2 is likely to contribute to the structural alterations observed in asthmatic airways, including fibroblast proliferation and angiogenesis [19].

TGF β is a pro-fibrotic cytokine that contributes to the structural changes of lung remodelling in asthma. Several studies show that in animal models of asthma, levels of TGF β are significantly increased in thickened smooth muscle airway [15,19]. Moreover, TGF β levels are increased in bronchoalveolar lavage fluids of patients with asthma [20].

In normal conditions, α SMA is mainly expressed in smooth muscle cells but also in myofibroblasts [21,22]. α SMA action is critical in the differentiation of fibroblasts into myofibroblasts in lung fibrosis; therefore, it is considered a key marker of lung remodelling in pathological conditions such as asthma or chronic obstructive pulmonary disease.

Myofibroblast emergence and accumulation are key characteristics of pulmonary remodelling and appear to arise by differentiation from fibroblasts. When fibroblasts differentiate into myofibroblasts, they produce high amounts of TGF β , which in turn could induce more neighbouring fibroblasts to differentiate through induction of α SMA upregulation [23]. Moreover, Bossè et al. demonstrated that FGF2 and TGF β synergistically induce bronchial smooth muscle cell proliferation [24].

In this study, lung remodelling activation was evaluated by analysing gene expression levels of TGF β , α SMA and FGF2 and it was found that infection potentiated the remodelling pathway activation in asthmatic mice. Further confirmation of this result was obtained by assessing proteins using immunohistochemistry. The results suggest that infection increases fibroblast differentiation into myofibroblasts as well as hyperplasia of bronchial smooth muscle, which contribute to worse airway remodelling. This observation is supported by the fact that antibiotic treatment significantly attenuated the lung remodelling pathway in treated compared with non-treated mice, suggesting that prompt antibiotic treatment of infected asthmatics that eradicates microorganisms could reduce the inflammatory response and modify the disease course.

TNF α gene expression was also analysed because it plays a crucial role in the induction of bronchial hyper-reactivity and remodelling. Results described by Cho et al. [25] suggested a direct role for this cytokine in remodelling. TNF p55/p75 receptor (TNF-R)-deficient mice had significantly reduced levels of structural remodelling changes (fibrosis and thickened smooth muscle) and extracellular matrix protein deposition compared with wild-type mice. Moreover the TNF-R-deficient mice had significantly reduced levels of TGF β -expressing bronchial cells. In the current study, we found an increase of TNF α levels in asthmatic mice, especially in those that were infected. Antibiotics were able to reduce TNF α gene expression, confirming that infection exerted a pro-inflammatory action during disease progression.

The gene expression data were confirmed with histological analysis of lung sections. Increased bronchial wall diameter measurements demonstrated that recurrent infectious stimuli induced remodelling processes. Prompt antibiotic treatment significantly attenuated this process and improved the disease course.

Asthma is characterised by excessive mucus production that contributes to airway narrowing. We observed goblet cell hyperplasia in the presence of infection, and antimicrobial therapy decreased goblet cell numbers and reduced airway obstruction progression.

Two types of antibiotics were employed to clarify whether the beneficial effect of remodelling attenuation was related to the antimicrobial or anti-inflammatory effect of antibiotics. It is known that some classes of antibiotics, such as macrolides, exert an anti-inflammatory effect [26]. Data from the current study indicate that there are no differences in the effect of antibiotics, therefore the antimicrobial action, reducing bacterial burden, may indirectly attenuate bronchial remodelling.

In conclusion, these data demonstrate that recurrent infections worsen asthma progression and enhance lung remodelling, which causes the disease to progress towards irreversible conditions. Therefore, it is conceivable that prompt and effective antibiotic treatment, reducing the bacterial burden, may be useful in ameliorating bronchial structural changes and improving the disease course in subjects with respiratory infection suffering from acute asthma attacks.

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Competing interests: None declared.

Ethical approval: The animal protocol was reviewed and approved by the Committee on Ethics in Animal Experiments of the Italian National Institutes of Health.

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