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Infliximab alleviates inflammation and *ex vivo* airway hyperreactivity in asthmatic E3 rats

Yan Cai[1,2], Yong-Xiao Cao[1], She-Min Lu[1], Cang-Bao Xu[3] and Lars Olaf Cardell[4]

[1] Department of Pharmacology, Xi'an Jiaotong University College of Medicine, Xi'an, Shaan-xi 710061, People's Republic of China

[2] Department of Pharmacy, the Second Affiliated Hospital of Xi'an Jiaotong University, Xi'an, Shaanxi 710004, People's Republic of China

[3] Department of Experimental Vascular Research, Institution of Medicine, Lund University, Lund SE-22184, Sweden

[4] Division of Ear, Nose and Throat Diseases, Department of CLINTEC, Karolinska Institute, Karolinska University Hospital, Huddinge B53, SE-141 86 Stockholm, Sweden

Running title: Infliximab eases inflammation and hyperreactivity

Correspondence to: Y. X. Cao; E-mail:yxy@xjtu.edu.cn

Abstract

Tumor necrosis factor-α (TNF-α) has been implicated in the pathogenesis of asthma, and neutralization of TNF-α is an effective therapy for inflammatory diseases. The present study tested the idea that a TNF-α antibody, infliximab, may be useful in the management of asthma. E3 rats were immunized with ovalbumin (OVA)/alum and received infliximab intra-peritoneally. Two weeks later, OVA–PBS was instilled intranasally daily for 7 days. Bronchoalveolar lavage fluids (BALFs), serum and lung homogenates were collected for analysis of cells and inflammatory mediators. Contractile responses of lobar-bronchus segments to agonists were functionally tested. Pulmonary tissues were investigated using histological examination. The results showed that the sensitized 'model E3 rats' exhibited an increase in the total amount of inflammatory cells, primarily eosinophils, in BALF and pulmonary tissue, as well as epithelial damage. Serum levels of IgE increased and so did the levels of nitric oxide, inducible nitric oxide synthase, TNF-α and IL-4, IL-5 and IL-13 in lung homogenate and serum. Furthermore, the contractile responses in bronchi induced by endothelin-1, sarafotoxin 6c and bradykinin increased and isoprenaline-induced relaxations decreased. All these changes induced by the sensitization procedure were reduced by the infliximab treatment. The results suggest that infliximab prevents the development of local airway inflammation and antagonizes changes of the bronchial smooth muscle receptor phenotype, thereby blocking the development of airway smooth muscle hyperreactivity of asthmatic rats.

Keywords: asthma, cytokine, inflammation, receptor, tumor necrosis factor-alpha

Introduction

Asthma is a chronic inflammatory disease characterized by airway hyperreactivity, edema and increased mucus secretion (1-3). Following exposure to allergens, inflammatory and structural cells such as macrophages, eosinophils, lymphocytes and epithelial cells are activated. This results in the release of immunomodulatory mediators triggering numerous inflammatory changes within the airways (4). Tumor necrosis factor-α (TNF-α), a well-known pro-inflammatory mediator, plays an important role in this process (5, 6). It causes an influx of neutrophils and eosinophils as well as bronchial hyperreactivity (7). The level of TNF-α expression has been reported to reflect the severity of the asthmatic disease. The analysis of patients who died of severe asthma has revealed obvious increases of TNF-α at both mRNA and protein levels (4). Inhalation of recombinant TNF-α enhances sputum neutrophilia and bronchial hyperreactivity in normal subjects (8).

Asthmatic patients exhibit a distinct pro-inflammatory mediator profile consisting of T_h2 cytokines, IgE and nitric oxide (NO). It is confirmed that these mediators play important roles in the development of the disease. For instance, IL-4 leads to an up-regulation of cell adhesion molecules on inflammatory endothelium resulting in the recruitment of inflammatory cells and the production of IgE by B cells. IL-5, another mediator increased in asthma, is important for the growth, differentiation and activation of tissue eosinophils, and IL-13 is involved in bronchial hyperreactivity, goblet cell hyperplasia and IgE synthesis (9, 10). These and other pro-inflammatory cytokines are also known to increase the transcription of inducible nitric oxide synthase (iNOS) in the airway epithelium with an ensuing increase in local NO production. NO is often used as a biomarker of ongoing airway inflammation (11). However, large amounts of NO may be directly detrimental to the airway epithelium (12).

We have previously demonstrated that long-term *in vitro* culture of isolated murine bronchial segments in presence of pro-inflammatory mediators, like TNF-α and IL-1β, up-regulates a multitude of contractile smooth muscle receptors (i.e. the bradykinin and 5-hydroxytryptamine 2A receptors) (13, 14) and TNF-α antibodies have the ability to abolish this amplification (13). The present study was designed to further evaluate the central role of TNF-α in pulmonary hyperreactivity by examining the effect of TNF-α antibody, infliximab, in an *in vivo* asthmatic model of E3 rats. To this end, an ovalbumin (OVA)/alum sensitization protocol was developed to make 'model rats' and infliximab was then administrated to test its effects.

Materials and methods

Animals

Male E3 rats aged 8–10 weeks were imported from Lund University, Sweden, and housed in a special facility at the Department of Pharmacology, Xi'an Jiaotong University College of Medicine. The rats were handled according to the guidelines provided by the Animal Center of Xi'an Jiaotong University College of Medicine and maintained on normal diet, with free access to food and water, in a climate-controlled environment.

Asthma model and infliximab treatment

The E3 rats were divided into three groups: control, model and infliximab. The rats in the model and infliximab group were immunized by intra-peritoneal injection of OVA–aluminum hydroxide (Alum) (1:50 mg) 1 ml per rat. Rats in the control group received equal volume of a PBS solution. Two weeks later, 100 µl OVA–PBS (containing 1 mg OVA) was instilled intranasally in the two sensitized groups. The rats in the sham-sensitized control group received the same amount of PBS. The intranasal instillations continued for 7 days (15). The rats in the infliximab group received infliximab (5 mg kg^{-1}) intra-peritoneally 1 h before each OVA–PBS challenges.

Peripheral blood

All the E3 rats were anaesthetized and sacrificed 24 h after the last OVA–PBS challenge. Their blood was harvested from the abdominal aorta. The serum was separated by centrifugation and then stored at −80°C for later analyses.

Bronchoalveolar lavage fluid

After the blood has been sampled, the whole lung was removed. The trachea was intubated with a polyethylene catheter and bronchoalveolar lavage fluid (BALF) was collected by washing with three separate aliquots of 2 ml of PBS (16). The first wash was centrifuged and the BALF supernatant was stored for NO analysis. The second and the third wash were also centrifuged and the obtained cell pellets were pooled with the cell pellets from the first wash. Then, all the cell pellets were suspended in 500 µl of PBS and total cell content was counted with a microscope. For differential counting, cells were smeared and then stained with Giemsa–Wright. Three hundred cells were counted on each slice and the percentage of eosinophils in each smear was calculated.

Histopathology

Immediately following the BALF collection, the intact left lungs from each rat was removed, fixed in 10% formalin and processed for routine histology in paraffin. Sections were prepared, stained with hematoxylin–eosin and examined by light microscopy.

Preparation of lung homogenates

The right lung was removed and placed in ice-cold homogenization buffer (0.05% Triton X-100 in PBS). A quick homogenization was followed by centrifugation (15 000 × g for 15 min at 4°C). The supernatant was used directly for quantification of L-arginase, nitric oxide synthase (NOS), NO and various cytokines.

Bronchial myograph system

The left lung was used for dissection of the lobar-bronchus. The bronchi were then cut into 1-mm segments and immersed into tissue baths containing 1 ml of Krebs solution (in millimolar: NaCl 119, $NaHCO_3$ 15, KCl 4.6, $CaCl_2$ 1.5, NaH_2PO_4 1.2, $MgCl_2$ 1.2, Glucose 5.6). The solution was continuously equilibrated with 5% CO_2 in O_2 resulting a pH of 7.4. Each lobar-bronchus segment was mounted on two L-shaped metal prongs. One prong was connected to a force-displacement transducer for continuous recording of isometric tension and the other prong was connected to a displacement device allowing adjustment of the distance between the two parallel prongs. Following equilibration, a pre-tension of 1 mN was applied to each segment and adjusted to this level of tension for at least 1 h. Each segment was contracted with K-Krebs solution (in millimolar: NaCl 63.6, $NaHCO_3$ 15, KCl 60, $CaCl_2$ 1.5, NaH_2PO_4 1.2, $MgCl_2$ 1.2, Glucose 5.6) to test the contractile function before agonist was added and its contraction was used as a reference. Concentration–response curves were obtained by cumulative administration of carbachol (CCh), endothelin-1 (ET-1), sarafotoxin 6c (S6c), bradykinin (BK) and des-Arg^9-bradykinin (des-Arg^9-BK). In order to study ET_A receptor mediated-contractions, the experiment started with the desensitization of the ET_B receptors by inducing a concentration–response curve to S6c. When the maximal contraction was reached, it was allowed a fade away until the contractile curves fell to the baseline, which was considered as a total desensitization (17). Thus, the subsequent administration of ET-1 resulted in a concentration–response curve representative for the ET_A receptor. Isoprenaline was cumulatively applied after pre-contraction with 5-hydroxytryptamine (5-HT) 10^{-5} M (18).

Measurement of NO production, NOS activity and cytokine levels

NO production was determined by measuring nitrate and nitrite concentrations using the Griess reaction. L-arginase was measured as described by Kropf *et al.* (19). NOS activity was detected by a NOS kit. TNF-α, IL-1β, IL-4, IL-5 and IL-13 in serum and lung homogenates were analyzed with commercial ELISA kits according to the manufacturer's instructions. So was IgE in serum. The protein content in BALF and lung homogenates were determined by the Biuret method (20) and then the values of NOS, TNF-α, IL-1β, IL-4, IL-5 and IL-13 in the lung homogenate were calculated in relation to its corresponding protein concentration; thus, they were expressed as mass (or unit) in per gram of protein.

Chemicals and detected kits

OVA, alum, CCh and 5-HT were obtained from Sigma (St Louis, MO, USA). ET-1 and S6c came from Auspep (Parkville, Australia). BK and des-Arg^9-BK were from Neosystem AB (Strasburg, France). Infliximab came from Centocor B.V (Leiden, the Netherlands). NOS assay kits were bought from Nanjing Jiancheng (Bioengineering Institute, China). TNF-α, IL-1β, IL-4, IL-5, IL-13 and IgE ELISA kits were supplied by Shanghai Xitang Bioengineering Institute (Shanghai, China).

Statistical analysis

All data were expressed as mean values ± SEM and analyzed with GraphPad Prism 4 software (version 4.03). Two-way analysis of variance (ANOVA) with Dunnett's post-test was used for comparisons between all treatment groups. P value <0.05 was considered as statistically significant and n equals the number of experiments performed. The maximal contractile response (E_{max}) was expressed as a percentage of the contraction induced by K-Krebs solution and the maximal dilatory response (R_{max}) induced by isoprenaline was expressed as a percentage of the 5-HT pre-contraction. The negative logarithm of the agonist concentration eliciting half the maximal response was referred to as pEC_{50}.

Results

IgE concentration in serum

IgE has a pivotal role in type I hypersensitivity reactions. In rats, a relation between serum IgE and the degree of airway inflammation has been reported (21). Accordingly, the present study exhibited that serum IgE levels (International units per milliliter) increased in the model rats in comparison with the control group (control: 7.7 ± 1.1; model: 20.6 ± 2.8; $n = 10$, $P < 0.01$), whereas the infliximab group presented significantly lower values than the model rats (12.4 ± 2.3; $n = 10$, $P < 0.05$) (Fig. 1).

Pulmonary recruitment of inflammatory cells

The number ($\times 10^3$) of total cells and eosinophils in BALF were much higher in model than control rats (control: total: 38 ± 2; eosinophils: 0.87 ± 0.11; model: total: 58 ± 3; eosinophils: 9.74 ± 1.1; $n = 10$, $P < 0.01$). Infliximab prevented the increase (total: 42 ± 3; eosinophils: 1.55 ± 0.28; $n = 10$, $P < 0.05$) (Fig. 2).

NO in serum, BALF and lung homogenates

Model rats exhibited higher NO levels (micromoles per liter) than control rats in both serum and lung homogenates (control: 69.9 ± 4.6, 35.2 ± 3.8; model: 94.0 ± 7.6, 57.0 ± 5.3; $n = 10$, $P < 0.05$ or $P < 0.01$). Infliximab decreased the NO levels at both locations (75.6 ± 2.5, 38.6 ± 2.7, $P < 0.05$ or $P < 0.01$). However, there was no significant difference in the NO concentrations found in BALF among the three groups (Fig. 3).

L-arginase and NOS activity

L-arginase and NOS activity were assessed in lung homogenates. Figure 4A showed that there was no significant difference of L-arginase among the groups. Total NOS and iNOS were analyzed. Total NOS represents the sum of iNOS and constitutive NOS (cNOS). iNOS releases far greater amounts of NO than cNOS and promotes oxidative reactions in response to epithelial injuries (12). The model group displayed higher iNOS (units per milligram protein) levels than the control and the infliximab groups (control: 0.10 ± 0.01, model: 0.14 ± 0.01, infliximab 0.10 ± 0.01; $n = 10$, $P < 0.05$). Somewhat surprisingly the infliximab group also exhibited lower total NOS (units per milligram protein) values than both the control and the model groups (model: 1.34 ± 0.08, infliximab: 1.05 ± 0.07; $n = 10$, $P < 0.05$) (Fig. 4B).

Cytokines in serum and lung homogenate

Measurements of TNF-α in serum and in lung homogenates were of special interest for the primary goal of this investigation. The concentrations of TNF-α in serum (picograms per milliliter) and in homogenate (nanograms per gram protein) were markedly elevated in the OVA-induced asthma model rats compared with the control group [control: 27.0 ± 2.2 (in serum), 7.6 ± 0.4 (in lung homogenates); model: 37.6 ± 2.0 (in serum), 11.2 ± 0.8 (in lung homogenates); $n = 10$, $P < 0.01$] and infliximab neutralized the increase brought about by the sensitization procedure (numbers: 29.8 ± 2.3 in serum 8.6 ± 0.2 in lung homogenates; $n = 10$, $P < 0.01$ or $P < 0.05$) (Fig. 5A).

IL-4, IL-5 and IL-13 are cytokines produced by T_h2 cells and they are of importance for the pathogenesis of asthma (9). All three cytokines were increased in lung homogenate (nanograms per gram protein) from the model rats (IL-4: control: 8.0 ± 0.3, model: 10.6 ± 0.9; IL-5: control: 7.2 ± 0.2, model: 10.3 ± 0.9; IL-13: control: 7.2 ± 0.2, model: 10.3 ± 0.7; $n = 10$, $P < 0.05$ or $P < 0.01$) and lowered by infliximab treatment (IL-4: 7.5 ± 0.5; IL-5: 8.0 ± 0.2; IL-13: 8.3 ± 0.2; $n = 10$, $P < 0.01$ or $P < 0.05$). In serum (picograms per milliliter), both IL-5 and IL-13 were increased (IL-5: control: 32.0 ± 2.8, model: 39.3 ± 1.1; IL-13: control: 22.7 ± 1.5, model: 31.3 ± 2.2; $n = 10$, $P < 0.05$ or $P < 0.01$), but only IL-5 was reduced significantly by infliximab treatment (34.2 ± 0.9; $n = 10$, $P < 0.05$). However, there was no statistical difference in the serum levels of IL-13 in the model and infliximab groups. No obvious difference in the levels of IL-4 in serum between the three groups (Fig. 5B–D).

Bronchial smooth muscle reactivity

The contractile reactivity of bronchial segments was investigated with myograph sestem. The results showed that K^+ induced similar contractions in each groups (data not shown). The contractile response of bronchial segments to muscarinic receptor agonist CCh was identical and there was no difference in E_{max} and pEC_{50} values in all three groups (Fig. 6A).

Both ET-1 and S6c evoked an increased contractile response in the model group compared with the control group [S6c: E_{max} (%): 102 ± 15 and 155 ± 11 in control and model, respectively; $n = 10$, $P < 0.05$ or $P < 0.01$; ET-1: E_{max} (%): 13 ± 6 and 36 ± 10 in control and model, respectively; $n = 10$, $P < 0.05$]. Infliximab prevented the contractile increase [S6c: E_{max} (%): 119 ± 7, ET-1: E_{max}(%): 14 ± 4; $n = 10$, $P < 0.01$]. (Fig. 6B and C).

Des-Arg^9-BK and BK are selective agonists for the bradykinin B_1 and bradykinin B_2 receptors, respectively. Des-Arg^9-BK did not induce contraction in any of the three groups. BK induced measurable contractions in all segments tested. The concentration–contractile curve of model

group was shifted to the left compared with control group with an increased pEC_{50} value (control: 6.23 ± 0.19, model: 7.05±0.08; $n = 10$, $P < 0.01$). Infliximab treatment restored the curve and pEC_{50} (6.08 ± 0.13; $n = 10$, $P < 0.01$). There was no significant change of E_{max} in control, model and infliximab groups (Fig. 6D).

Isoprenaline relaxed the pre-contracted bronchial segments in a concentration-dependent way. The relaxation curve was shifted to the right by the sensitization procedure (pEC_{50}: control 6.34 ± 0.13 and model 5.94 ± 0.15, $n = 10$, $P < 0.05$) and the R_{max} value was reduced [R_{max}(%):control 74 ± 6 and model 45 ± 6; $n = 10$, $P < 0.01$] These effects were antagonized by the infliximab treatment [pEC_{50}: 6.58 ± 0.27; $n = 10$, $P < 0.01$; R_{max} (%): 62 ± 6; $n = 10$, $P < 0.05$] (Fig. 7).

Histopathology

Histopathological analysis of lung parenchyma revealed a marked increase in inflammatory cells and damage to the epithelium in the model group in comparison with the control group. Eosinophils could be seen both in the alveoli and in the pulmonary interstitium of the model rats and there were much less infiltrative changes and more complete epithelium in the infliximab-treated group (Fig. 8).

Discussion

The current study presents a rat model for evaluation of airway inflammation and its effects on bronchial smooth muscle activity. The chosen E3 rat, used in the our experiments, exhibited changes in response to the present model that could clearly be associated with the development of both inflammation and airway smooth muscle hyperreactivity in the previous study, which allowed it to be an new strain of asthma model rats. (18). The model is based on common principles for sensitization (22), an initial intra-peritoneal OVA/alum followed 2 weeks later by intranasal OVA instillations for seven consecutive days. The OVA/alum model E3 rats exhibited other signs of local airway inflammation, like an increase of inflammatory cells in BALF and pulmonary tissue, enhanced levels of serum IgE and raised levels of traditional pro-inflammatory mediators (NO, iNOS, TNF-α and T_h2 cytokines) in lung homogenate and serum. A histological investigation of the lung parenchyma revealed signs of eosinophils infiltration. When infliximab was administrated, most changes were markedly reduced. The IgE concentration in serum was diminished along with the recruitment of inflammatory cells. The levels of NO, TNF-α and T_h2 cytokines were decreased in serum and

lung. Infliximab also depressed the development of airway smooth muscle hyperreactivity and prevented the inflammatory damage. Infliximab is a mAb that neutralizes TNF-α. In our study, PBS was chosen as a negative control instead of non-specific IgG. It has been previously shown that non-specific IgG and saline exhibited similar effects in the asthmatic model (23), Therefore, it is reasonable to believe that the effectiveness of infliximab observed in the present study is due to neutralization of the biological activity of TNF-α.

Chronic airway inflammation is associated with the development of airway hyper-responsiveness (24, 25). The release of different mediators, such as TNF-α, during the inflammatory process, is known to cause both functional and structural alterations of the airways (14, 26). Since our first goal was to evaluate the potential role of a TNF-α antibody in the management of asthma, it was exciting to notice that the levels of TNF-α in serum and in lung homogenates were markedly elevated in the OVA-induced asthma model rats compared with the control group and that treatment with infliximab prevented this increase from taking place.

TNF-α is a crucial pro-inflammatory cytokine in the pathogenesis of asthma. Once released in the airways, TNF-α can induce an enhanced release of pro-inflammatory/chemotactic mediators and an up-regulation of adhesion molecules, thus facilitating the migration of eosinophils and neutrophils (27). These cells then become primed for mediator secretion, something that ultimately leads to the development of chronic inflammation and irreversible airway remodeling (28). TNF-α has also been implicated in the regulation of airway smooth muscle contraction via its ability to modulate the release of other cytokines/chemokines and by affecting the expression of various adhesion molecule (4, 29).

Anti-TNF-α therapy is wildly used clinically today for the treatment of rheumatoid arthritis, psoriasis, inflammatory bowel disease and other chronic inflammatory diseases. It has been suggested that infliximab might also be effective for the treatment of severe asthma in humans (30), but the outcomes of clinical trials on other anti-TNF-therapy in asthma vary and issues about their risk-benefit ratio have been raised (31). It is therefore important to better understand the mechanisms behind the actions of anti-TNF-α antibodies to be able to identity potential beneficiates of the therapy. Results from our asthmatic model E3 rats agree well with other results from a murine model of acute asthma showing that similar concentrations of infliximab decreases BALF cell count and a range of inflammatory cytokines in OVA/alum-sensitized mice (33). Sensitization with house dust extract also increased pulmonary inflammation as measured from BALF as well as *in vivo* reactivity to metacholine (32), but

surprisingly, despite the reduction in airway hyperreactivity and BALF cell count, anti-TNF-α antibody did not decrease T_h2 cytokines in BALF, whereas IL-4, IL-5 and IL-13 in lung homogenate were all significantly decreased by infliximab in our studies. This could be due to the differences between lung homogenate and BALF or the difference in sensitization protocols and treatment regimes used since these are shown to be critical for obtaining optimal effect of anti-TNF-α treatment (32).

As described above, many pre-clinical studies have focused on the anti-TNF-α therapy. Different from other researches, we studied the *ex vivo* bronchial smooth muscle reactivity to different agonists but not the *in vivo* lung function tests. As to AHR, inflammatory cytokines and mediators, airway remodeling, neural reflexes and dysfunction of airway smooth muscle have been postulated to contribute to its development in patients with asthma (33). There are studies showing that *in vivo* airway hyperreactivity is in accordance with the *ex vivo* smooth muscle reactivity (34, 35), However, one study showed that *in vivo* airway responsiveness to acetylcholine in asthma mice is increased, but *ex vivo* tracheal smooth muscle contractile responsiveness to acetylcholine does not alter (26). These contradictory data suggest that in different models or animal strains, the contributory factors to *in vivo* AHR varies and the smooth muscle changes are not the only factors. It is well known that *in vivo* techniques may reflect the impact of mucus production, mucosal edema or other changes in the upper and/or lower airways. However, the *in vivo* interactions between pulmonary and systemic responses might be very complicated for the interpretation of the data obtained from *in vivo* lung functions and bronchial reactivity measurements. Furthermore, pharmacological investigations are limited by systemic side effects and pharmakokinetic issues. In fact, *in vivo* and *ex vivo* can show their respective trait.

In our experimental set-up, an obvious increased smooth muscle reactivity was observed, which can partly reflex airway hyper-responsiveness. What's more, we focused on the phenotype changes of M receptor, ET receptor(ET_A and ET_B), BK receptor(BK_1 and BK_2) and β receptor in the bronchial smooth muscle of the asthma model as AHR associates with airway dysfunction of airway smooth muscle including receptor expressions (29). In the present study, the bronchi from E3 model rats had an augmented response to the three contractile agents, ET-1, S6c and BK and a reduced response to the dilator agent, isoprenaline, showing that there is some changes of receptors in bronchial smooth muscle cells which may be dependent on intracellular pathways (14, 36) and further researches can be performed to investigate these mechanisms. Airway inflammation and mediators such as TNF-α during

asthma can facilitate the hyperreactivity. Infliximab can prevent the release of pro-inflammatory/chemotactic mediators and inflammatory process, further relieves the hyper-responsiveness. *In vitro* incubation of mice tracheal segments with TNF-α increased contractile responses to bradykinin (37) and IL-1β-induced increase in bradykinin receptors was suppressed by treatment with infliximab (13). The present study confirmed these *in vitro* results that infliximab not only restores the contractile response mediated by bradykinin B_2, ET_A and ET_B receptors but also prevents impaired relaxatory response to the beta-agonist isoprenaline.

In conclusion, our results not only support the former studies that blocking TNF-α inhibits inflammation in asthmatic animals but also show that infliximab antagonizes changes of the bronchial smooth muscle receptor phenotype, thus alleviates the development of airway hyperreactivity, providing a support for anti-TNF-α antibody as a novel and valuable asthma therapy.

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Figure legends

Figure 1

Serum IgE of E3 rats in sham-sensitized (control), sensitized with OVA/alum (model) and treated with infliximab (infliximab). Results are represented as mean ± SEM; $n = 10$; $*P < 0.05$, $**P < 0.01$ (model versus control and infliximab).

Figure 2

Recruitment of inflammatory cells to the lung expressed as the total number of inflammatory cells and eosinophil number in BALF based on a differential count in three groups of rats; sham-sensitized (control), sensitized with OVA/alum (model) and treated with infliximab (infliximab). Results are given as mean ± SEM; $n = 10$; $*P < 0.05$, $**P < 0.01$ (model versus control and infliximab).

Figure 3

NO level of lung homogenate, serum and BALF in sham-sensitized (control), sensitized with OVA/alum (model) and treated with infliximab (infliximab). Results are given as mean ± SEM; $n = 10$; $*P < 0.05$, $**P < 0.01$ (model versus control and infliximab).

Figure 4

L-arginase and NOS activity in lung homogenates. L-arginase was measured according to Kropf (1 U: the amount of enzyme needed when 1 μM urea generated) (A) and iNOS and total NOS (B) in three groups of rats; sham-sensitized (control), sensitized with OVA/alum (model) and treated with infliximab (infliximab). Results are given as mean ± SEM; $n = 10$; $*P < 0.05$ (model versus control and infliximab).

Figure 5

Cytokines in serum and lung homogenate. The columns represent the levels of TNF-α (A), IL-4 (B), IL-5 (C) and IL-13 (D) in three groups of rats: sham-sensitized (control), sensitized with OVA/alum (model) and treated with infliximab (infliximab). Results are given as mean ± SEM; $n = 10$; $*P < 0.05$, $**P < 0.01$ (model versus control and infliximab).

Figure 6

Concentration–effect curves for four contractile agents; Cch (A), S6c (B), ET-1 (C) and BK (D) obtained on lobar-bronchial segments derived from three groups of rats; sham-sensitized (control), sensitized with OVA/alum (model) and treated with infliximab (infliximab). Each point represents the mean of all segments tested with error bars representing SEM from 10 animals. $*P < 0.05$, $**P < 0.01$ (model versus control and infliximab).

Figure 7

Concentration–relaxation curves induced by isoprenaline on 5-HT (10^{-5} M) pre-contracted lobar-bronchial segments derived from three groups of rats; sham-sensitized (control), sensitized with OVA/alum (model) and treated with infliximab (infliximab). Each point represents the mean of all segments tested. Bars represent SEM from 10 animals. $*P < 0.05$, $**P < 0.01$ (model versus control and infliximab).

Figure 8

Morphological changes induced by sensitization and infliximab treatment. Pulmonary sections were stained with hematoxylin–eosin representing three groups of rats; sham-sensitized (A and B), sensitized with OVA/alum (C and D) and treated with infliximab (E and F). Arrows in C indicate where there are epithelial injury and an incrassate wall of bronchus. Arrows in D indicate where there are a marked increase in inflammatory and eosinophils in the alveoli and in the pulmonary interstitium. There were much less infiltrative changes and more complete epithelium in the infliximab-treated group.

Figure 1

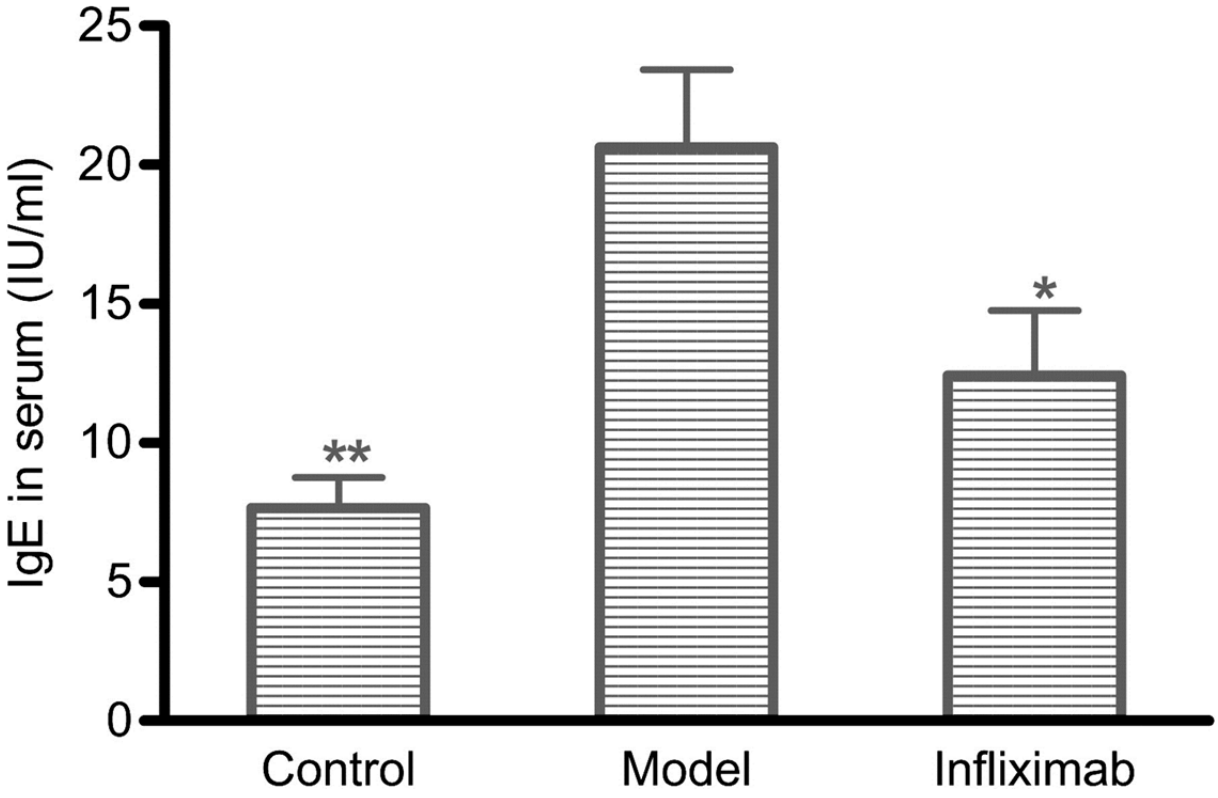

Figure 2

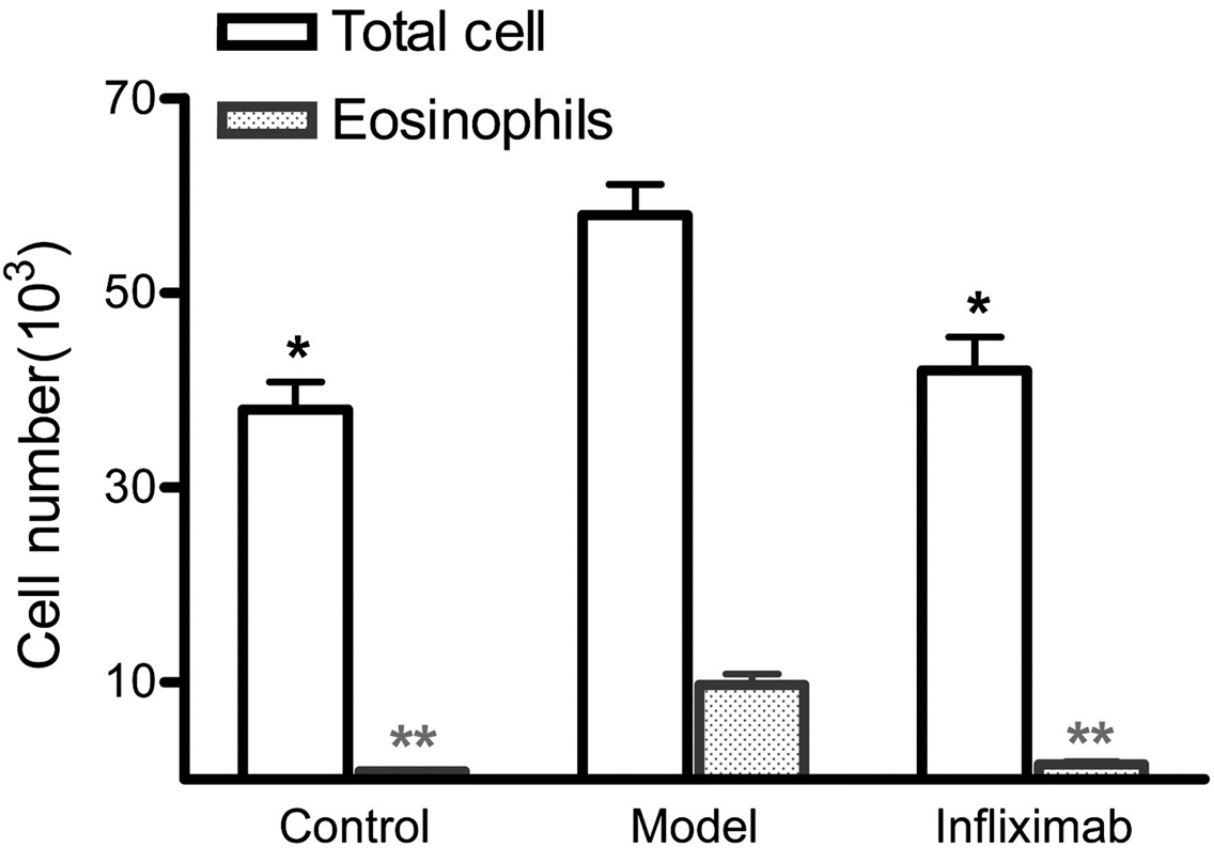
Total cell
Eosinophils
70
50
30
10
Cell number(10^3)
*
**
*
**
Control
Model
Infliximab

Figure 3

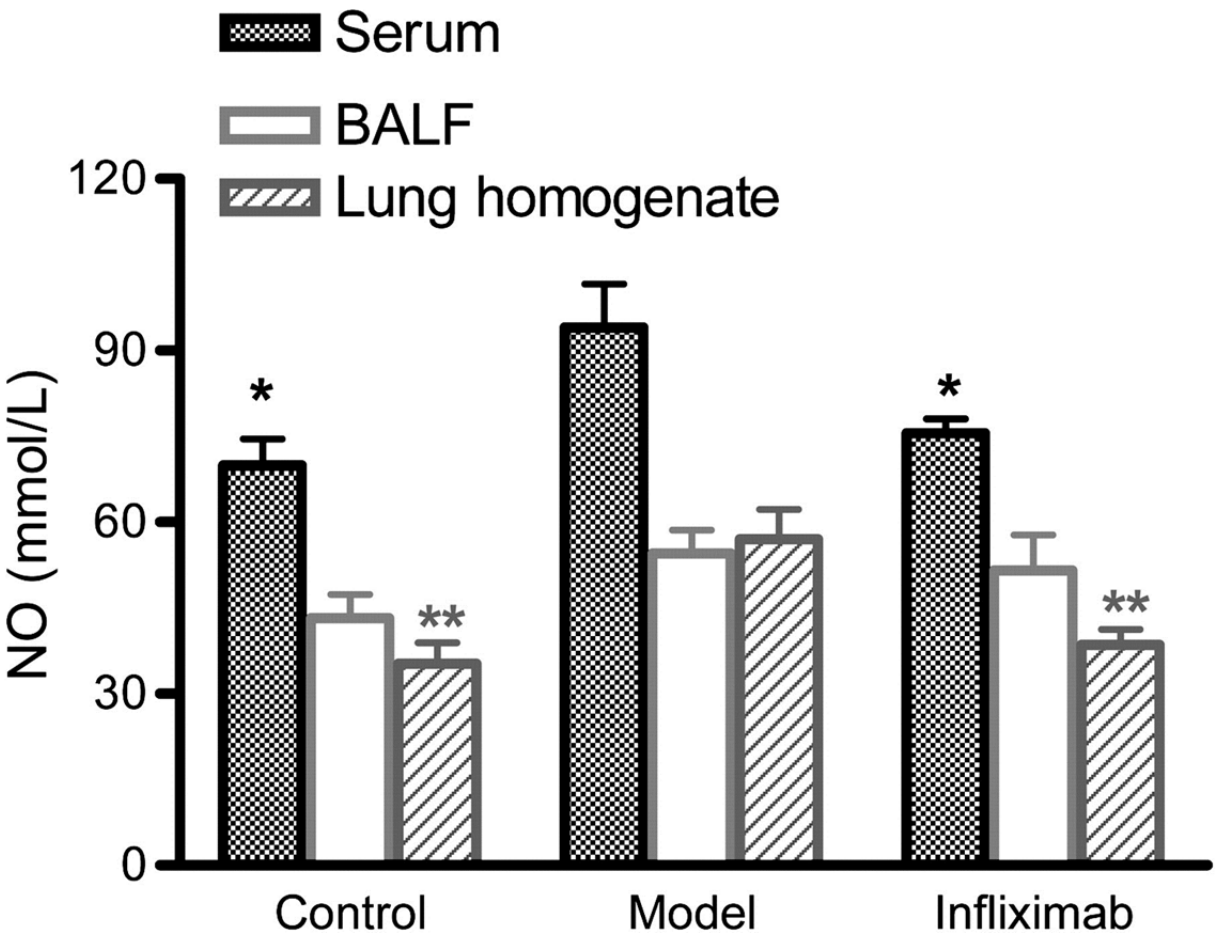
Serum
BALF
Lung homogenate
NO (mmol/L)
120
90
60
30
0
*
**
*
**
Control
Model
Infliximab

Figure 4

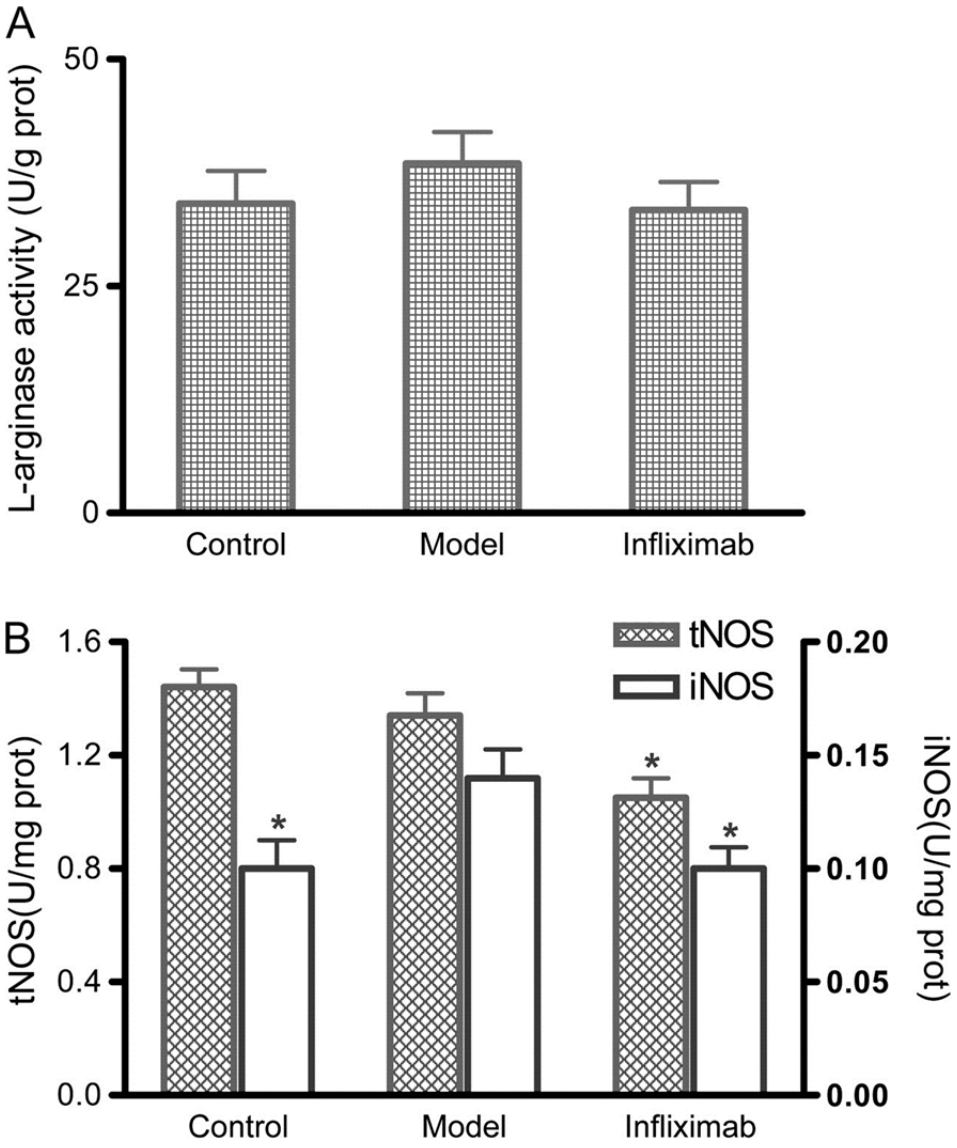
A
L-arginase activity (U/g prot)
50
25
0
Control
Model
Infliximab
B
tNOS
iNOS
tNOS(U/mg prot)
iNOS(U/mg prot)
1.6
1.2
0.8
0.4
0.0
0.20
0.15
0.10
0.05
0.00
*
*
*
Control
Model
Infliximab

Figure 5

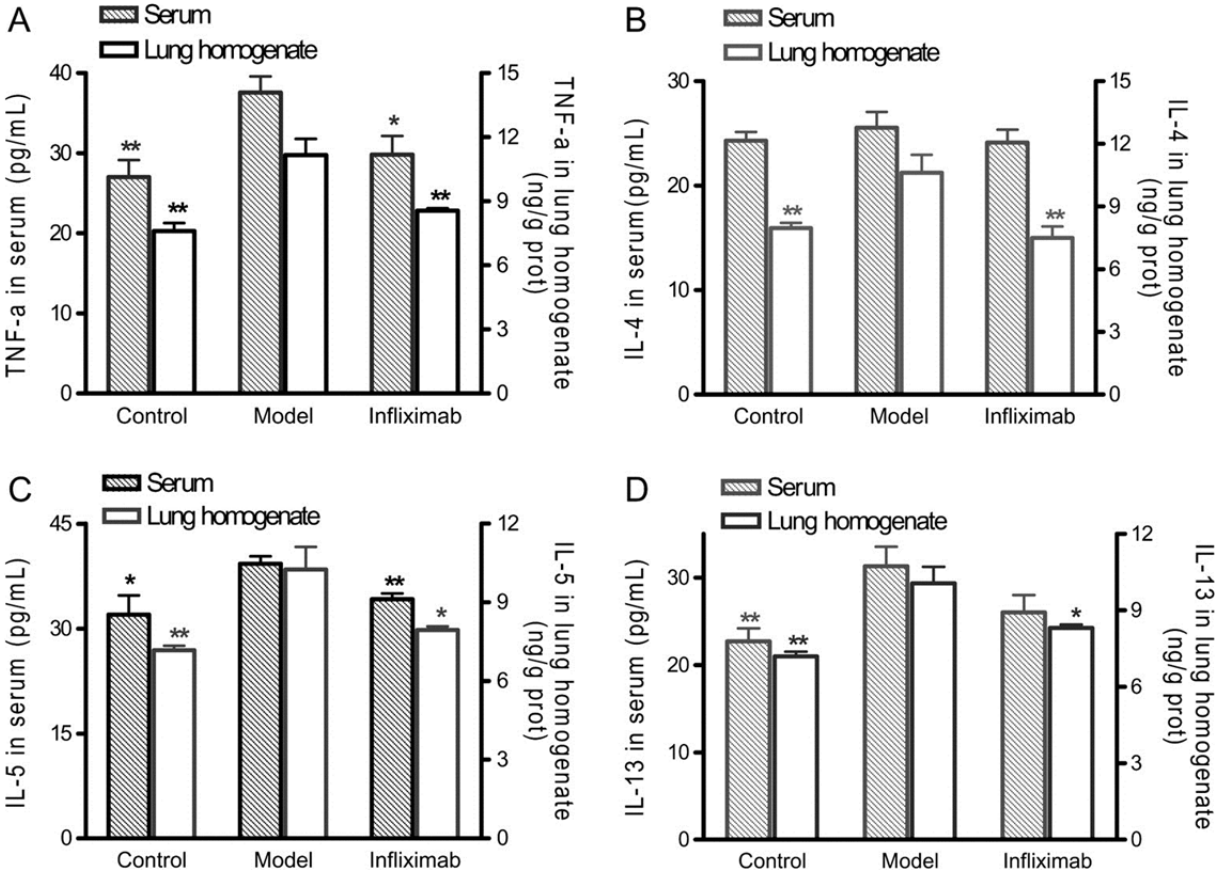
A
Serum
Lung homogenate
TNF-a in serum (pg/mL)
TNF-a in lung homogenate (ng/g prot)
Control
Model
Infliximab
B
IL-4 in serum(pg/mL)
IL-4 in lung homogenate (ng/g prot)
C
IL-5 in serum (pg/mL)
IL-5 in lung homogenate (ng/g prot)
D
IL-13 in serum (pg/mL)
IL-13 in lung homogenate (ng/g prot)

Figure 6

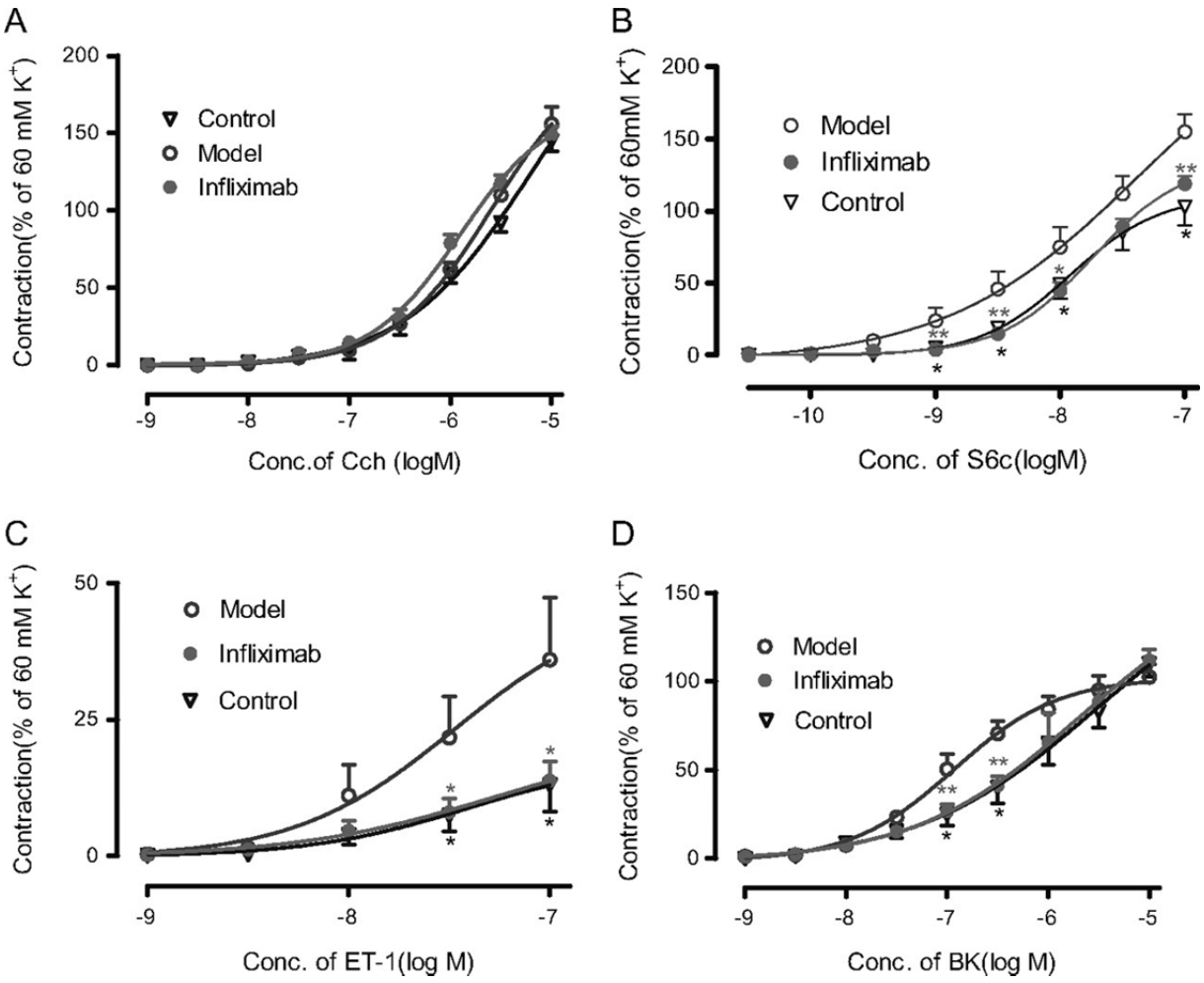
A
Contraction(% of 60 mM K+)
200
150
100
50
0
Control
Model
Infliximab
-9
-8
-7
-6
-5
Conc.of Cch (logM)
B
Contraction(% of 60mM K+)
Model
Infliximab
Control
-10
-9
-8
-7
Conc. of S6c(logM)
C
Contraction(% of 60 mM K+)
50
25
0
Model
Infliximab
Control
Conc. of ET-1(log M)
D
Contraction(% of 60 mM K+)
150
100
50
0
Model
Infliximab
Control
Conc. of BK(log M)

Figure 7

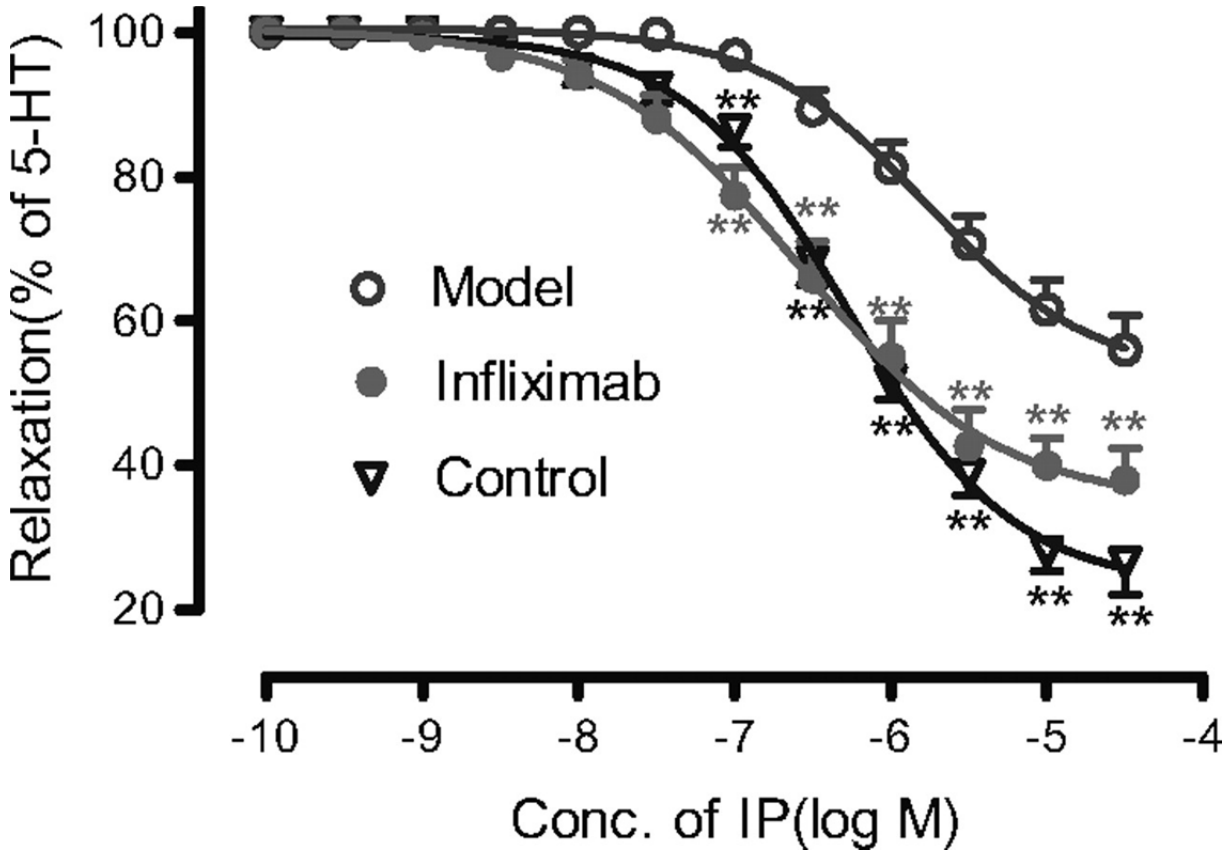
Relaxation(% of 5-HT)
100
80
60
40
20
Model
Infliximab
Control
**
-10
-9
-8
-7
-6
-5
-4
Conc. of IP(log M)

Figure 8