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Role of adiponectin in sphingosine-1-phosphate induced airway hyperresponsiveness and inflammation

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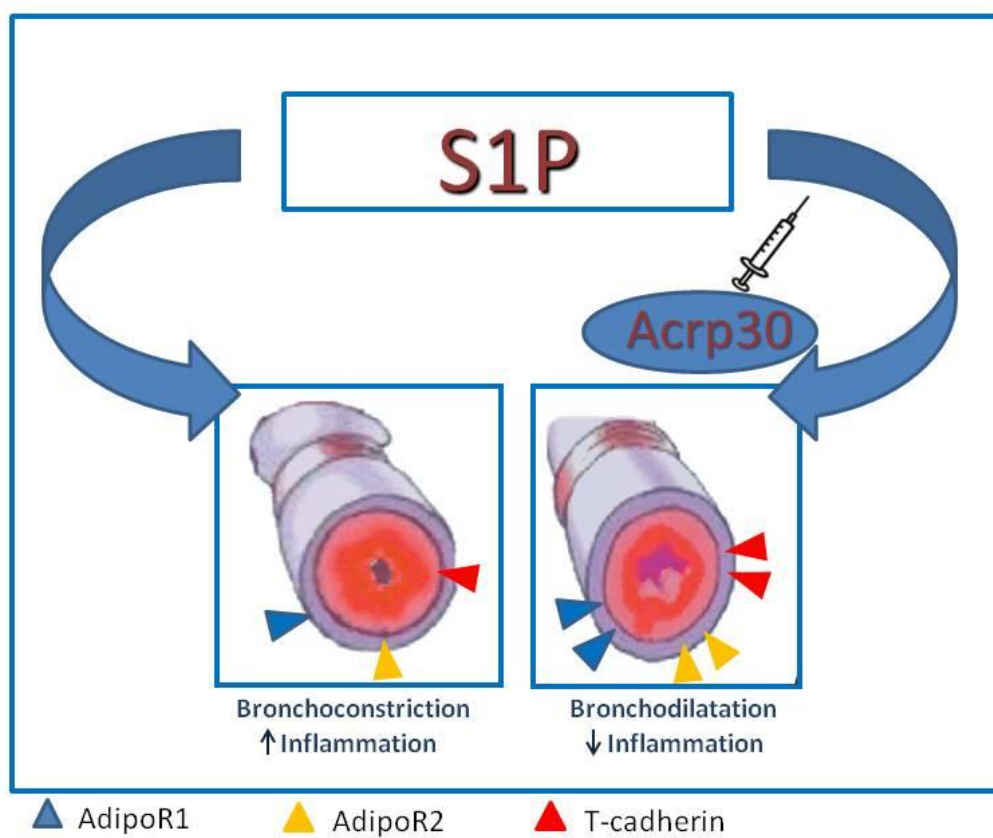
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Graphical abstract



Abstract

Epidemiological data suggest that obesity represent an important risk factor for asthma, but the link between excess fat and airway hyperresponsiveness (AHR) and inflammation is not fully understood. Recently, a key role in physiopathologic conditions of lungs has been given to adiponectin (Acrp30). Acrp30 is one of the most expressed adipokines produced and secreted by adipose tissue, showing an intriguing relationship with metabolism of sphingolipids. Sphingosine-1-phosphate (S1P) has been proposed as an important inflammatory mediator implicated in the pathogenesis of airway inflammation and asthma. In the present study we analyze the effects of recombinant Acrp30 administration in an experimental model of S1P-induced AHR and inflammation. The results show that S1P is able to reduce endogenous Acrp30 serum levels and that recombinant Acrp30 treatment significantly reduce S1P-induced AHR and inflammation. Moreover, we observed a reduction of Adiponectin receptors (AdipoR1, AdipoR2 and T-cadherin) expression in S1P treated mice. Treatment with recombinant Acrp30 was able to restore Acrp30 serum levels and adiponectin receptors expression. These results could indicate the ability of S1P to modulate the Acrp30 action, by modulating not only the serum levels of the protein, but also its receptors. Taken together, these data suggest that adiponectin could represent a possible biomarker in obesity-associated asthma.

Keywords: airway hyperresponsiveness; inflammation; S1P; adiponectin; adiponectin receptors

Chemical compounds studied in this article

Sphingosine 1-phosphate (PubChem CID: 5283560)

1.Introduction

Asthma has been viewed for a long time as a single disease entity, but currently it is considered a syndrome characterized by intermittent airway obstruction, bronchial hyperreactivity and chronic airway inflammation (1). The rapid increase of asthma prevalence in recent years has a considerable impact on public health. Furthermore, obesity represents a risk factor not only for the onset but also for the severity of asthma in adult as well as in children (2-5). Until now, the link between excess fat and asthma is not fully understood but deregulation of adipokines secretion by adipose tissue plays a crucial role (6). Adiponectin (Acrp30) is one of the most expressed adipokines produced and secreted by adipose tissue. Acrp30 plays a key role in metabolic and inflammatory disorders (6); recently, a role for Acrp30 has been evidenced also in physiopathologic conditions of lung (7). *In vivo*, excess fat induces airway hyperresponsiveness (AHR), enhances allergen or ozone-induced AHR (8) but the precise mechanisms linking obesity with airway inflammation and AHR have not been understood. Acrp30 acts through its receptors (AdipoR1 and AdipoR2 and T-cadherin) widely expressed in several organs, tissues and cell lines (9, 10, 11). Recently, an intriguing potential relationship has been evidenced between Acrp30 and metabolism of sphingolipids. Both AdipoR1 and AdipoR2 stimulate ceramidase to degrade ceramide to sphingosine, converting it into sphingosine 1-phosphate (S1P). S1P has been proposed as an important inflammatory mediator implicated in the pathogenesis of airway inflammation and asthma. At present, there is a number of clinical and preclinical evidences that support a role for S1P in asthma (12-15). Elevated S1P levels have been reported in lung of patients with allergic asthma (16-20). *In vivo*, S1P promotes the contraction of airway smooth muscle cells, it induces AHR by S1PR1 and/or S1PR3 (21), and regulates activation and function of mast cells, eosinophils and dendritic cells. *Viceversa*, inhalation of a SphK1 inhibitor mitigated asthma in rodent models (22). In the present study, in order to further analyze the role of Acrp30 in AHR and its interaction with sphingolipid metabolism, we examined the effects of recombinant Acrp30 administration in an experimental rodent model of S1P-induced AHR.

2.Materials and Methods

2.1 Acrp30 recombinant production

Total mRNA from visceral adipose tissue was extracted and retrotranscribed into cDNA (Qiagen); the full-length Acrp30 was amplified using PCR primers (5'-ACTGAGGATCCGATCCAGGTCTTATTGGTCCTAAGGGAGAC-3'; 5'-CGATAGCGGCCGCTTAGCCTTGGATTCCCGGAAAGCCTCG-3') which introduce a BamHI restriction site at the 5' end, and a stop codon and NotI site at the 3' end. PCR products were

digested with BamHI and NotI (New England Biolabs) and ligated into vector pET15b (EMD Chemicals) to create plasmid pET15b-His-(AdipoQ). Positive clones were sequenced to verify the introduction of the correct Acrp30 cDNA sequence. Acrp30 was purified using Hig-tag proteins purification kit (Macherey-Nagel) according to instructions and concentrated using a 8 kDa cutoff Amicon protein concentrator (Millipore).

2.2 Animals

Balb/c mice were purchased by Charles River (Lecco, Italy). The animals were housed in a controlled environment and provided with standard rodent chow and water. All mouse strains (20-25 g) were housed with a 12h light dark cycle and were allowed food and water *ad libitum*. Animal care was in compliance with Italian regulations on protection of animals used for experimental and other scientific purposes (DLgs 26/2014, application of the EU Directive 2010/63/EU) as well as with the EEC regulations (O.J. of E.C. L 358/1 12/18/1986). All studies were performed in accordance with European Union regulations for the handling and use of laboratory animals and were approved by the local committee.

2.3 Experimental protocol

Mice received subcutaneous (s.c.) injection of 0.1ml of S1P (10ng; Enzo Life Science, Italy) dissolved in sterile saline containing Bovine Serum Albumin (BSA 0,001%) according to the manufacturer's instructions. Briefly, S1P is dissolved in sterile saline containing BSA fatty acid free at the concentration of 1mg/ml. The stock solution obtained has been used to perform the serial dilutions in sterile saline (the final concentration of BSA is 0.001%). S1P or vehicle (control group) was administered at 0 and 7 days (Fig. 1 A and B). In S1P treated animals, Alzet micro-osmotic pumps were implanted subcutaneously in the intrascapular region at day 0. The pumps daily infused solutions (Acrp30 or saline) 0.5 μ L/h for 10 consecutive days (Fig. 1B). Mice from each experimental group were anesthetised and subjected to euthanasia at different time points. The details of both experimental protocols are reported in Figure 1.

2.4 Tissue Preparation and Bronchial Reactivity Assay

Mice were sacrificed at 21 days. Bronchi were rapidly dissected and cleaned from fat and connective tissue. Rings of 1-2mm length were cut and placed in organ baths (2,5ml) filled with oxygenated (95% O₂–5% CO₂) Krebs solution at 37°C and mounted to isometric force transducers (type 7006, Ugo Basile, Comerio, Italy) and connected to a Powerlab 800 (ADInstruments). Rings were initially stretched until a resting tension of 0.5g was reached and allowed to equilibrate for at least 30 min during which tension was adjusted, when necessary, to a 0.5g and bathing solution was

periodically changed. In a preliminary study a resting tension of 0.5g was found to develop the optimal tension to stimulation with contracting agents. In each experiment bronchial rings were previously challenged with acetylcholine (10^{-6} mol/L) until the responses were reproducible. Subsequently, bronchi were challenged with carbachol (10^{-8} - 10^{-5} M).

2.5 Lung Reactivity Assay

Lung reactivity was assessed, at day 21, using an isolated and perfused mouse lung model (15). Lungs were perfused in a non-recirculating fashion through the pulmonary artery at a constant flow of 1 ml/min resulting in a pulmonary artery pressure of 2-3 cm H₂O. The perfusion medium used was RPMI 1640 lacking phenol red (37° C). The lungs were ventilated by negative pressure (-3 and -9 cm H₂O) with 90 breath min⁻¹ and a tidal volume of about 200μl. Every 5 min a hyperinflation (-20 cm H₂O) was performed. Artificial thorax chamber pressure was measured with a differential pressure transducer (Validyne DP 45-24) and airflow velocity with pneumotachograph tube connected to a differential pressure transducer (Validyne DP 45-15). The lungs respired humidified air. The arterial pressure was continuously monitored by means of a pressure transducer (Isotec Healthdyne) which was connected with the cannula ending in the pulmonary artery. All data were transmitted to a computer and analysed with the Pulmodyn software (Hugo Sachs Elektronik, March Hugstetten, Germany). The data were analysed through the following formula: $P = V \cdot C^{-1} + R_L \cdot dV \cdot dt^{-1}$, where P is chamber pressure, C pulmonary compliance, V tidal volume, R_L airway resistance. The airway resistance value registered was corrected for the resistance of the pneumotacometer and the tracheal cannula of 0.6 cm H₂O s ml⁻¹. Lungs were perfused and ventilated for 45 min without any treatment in order to obtain a baseline state. Subsequently, lungs were challenged with carbachol (10^{-8} - 10^{-3} M). Repetitive dose response curve of carbachol was administered as 50μl bolus, followed by intervals of 15 min, in which lungs were perfused with buffer only.

2.6 Total serum Acrp30 assay

Total serum Acrp30 was measured by enzyme-linked immunosorbent assay (ELISA) method utilizing a polyclonal antibody, in house produced, versus a human Acrp30 amino acid region (H2N-ETTTQGPVLLPLPKG-COOH) as previously reported (7). A human recombinant Acrp30 was used as the standard (Phoenix Pharmaceuticals, Burlingame, CA). Each serum sample was tested three times in triplicate.

2.7 Protein extraction and Western Blotting

Mice tissues were lysed and homogenized in RIPA buffer (20mM Tris-HCl pH 7.5, 150 mM NaCl, 10% glycerol, 1 mM Na₃VO₄, 1 mM NaF, 1% Triton X100, 0.1% SDS, 1% Na deoxycholate) containing 1mM PMSF and protease inhibitors. Proteins were quantified by the Bradford method. 25 µg of proteins extracted were dissolved in 1X Laemmli buffer and separated using 10% SDS-PAGE gel and the electrophoresis was performed as previously described (7). Incubation with AdipoR1 (Novus Biologicals, CO, USA), AdipoR2 (Novus Biologicals, CO, USA), T-cadherin (Abcam, UK) primary antibodies was performed according to the manufacturer's instructions. Membranes were normalized after incubation with GAPDH primary antibody (Sigma-Aldrich, MO, USA). Finally, the blots were developed and pixel quantization was performed with Image J software (<http://rsbweb.nih.gov/ij/>). Each sample was tested two times in triplicate.

2.8 RNA Extraction and Real-Time PCR

Lung and adipose tissues were harvested and immediately frozen at -80°C. RNA was isolated using TRIzol (Invitrogen Life Technologies, CA, USA) according to the manufacture's instructions and quantified by absorbance reading at 260 and 280 nm. Real Time quantitative PCR was performed using standard protocols with a 7900HT Fast Real-time system instrument (Applied Biosystems, CA, USA) using primers designed with PRIMER 3 software. The primers and PCR protocol are available on request. We used the comparative Ct method to quantify mRNAs. The GAPDH was used as a housekeeping gene. Gene expression data were obtained using the Δ Ct method. Each mRNA sample was tested two times in triplicate in all tissues.

2.9 Cytokines measurements

Pulmonary IL-4 (R&D system, UK) and IL-13 (eBioscience, CA, USA) expression was determined by ELISA according to the manufacturer's instruction. Lung tissues were homogenized in 500 µl of lysis buffer (20 mM HEPES, 0.4 mM NaCl, 1.5 mM MgCl₂, 1 mM EGTA, 1 mM EDTA, 1% Triton X-100, and 20% glycerol) with protease inhibitors (1 mM DTT, 0.5 mM PMSF, 15 µg/ml Try-inhibitor, 3 µg/ml pepstatin-A, 2 µg/ml leupeptin, and 40 µM benzamidine). The supernatant recovered was used for IL-4 or IL-13 measurement.

2.10 Histochemistry

Lungs were perfused and fixed in 10% phosphate-buffered formalin. Tissue was embedded in paraffin and cut in 5 µm sections for histological analysis. The sections were stained with a modified HE protocol (23) and the number of eosinophils per mm² of the peribronchial tissue was

measured. Samples were analyzed with a Leica microscope using a 40x and 100x oil-immersion objective.

2.11 Statistical analysis

Data are expressed as means $\pm$ SE. Comparisons between control and S1P-treated mice were compared with 2-tailed unpaired Student t-test. Multiple comparisons among control, S1P-treated mice, and S1P+Adiponectin-treated mice were performed by ANOVA test, followed by Bonferroni post-test. Analyses were performed using the StatView 5.0.1.0 and GraphPad Prism 4 software. The statistical significance was established at $p < 0.05$.

3. Results

3.1 S1P effect on Acrp30 expression in serum and adipose tissue

Mice treated with vehicle or S1P were sacrificed at different time points (7, 14 and 21 days after challenge). Acrp30 expression was quantified in the serum. Statistical analysis revealed that Acrp30 expression was significantly reduced in S1P treated mice sacrificed at day 21 compared to vehicle treated mice (3 ± 0.13 vs 3.7 ± 0.5 $\mu\text{g/ml}$) (Fig. 2A). On the contrary, we didn't find any statistical difference in the expression of Acrp30 in adipose tissue harvested from S1P treated mice when compared to control mice (Fig. 2B).

3.2 AdipoRs expression in lung and adipose tissues of S1P treated mice

In order to further clarify the role of Acrp30 in S1P induced effects on lung function, AdipoRs expression was analyzed in lung tissues harvested at different time points e.g. 7, 14 and 21 days from the first S1P administration. Western Blotting analysis revealed that AdipoR1, AdipoR2 and T-cadherin were reduced in S1P treated mice compared to control mice only 21 days after the S1P challenge (Fig. 3A-C). In order to assess if the altered expression of AdipoRs induced by S1P in the serum was present in adipose tissues also, AdipoRs expression was evaluated in visceral adipose tissues harvested from S1P treated mice. Western blotting analysis revealed that AdipoR1 was significantly increased in S1P-treated mice at day 21 (Fig.4A), while AdipoR2 did not show any significant difference at all time points analyzed (Fig.4B).

3.3 Effect of Acrp30 on bronchial and lung reactivity

In S1P treated mice we observed a significant increase of bronchial and lung reactivity. In order to assess a correlation between decreased expression of endogenous Acrp30 and hyperresponsiveness

present in S1P treated mice, we evaluated the effect of the recombinant Acrp30 administration on bronchial reactivity and lung resistance. Both in vitro, on bronchi (Fig 5A) and in ex vivo, on isolated and perfused lungs (Fig 5B) results showed that Acrp30 daily administration for 10 consecutive days significantly reduce S1P-induced hyperresponsiveness.

3.4 Effect of Acrp30 on lung and adipose tissue AdipoRs expression

Acrp30 administration in S1P treated mice significantly increased AdipoR1, AdipoR2 and T-cadherin expression in lung tissues, with respect to S1P treated mice ($p<0.05$) (Fig. 6A, B and C). Real time PCR confirmed the western blotting results, showing a significant increase of AdipoRs and T-cadherin mRNA expression with respect to S1P treated mice (Fig. 6D, E and F). Western blotting analysis from adipose tissues revealed that Acrp30 treatment significantly increased AdipoR1 expression with respect to S1P treated mice ($p<0.05$) (Fig. 7A) while AdipoR2 expression does not show significant difference between two groups (see Fig. 7B). Real time PCR confirmed the increase of AdipoR1 expression in Acrp30 treated mice with respect to S1-P treated mice (Fig. 7C). On the contrary, Acrp30 did not induce any statistical difference in the expression of AdipoR2 at mRNA level (Fig. 7D).

3.5 Effect of Acrp30 on cytokines levels and number of eosinophils

In order to investigate the role of Acrp30 in inflammation, pro-inflammatory cytokine (IL-4 and IL-13) levels assays was performed. In pulmonary tissues, S1P treatment caused a significant increase of both assayed cytokines with respect to vehicle group. Pro-inflammatory cytokine levels, IL-4 (Fig. 8A) and IL-13 (Fig. 8B), were significantly reduced by Acrp30 treatment. A significant accumulation of eosinophils was present in peribronchial region in the animals exposed to S1P and was markedly lowered following Acrp30 treatment (Fig. 8C-F). These data confirmed a role of Acrp30 in inflammatory processes linked to AHR.

Discussion

Our results show that S1P-treated mice had a 19% reduction in endogenous adiponectin serum levels with respect to vehicle treated mice, coupled to a significant increase in the airway responsiveness and Th2 cytokines expression in the lungs. Treatment of mice with recombinant Acrp30 significantly reduced the S1P-induced airway AHR and inflammation.

Epidemiological data indicate an increased prevalence of asthma in obese subjects (24-28) and that in obesity, adipokine levels are largely modified (29); this deregulation could represent a possible biological mechanism that correlates adipose tissue to the lung dysfunction (30).

In our study, the S1P-induced reduction in serum adiponectin levels does not reflect the reduction of protein expression in adipose tissue, the primary source of adiponectin; in fact, adiponectin mRNA expression in adipose tissue was not modified in S1P-treated mice. Since we evaluated mRNA expression levels only at 21st day, we hypothesized that S1P, as documented in literature, induced a reduction in protein expression in the adipose tissue, but we were not able to appreciate this reduction at our end point. A role for S1P in airway inflammation and AHR has been documented in several animal model of asthma, including ours (15, 17, 31). Several studies documented a role of adiponectin and AdipoR1 and AdipoR2, in stimulating a ceramidase activity in the conversion of ceramide to its anti-apoptotic metabolite, S1P (32-34). Therefore, the bioactive sphingolipid metabolite S1P, has been chosen to study the possible involvement of adiponectin in obesity and asthma. At the airway level, in ex vivo and in vitro studies, a daily Acrp30 administration through continuous infusion significantly reduced the S1P induced AHR. Moreover, proinflammatory cytokine (IL-4 and IL-13) levels and tissue eosinophils were also significantly reduced by recombinant Acrp30 in S1P treated mice. These data are in agreement with several studies in literature. In particular, Shore et al. have showed the adiponectin capacity to attenuate airway inflammation and hyperresponsiveness in OVA sensitized mice (25). While, an interesting study showed that adiponectin deficiency increases allergic airway inflammation and pulmonary vascular remodeling (34). Finally, several studies documented the ability of adiponectin to inhibit cytokines (TNF- α , IL-6 and NF- κ B) (36-38) and to induce anti-inflammatory cytokines (IL-10 and IL-1 receptor antagonist) (38-40).

Adiponectin actions are mediated by its receptors, AdipoR1, AdipoR2 widely expressed in adipose tissue and in several other organs and tissues (lung, kidney, muscle, liver, brain, etc.) (9,10). A third adiponectin binding protein, T-cadherin, is largely expressed in cardiovascular system and in brain, lung, kidney and muscle (11, 41). Therefore, in our study we examined the AdipoR1 and AdipoR2 expression in lung and adipose tissues, while T-cadherin expression in lung tissues only.

Our results showed a significant reduction in AdipoR1, AdipoR2 and T-cadherin expression in lung tissue of S1P- treated mice. The reduction in adiponectin binding proteins expression, together with the reduction of adiponectin serum levels, suggests that S1P-treatment might reduce the positive effects of adiponectin on the lungs by attenuating the components involved in adiponectin signaling pathways. These data are in agreement with Shore et al. that showed a significant reduction in lung expression levels of AdipoRs in allergen exposed mice (5). Otherwise, in our model, Acrp30 administration restored adiponectin serum levels and adiponectin receptors expression with respect to vehicle treated mice. These data indicate the ability of S1P to modulate the adiponectin action, by modulating not only adiponectin serum levels but also its binding proteins.

Interestingly, in adipose tissue AdipoR1 expression was significantly increased in S1P treated mice with respect to vehicle, while no differences were found in AdipoR2 expression. Yamauchi T et al., using AdipoR1- and AdipoR2-knockout mice, showed apparent functional differences between AdipoR1 and AdipoR2 in adiponectin signaling. In particular, in the liver, AdipoR1 is more tightly involved in metabolic process through AMPK pathways activation, while AdipoR2 is more closely involved in inflammatory inhibition through PPAR- α pathways activation (42). Moreover, a recent *in vitro* study evidenced that in lung cells the anti-inflammatory role of adiponectin is mediated by AdipoR1, while AdipoR2 seems not be involved (39). Therefore, it not clear which are the exact functional roles of the different adiponectin receptors. These great differences in the role and involvement of adiponectin receptors according not only to the physio-pathologic processes, but also to the specific tissues and organs, could explain the different modulation of the receptors between lung and adipose tissue in our experimental model.

In summary, our results indicate that S1P is able to decrease endogenous levels of adiponectin and that exogenous adiponectin administration inhibit AHR and inflammation in a model of S1P induced AHR through lung AdipoRs modulation. Taken together, these data suggest that adiponectin could represent a possible biomarker in obesity-associated asthma. However, to further clarify the role of adiponectin in asthma and its interaction with sphingolipid metabolism, we need to investigate molecular and cellular mechanisms.

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Conflict of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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FIGURE CAPTIONS

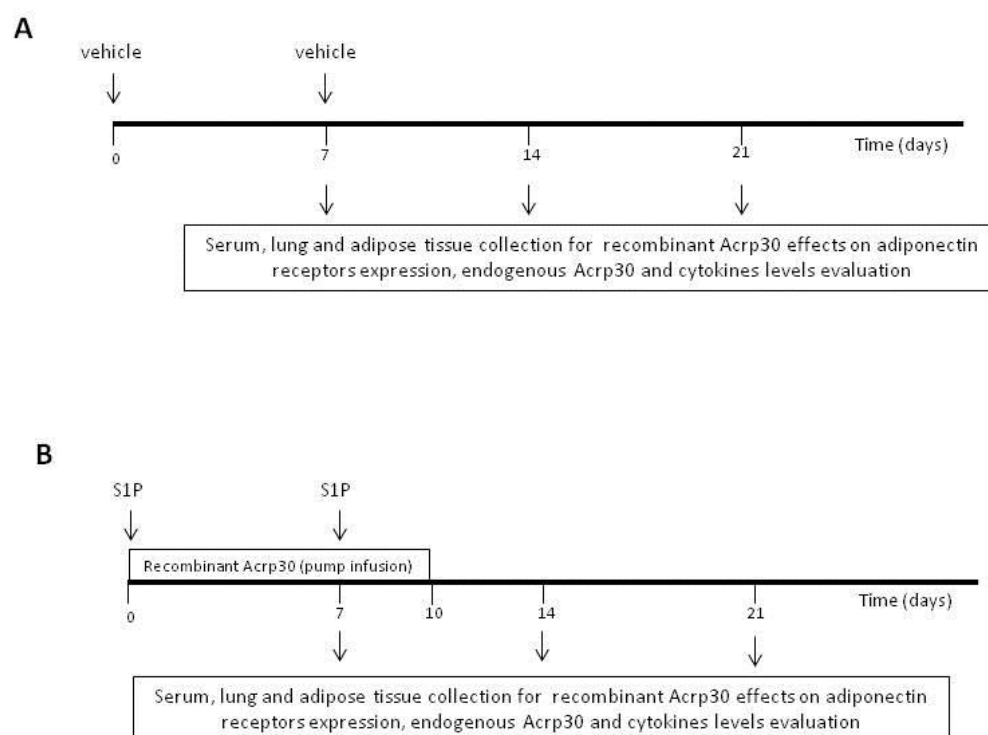


Figure 1

Fig. 1: Schematic representation of experimental protocols

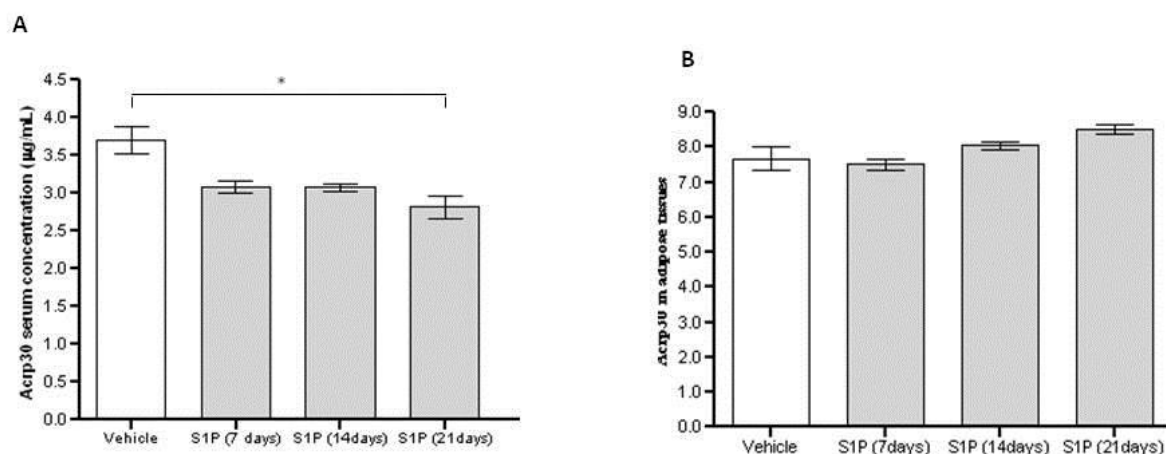


Figure 2

Fig 2: Endogenous Acrp30 levels in serum and adipose tissue from treated mice. Graphical representation of total serum Acrp30 concentration in naive and treated mice (A) and Acrp30 expression in adipose tissue (B). Values represent means $\pm$ SE of experiments performed three times in triplicate. Statistical differences are indicated with $*(p<0.05)$.

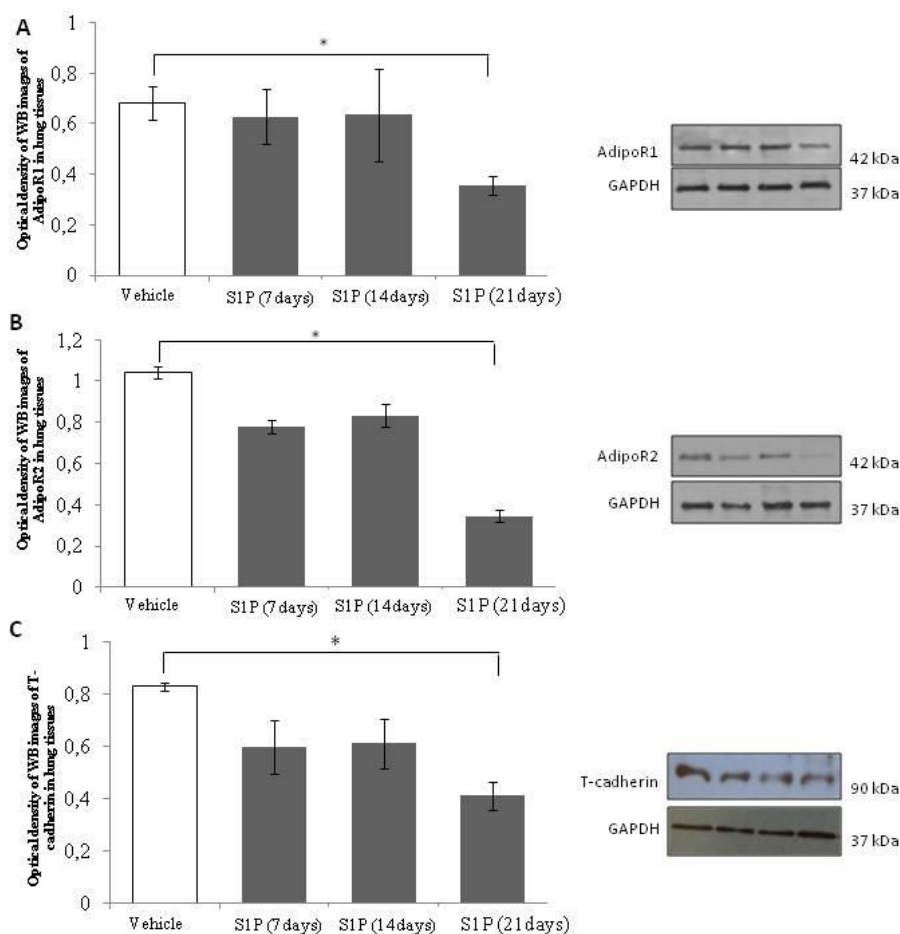


Figure 3

Fig. 3: AdipoR1, AdipoR2 and T-cadherin expression in lung tissue of mice treated with S1P. Graphical representation of pixel quantization of AdipoR1 (A) AdipoR2 (B) and T-cadherin (C) in lung tissue and representative WB image of AdipoR1, AdipoR2, T-cadherin relative to GAPDH. Data are expressed as mean of two independent experiments performed in triplicate. Statistical differences are indicated with $*(p<0.05)$.

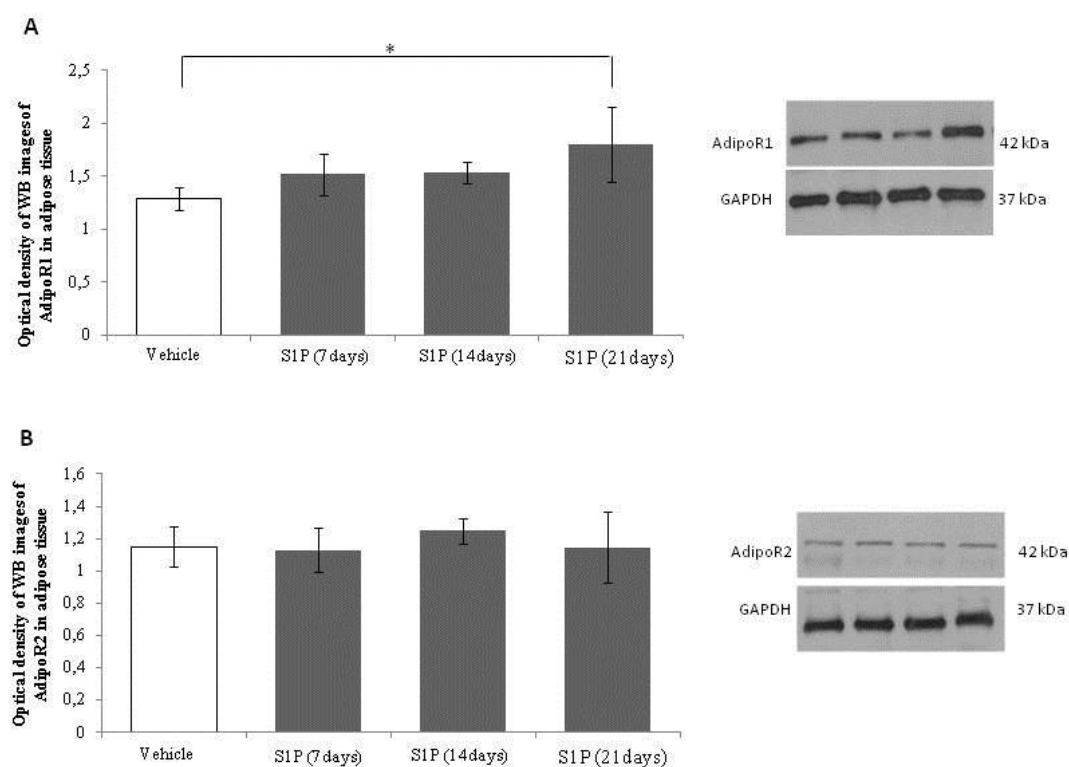


Figure 4

Fig. 4: AdipoR1 and AdipoR2 expression in adipose tissue of mice treated with S1P. Graphical representation of pixel quantization of AdipoR1 (A), AdipoR2 (B) in adipose tissue and representative WB image of AdipoR1, AdipoR2 relative to GAPDH. Data are expressed as mean of two independent experiments performed in triplicate. Statistical differences are indicated with $*(p<0.05)$.

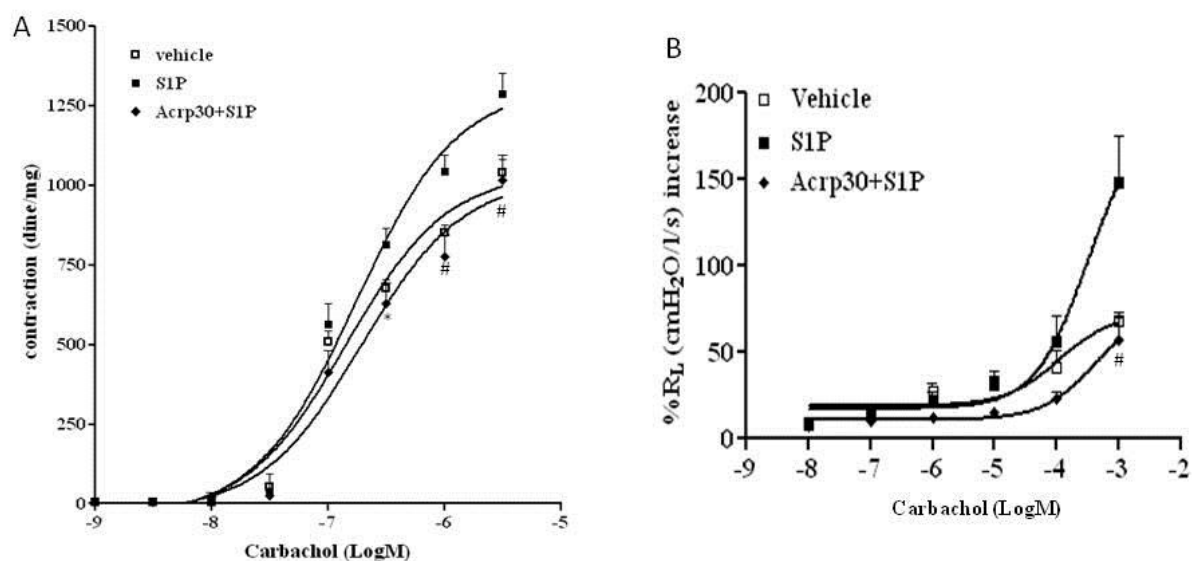


Figure 5

Fig. 5: Acrp30 effects on bronchial reactivity and airway hyperresponsiveness induced by S1P. Dose response curves to cumulative administration of carbachol in isolated bronchi (A) and in isolated perfused mouse lungs (B). Data are means $\pm$ SE (n = 6). Statistical differences are indicated with * ($p < 0.05$) and # ($p < 0.001$).

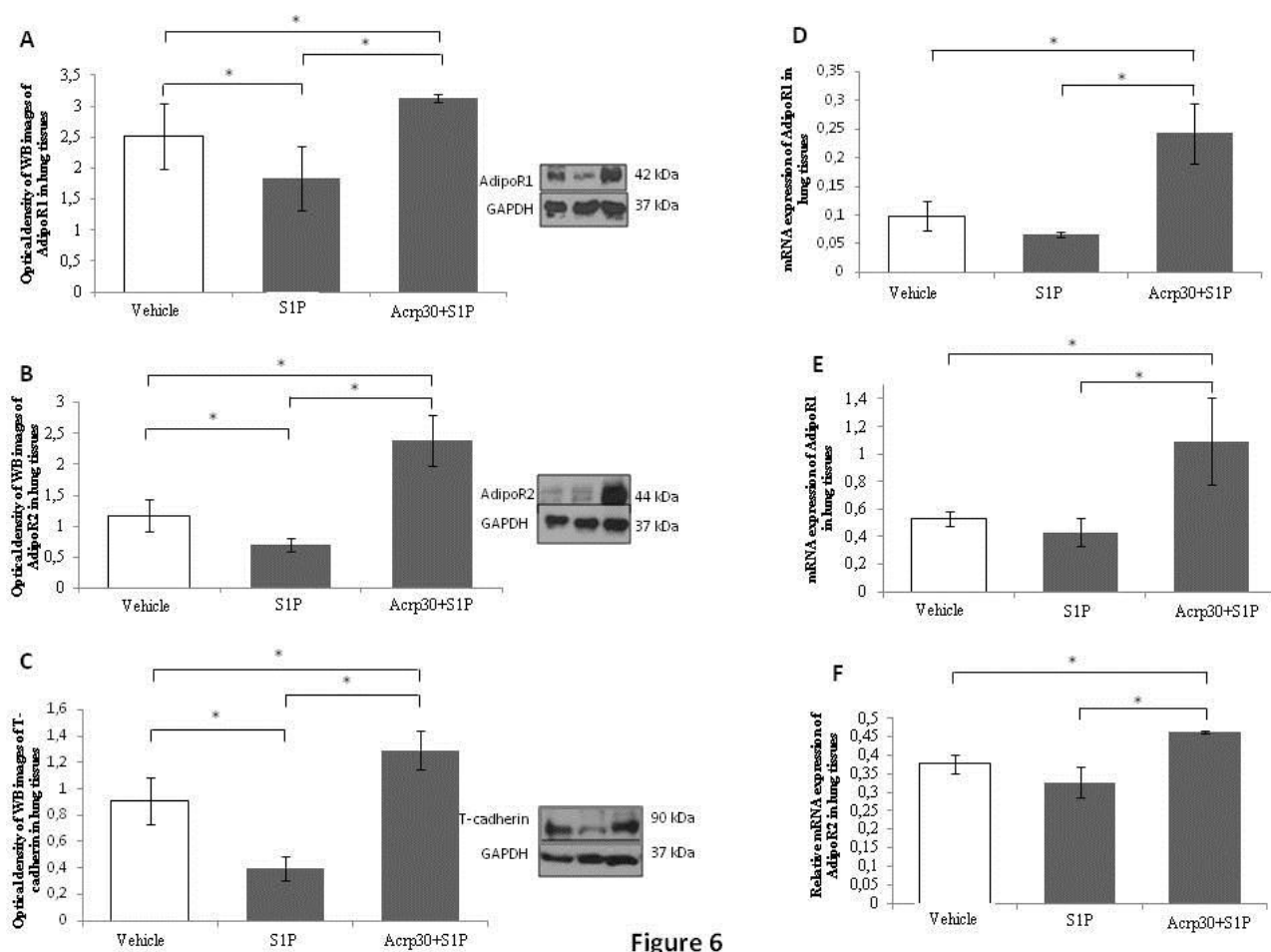


Figure 6

Fig. 6: AdipoR1, AdipoR2 and T-cadherin expression in lung tissue after Acpr30 administration. Graphical representation of pixel quantization of AdipoR1 (A) and AdipoR2 (B) and T-cadherin (C) in lung tissue and representative WB image of AdipoR1, AdipoR2 and T-cadherin relative to GAPDH. Real Time PCR analysis of AdipoR1 (D) and AdipoR2 (E) and T-cadherin (F) relative to GAPDH expression in lung tissue; data are expressed as mean of $2^{-\Delta Ct}$ of two independent experiments performed in triplicate. Statistical differences are indicated with $*(p < 0.05)$.

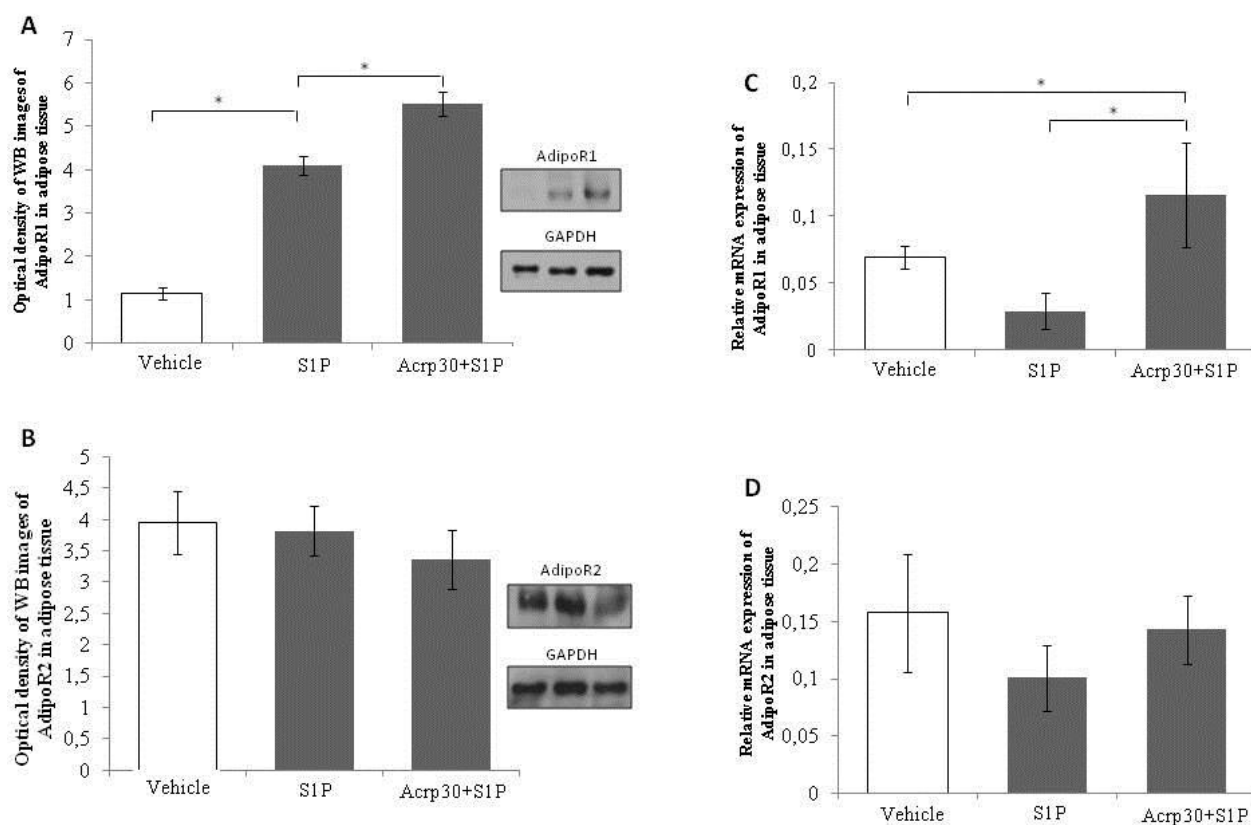


Figure 7

Fig. 7: AdipoR1, AdipoR2 expression in adipose tissue of mice treated with S1P. Graphical representation of pixel quantization of AdipoR1 (A), AdipoR2 (B) in adipose tissue and one representative WB image of AdipoR1, AdipoR2 relative to GAPDH. Real Time PCR analysis of AdipoR1 (D), AdipoR2 (E) relative to GAPDH expression in adipose tissue; data are expressed as mean of $2^{-\Delta C_t}$ of two independent experiments performed in triplicate. Statistical differences are indicated with $*(p < 0.05)$.

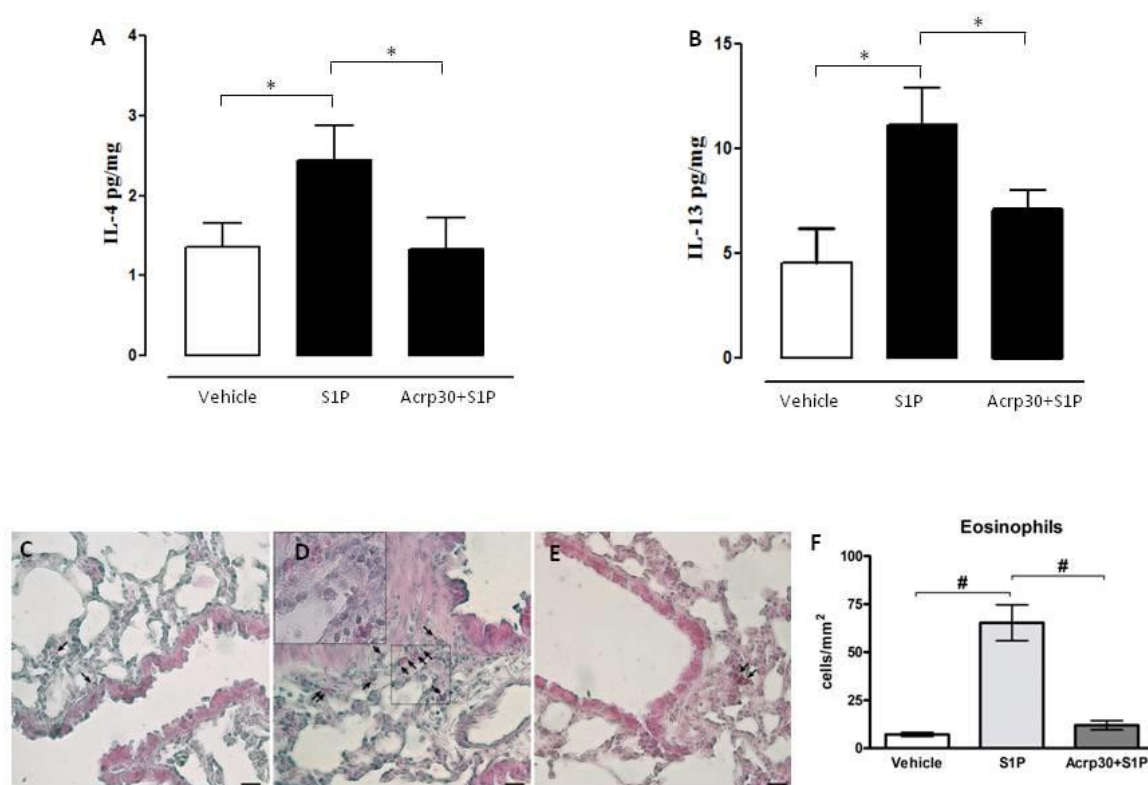
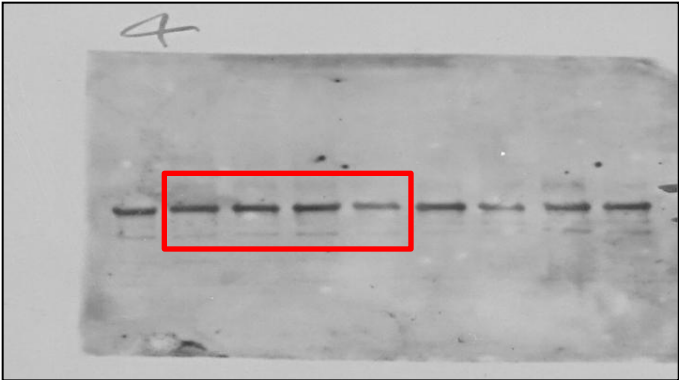


Figure 8

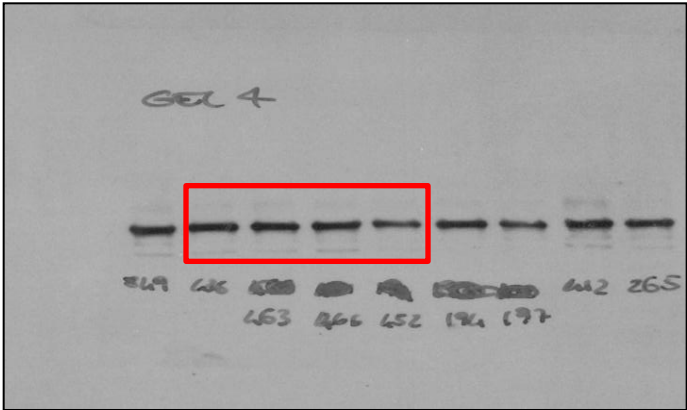
Fig. 8: Cytokines levels and eosinophils in lung tissues. Graphical representation of IL-4 (A) and IL-13 (B) levels in lung tissues. Data are expressed as pg/mg of tissue. Modified HE staining showing eosinophils in the peribronchial tissue (C-E). Eosinophils with red cytoplasm are visible (arrows). In comparison with control animals (C), a significant accumulation of eosinophils was present in S1P animals (D) and was markedly reduced with Acrp30 treatment (E). The area in the square in (D) is shown at higher magnification in the inset. Scale bars, 20µm. The number of eosinophils per mm² of the peribronchial tissue (F). Statistical differences are indicated with $^*(p<0.05)$ and $^{\#}(p<0.001)$.

WB figure 3

AdipoR1

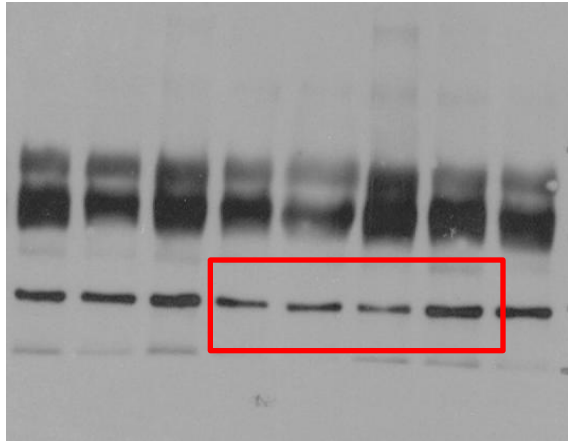


GAPDH

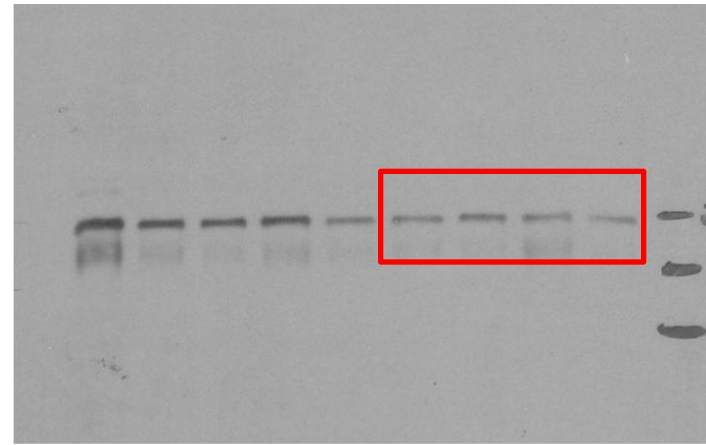


WB figure 4

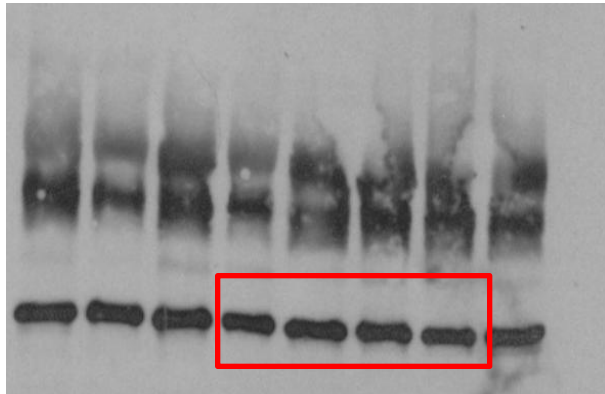
AdipoR1



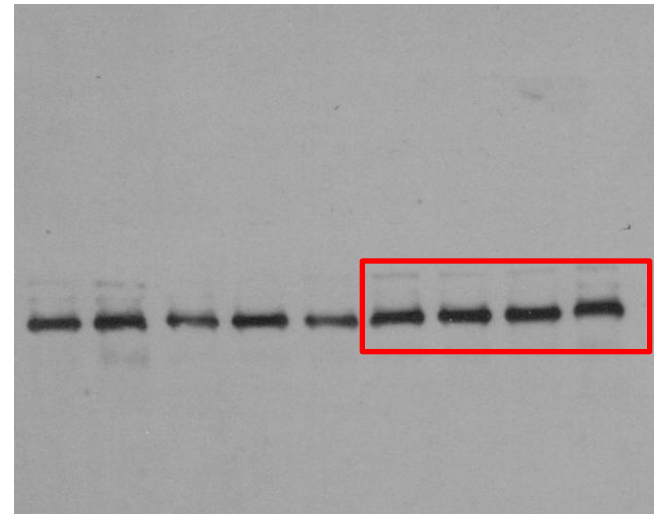
AdipoR2



GAPDH

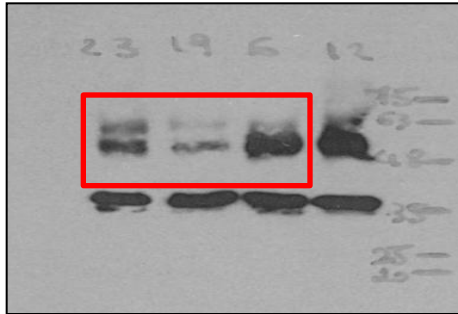


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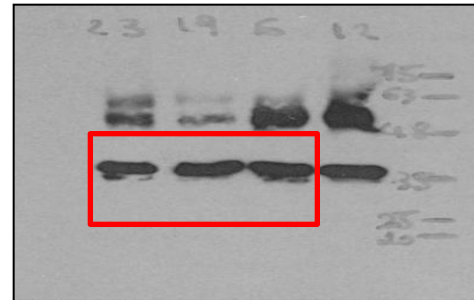


WB figure 6

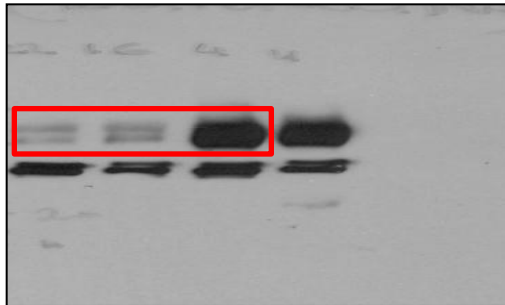
AdipoR1



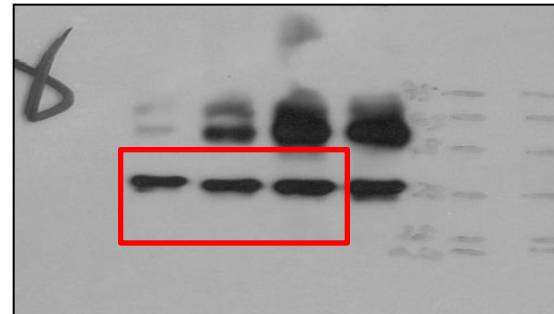
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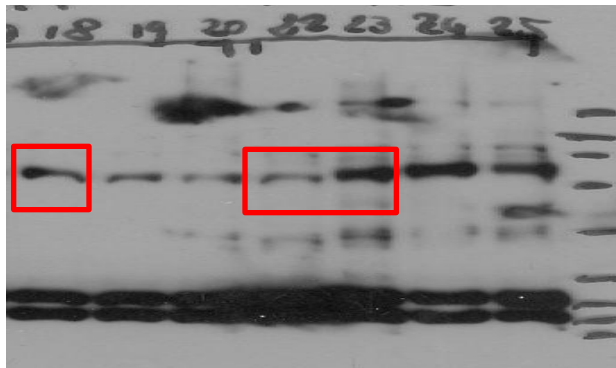
AdipoR2



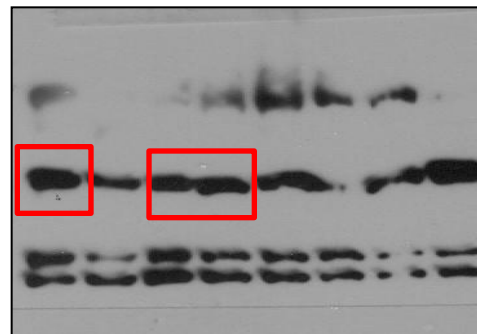
GAPDH



T-cadherin

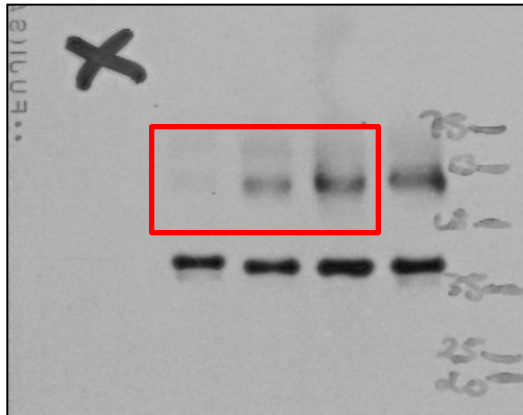


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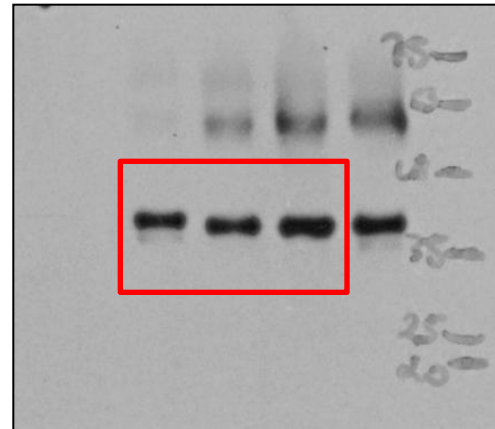


WB figure 7

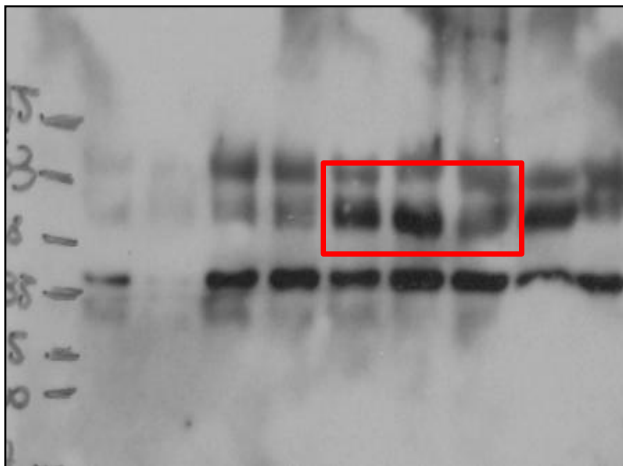
AdipoR1



GAPDH



AdipoR2



GAPDH

