Neurokinin-1 receptor, a new modulator of lymphangiogenesis in obese-asthma phenotype

Renata Ramalho, Joana Almeida, Rubén Fernandes, Raquel Costa, Ana Pirraco, Luísa Guardão, Luís Delgado, André Moreira, Raquel Soares

A B S T R A C T

Aims: Obesity and asthma are widely prevalent and associated disorders. Recent studies of our group revealed that Substance P (SP) is involved in pathophysiology of obese-asthma phenotype in mice through its selective NK1 receptor (NK1-R). Lymphangiogenesis is impaired in asthma and obesity, and SP activates contractile and inflammatory pathways in lymphatics. Our aim was to study whether NK1-R expression was involved in lymphangiogenesis on visceral (VAT) and subcutaneous (SAT) adipose tissues and in the lungs, in obese-allergen sensitized mice.

Main methods: Diet-induced obese and ovalbumin (OVA)-sensitized Balb/c mice were treated with a selective NK1-R antagonist (CJ 12,255, Pfizer Inc., USA) or placebo. Lymphatic structures (LYVE-1 +) and NK1-R expression were analyzed by immunohistochemistry. A semi-quantitative score methodology was used for NK1-R expression.

Key findings: Obesity and allergen-sensitization together increased the number of LYVE-1 + lymphatics in VAT and decreased it in SAT and lungs. NK1-R was mainly expressed on adipocyte membranes of VAT, blood vessel areas of SAT, and in lung epithelium. Obesity and allergen-sensitization combined increased the expression of NK1-R in VAT, SAT and lungs. NK1-R antagonist treatment reversed the effects observed in lymphangiogenesis in those tissues.

Significance: The obese-asthma phenotype in mice is accompanied by increased expression of NK1-R on adipose tissues and lung epithelium, reflecting that SP released during inflammation may act directly on these tissues. Blocking NK1-R affects lymphangiogenesis, implying a role of SP, with opposite physiological consequences in VAT, and in SAT and lungs. Our results provide a clue for a novel SP role in the obese-asthma phenotype.

Keywords:
Obesity
Asthma
Allergen-challenge
NK1-R
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Introduction

The lymphatic vascular system is critical for reabsorption of extravasated fluid and dietary fat into the circulation. By transporting inflammatory cells to lymphoid organs, it also plays a critical role in inflammation, infection and immunity. In comparison to angiogenesis, the molecular mechanisms underlying lymphatic vessels development are less well characterized. Vascular endothelial growth factor (VEGF)-C is a member of the VEGF family, mainly involved in lymphangiogenesis that closely correlates with metabolic and lipid parameters (Detoraki et al., 2009; Wada et al., 2011), implying a strong association between lymphangiogenic process and adipose tissue metabolism. Furthermore, lymphatic fluid associates with fat deposition, and lymphatic vascular defects may lead to adult-onset obesity (Lijnen et al., 2009).

On the other hand, asthma is a chronic inflammatory disease, where airway edema occurs whenever plasma leakage from blood vessels exceeds lymph vessels drainage capacity. Mast cells, macrophages and eosinophils, which migrate into the airways, are able to release huge amounts of angiogenic and lymphangiogenic growth factors, rendering these cells crucial promoters of lymphangiogenesis (Detoraki et al., 2010). Nevertheless, data regarding the distribution and role of lymphatic vessels in the lung during inflammatory conditions remains rather scarce.

Obesity and asthma are widely prevalent and associated disorders (Shore, 2006). Obesity is recognized as a risk factor for asthma

development in both children and adults (Beuther and Sutherland, 2007). Also, a distinct asthma phenotype has been identified in obese subjects. This phenotype is characterized by increase asthma severity, impaired glucocorticoid response, reduced response to standard medication and metabolic and immune imbalances related to the obese state (Lessard et al., 2008; Stream and Sutherland, 2012). Epidemiological studies reveal that the association between obesity and asthma has been challenging for both researchers and clinicians. Nevertheless, the mechanistic basis of this association remains an open field for investigation (Farah and Salome, 2012).

Substance P is a neuropeptide originally discovered in 1931 by Ulf von Euler and John H. Gaddum (Devane, 2001), involved in neurogenic inflammation by acting through its selective NK1 receptor (NK1-R) (Datar et al., 2004). In addition to the previously recognized effects on asthma (bronchonstrictor agent, inductor of mucus secretion, elicitor of airway responsiveness, pro-inflammatory mediator) (Chu et al., 2000; Groneberg et al., 2006) and obesity (orexigenic, weight gain promoter) (Karagiannides et al., 2011; Karagiannides et al., 2008), we recently studied SP as a potential mechanism behind the obese-asthma phenotype. Accordingly, we showed that serum SP is increased in obese allergen-sensitized mice (Ramalho et al., 2012) and that targeting NK1-R with a selective antagonist (CJ 12,255) improves both metabolic (weight gain, insulinemia, glycemia, and adipocyte areas) and lung-related parameters (allergen sensitization and peribronchial inflammation) in obese sensitized and allergen-challenged mice (Ramalho et al., 2013).

Recent evidence indicates that neuropeptides such as SP strongly affect lymphatic vessels contractility (Kurowska-Stolarska et al., 2011). Although SP has been shown to bind to NK1 receptor in lymphatic endothelial cells (Rayner and Van Helden, 1997), there is no information concerning the role of this neuropeptide in lymphangiogenesis.

Given the previous established role of SP in diet-induced obesity as well as in allergic asthma, and taking into account that lymphangiogenesis is relevant both during adipose tissue expansion and in asthma, the aim of the present study was to examine the role of SP in lymphangiogenesis, in the adipose tissue and lung of a diet-induced and allergen-challenged mouse model.

Material and methods

Animals and study design

Balb/c mice aged 6–8 weeks old were used in this study. The mice were purchased from Charles-River (USA) and kept in a controlled environment (temperature: 22–24 °C; humidity: 55%; air renovations/hour: 15; light cycle (hour): 12/12) with *ad libitum* access to solid food and water. Mice were randomly divided into 6 groups (6 independent experiments, n = 6–8 *per group*). The control groups were obese, lean, OVA-lean and OVA-obese. The two other groups were NK1-R antagonist (CJ 12,255) treated mice: OVA-Lean + CJ and OVA-obese + CJ. All *in vivo* manipulations were handled by trained technicians and in agreement with the Portuguese Act 1005/92 (number 3, iii) and European Community guidelines (86/609/EEC) for the use of experimental animals. This study was approved by the Portuguese National Authority for Animal Health.

Mice in the "obese" groups were fed a high fat diet (45 kcal % fat, 4.73 kcal/g, Research Diets #D12451) for 17 weeks. "Lean" mice received normal diet (11 kcal% fat w/corn starch, Research Diets #D12328) for the same period. Final weight was lean 25.2 ± 0.98 g, obese 30.0 ± 1.3 g, OVA-lean 25.3 ± 0.5 g, OVA-obese 30.5 ± 1.0 g, OVA-lean + CJ 24.5 ± 1.4 g and OVA-obese + CJ 24.2 ± 1.5 g.

Animals were systemically sensitized by means of intraperitoneal injections of 10 μg ovalbumin (OVA, #LS003048, Worthington, NJ, USA) adsorbed to 1.5 mg of aluminum hydroxide $Al(OH)_3$ (AlumImject, Pierce, Rockford, IL, USA) as adjuvant on days 1, 14, and 21. Animals received two consecutive local aerosol challenges of 1% OVA (in phosphate-buffered saline, PBS) for a period of 20 min on days 26 and 27 (2 × challenge). Challenge was repeated on days 33 and 34, prior to mice sacrifice. Non-sensitized animals were treated with intraperitoneal PBS and aerosol OVA (Ramalho et al., 2012).

To antagonize the NK1-R, we used the selective antagonist 6-diphenylmethyl-5-(5-isopropyl-2-methoxybenzylamino)-1-azabicyclo (2.2.2)octane-3-carboxylic acid, also known as CJ 12,255 (Pfizer Inc., Groton, CT, USA). One day after the last aerosol challenge, mice in the "CJ" group received a daily intraperitoneal injection of 300 μg CJ 12,255, and control groups received saline solution, for 7 consecutive days prior to sacrifice.

After sacrifice with intraperitoneal anesthetic overdose of sodium pentobarbital 6 mg/ml, lungs and adipose tissues were removed, fixed with 4% paraformaldehyde and embedded in paraffin. Sections of 4–5 μm from all tissues were stained with hematoxylin and eosin and prepared for immunohistochemistry.

Immunohistochemistry of lymphatic vessels

To study lymphatic structures we selected LYVE-1, the hyaluronan receptor in lymphatic endothelial cell (LECs) and a CD44 homologue. Immunohistochemistry was performed with LYVE-1: ab33682 rabbit anti-mouse polyclonal antibody (Abcam plc, Cambridge, UK) and incubated with biotinylated goat anti-rabbit secondary antibody IgG-B: sc-2040 (Santa Cruz Biotechnology, Inc., EUA). VECTASTAIN Elite ABC Kit (Vector Laboratories, Inc., EUA) and 3,3′-diaminobenzidine (Abcam plc, UK) were used for signal amplification. Control blood vessels were stained with anti-mouse monoclonal antibody CD31 (PECAM-1): 89C2 (Cell Signaling Technology, Inc., EUA) with goat anti-rabbit IgG-B: sc-2040 (Santa Cruz Biotechnology, Inc., EUA). LYVE-1 + lymphatics were identified by presence of staining. Areas without staining were considered as negative for lymphatics. Number of LYVE-1 + lymphatic vessels was counted in 7–10 randomly selected tissue sections per mouse.

Immunohistochemistry of NK1-R

Immunohistochemistry was performed using NK1-R rabbit anti-mouse antibody (Novus Biologicals, LLC) and biotinylated goat anti-rabbit secondary antibody. VECTASTAIN Elite ABC Kit (Vector Laboratories, Inc., EUA) and 3,3′-diaminobenzidina (Abcam plc, UK) were used for signal amplification. We used a semi-quantitative score methodology to quantify NK1-R expression. Percentage of stained structures (P) was scored as 0 (<10%), 1 (10–15%), 2 (25–50%), 3 (50–75%) or 4 (>75%). The score system for intensity (I) of staining was 1 (weak staining), 2 (moderate staining) or 3 (strong staining). The immunohistochemical score for NK1-R was obtained by multiplying the percentage of stained structures by the intensity (P × I = maximum 12). Areas without staining were considered as negative for NK1-R.

In order to exclude false-positive or false-negative signals, the specificity of primary and secondary antibodies were tested prior to the experiment, and tissue preparation was performed with especial attention to fixation conditions, temperature and duration of incubation in primary antibody and secondary antibody solution, and duration of the rinsing steps between antibody solutions. Negative controls for the immunostaining were carried out by omission of the primary antibody.

Statistical analysis

Data are presented as mean ± SEM, unless otherwise stated. All tests were performed using Graphpad Prisma version 5.00 (Graphpad Software, San Diego, CA, USA). Student's *t*-test and Mann–Whitney *U*-test were used to analyze number of LYVE-1 + lymphatic vessels and NK1-R expression, respectively. All groups were compared with

Analysis of Variance (ANOVA) with Bonferroni correction for multiple comparisons.

Results

LECs density in VAT, SAT and lungs of obese and/or allergen sensitized mice

We first evaluated the density of LECs in the two adipose tissue stores and lungs in obese and OVA-sensitized mice.

Both obesity and allergen-sensitization conditions increased the number of LYVE-1 + lymphatics in VAT (Fig. 1, A: lean *versus* obese, $p = 0.025$; obese *versus* OVA-obese, $p = 0.049$; and tendency in OVA-lean). In addition, obesity further enhanced the number of LYVE-1 + lymphatics induced by allergen sensitization in this tissue (Fig. 1, A: OVA-lean *versus* OVA-obese, $p = 0.038$).

Strikingly, obesity and allergen-sensitization presented opposite effect in the number of lymphatics in SAT. Obesity resulted in a significant reduction in the number of LECs. OVA-sensitized mice exhibited also a decrease in LECs in lean mice and a tendency toward a reduction in obese sensitized mice (Fig. 2, A: lean *versus* obese, $p = 0.025$; lean *versus* OVA-lean, $p < 0.001$). The association of obesity and allergen-sensitization led to a tendency for reduced number of LYVE-1 + lymphatics (Fig. 2, A).

Obesity and allergen-sensitization independently decreased the number of LYVE-1 + lymphatics in lungs (3, A: lean *versus* obese, $p = 0.0255$; lean *versus* OVA-lean, $p = 0.0028$; and obese *versus* OVA-obese, $p = 0.053$). The association of obesity further reduced

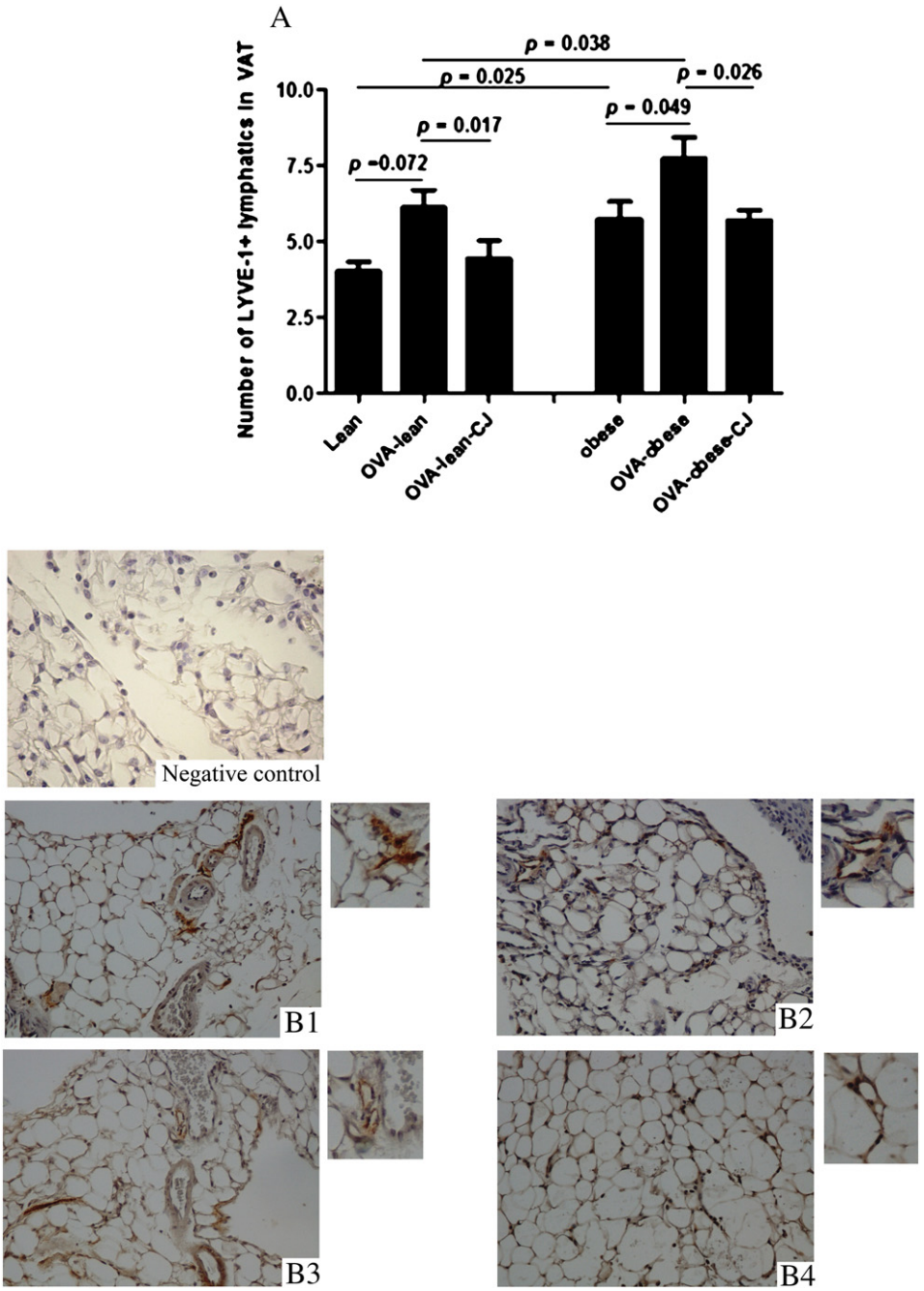

Fig. 1. LYVE-1 + lymphatics in VAT. A — Number of LYVE-1 + lymphatics in VAT; animals *per group* 6–8; CJ indicates groups treated with NK1-R antagonist. B — Immunohistochemistry for LYVE-1 on VAT (LYVE-1 +, 200×); B1, OVA-lean; B2, OVA-lean-CJ; B3, OVA-obese; B4, OVA-obese-CJ; photomicrographs presented are representative of the respective group. Magnifications are shown in right side insights.

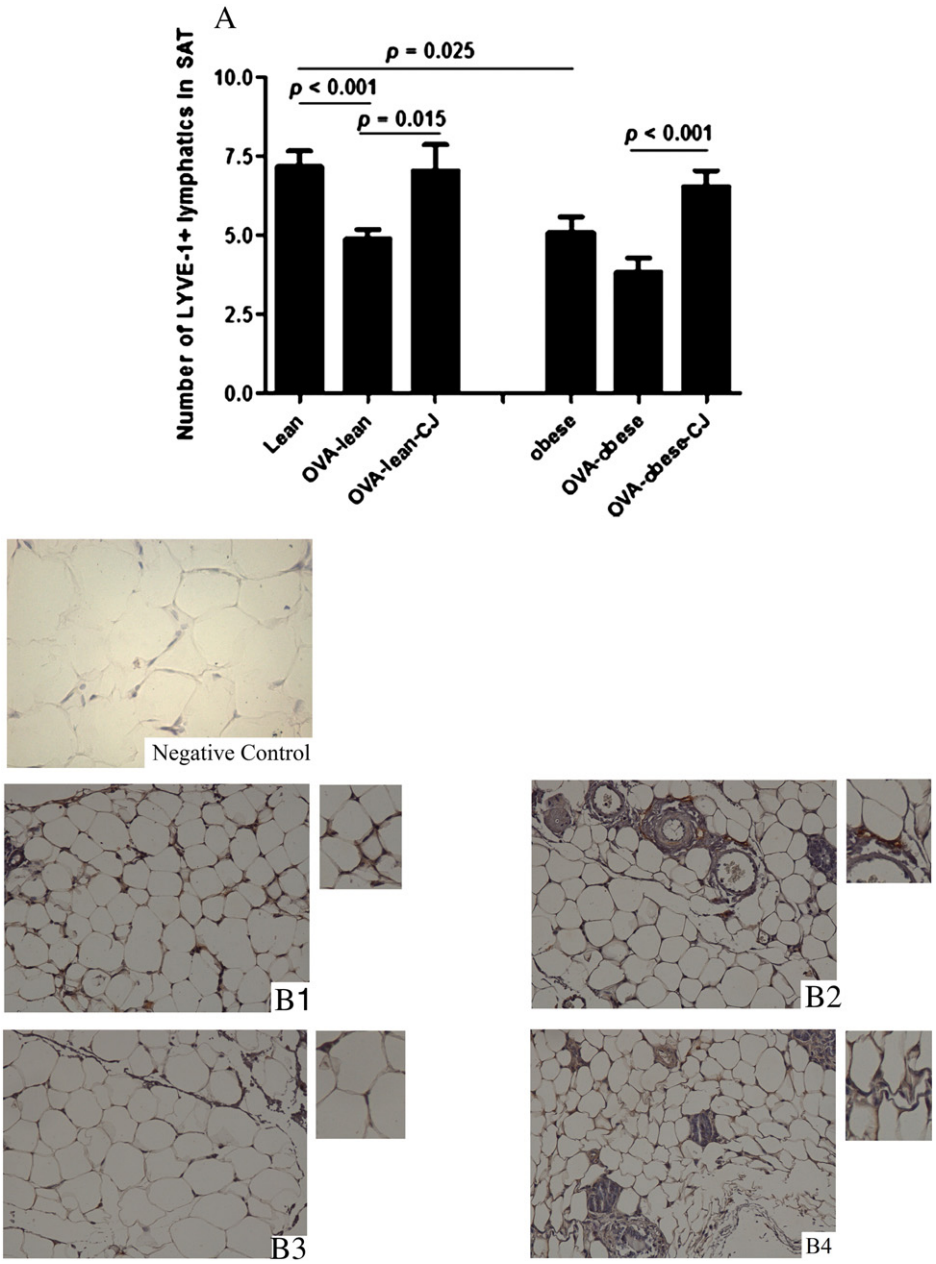

Fig. 2. LYVE-1 + lymphatics in SAT. A — Number of LYVE-1 + lymphatics in SAT; animals *per group* 6–8; CJ indicates groups treated with NK1-R antagonist. B — Immunohistochemistry for LYVE-1 on SAT (LYVE-1 +, 200×); B1, OVA-lean; B2, OVA-lean-CJ; B3, OVA-obese; B4, OVA-obese-CJ; photomicrographs presented are representative of the respective group. Magnifications are shown in right side insights.

the number of LYVE-1 + lymphatics present in the lungs of allergen sensitized mice (Fig. 3, A: OVA-lean *versus* OVA-obese, $p = 0.052$).

Number of LYVE-1 + LECs were different among the groups (VAT: $p < 0.0001$, $R^2 = 0.099$; SAT: $p < 0.0001$, $R^2 = 0.123$; lungs: $p < 0.0001$, $R^2 = 0.215$).

Expression of NK1-R on VAT, SAT and lungs

To examine whether SP played any role in the observed changes in the number of LECs upon obesity or allergen sensitization, we next examined its receptor expression in the different tissues.

Immunohistochemical analysis of VAT showed that NK1-R was mainly expressed on adipocyte membranes throughout the tissue (Fig. 4, B). Both obesity and allergen-sensitization increased expression of NK1-R (Fig. 4 A, lean *versus* obese, $p = 0.023$; lean *versus* OVA-lean, $p = 0.002$; obese *versus* OVA-obese, $p = 0.0091$). Interestingly, obesity enhanced NK1R expression induced by allergen sensitization (Fig. 4 A, OVA-lean *versus* OVA-obese, $p = 0.059$), implying an additive effect between both conditions.

In SAT NK1-R expression was restricted to blood vessel areas (Fig. 5, B). In this tissue, allergen-sensitization increased NK1-R expression both in lean and in obese-sensitized mice (Fig. 5, A: lean *versus* OVA-lean, $p < 0.001$; obese *versus* OVA-obese, $p < 0.001$), but obesity *per se* did not affect this expression. Nevertheless, obesity and allergen-sensitization combined resulted in increased NK1-R expression in SAT (Fig. 5, A: OVA-lean *versus* OVA-obese, $p = 0.015$).

NK1-R was also expressed in lungs, mainly in lung epithelium and predominantly upon allergen-sensitization (Fig. 6, B; Fig. 1, A: lean *versus* OVA-lean, $p < 0.001$; obese *versus* OVA-obese, $p < 0.001$). Obesity *per se* did not affect NK1-R expression on lung epithelium (Fig. 6, B3), but it rendered this tissue much more detached and disorganized in obese mice, indicating that obesity negatively affected lung structures.

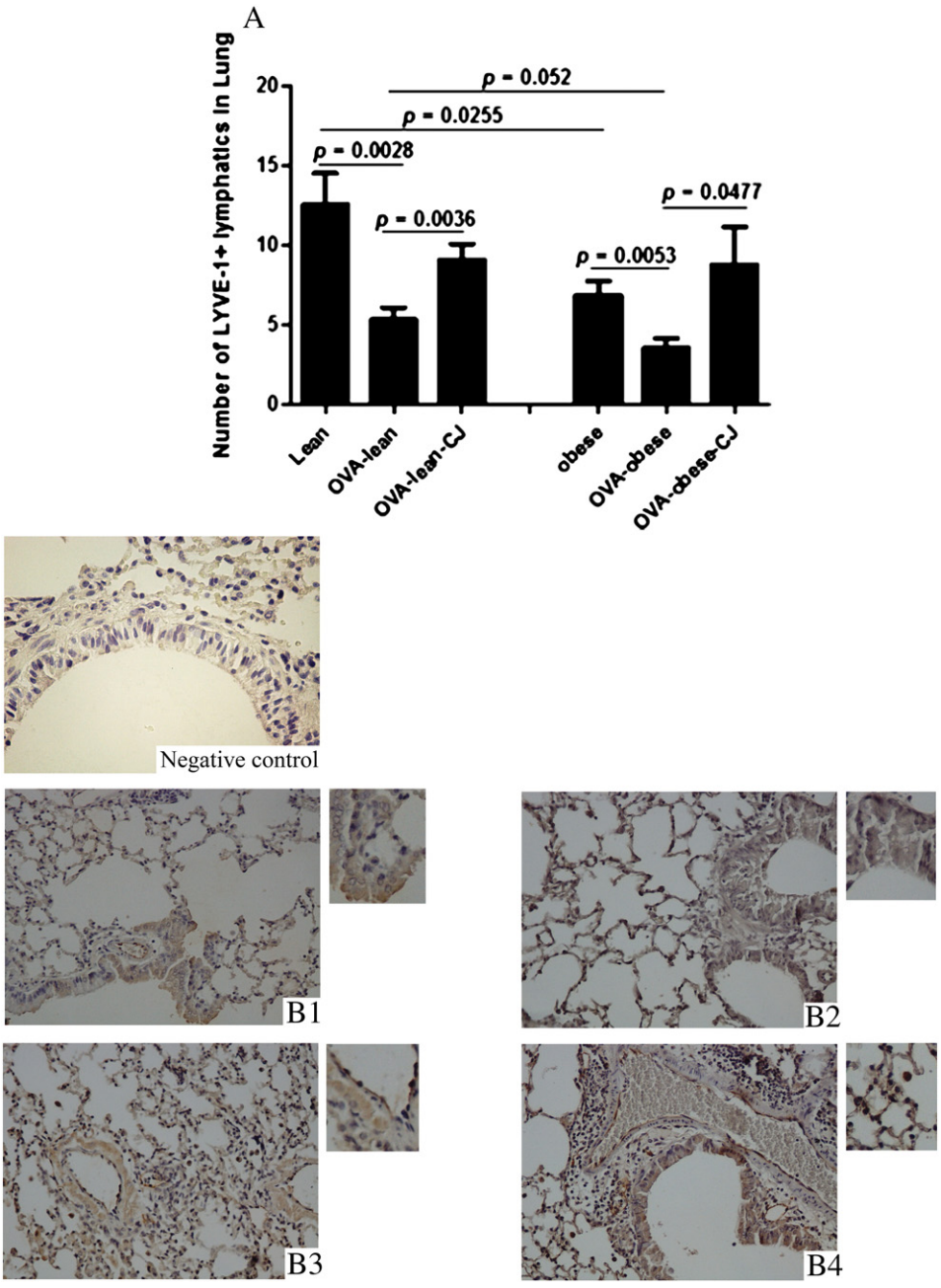

Fig. 3. LYVE-1 + lymphatics in lungs. A — Number of LYVE-1 + lymphatics in lungs; animals *per group* 6–8; CJ indicates groups treated with NK1-R antagonist. B — Immunohistochemistry for LYVE-1 in lungs (LYVE-1+, 200×); B1, OVA-lean; B2, OVA-lean-CJ; B3, OVA-obese; B4, OVA-obese-CJ; photomicrographs presented are representative of the respective group. Magnifications are shown in right side insights.

Interestingly, allergen-sensitization led to NK1-R overexpression in lung epithelium of obese mice when compared to lean ones (Fig. 6, A: OVA-lean *versus* OVA-obese, $p = 0.015$), indicating a synergistic effect between the two conditions in SP signaling.

We then confirmed these findings by treating mice with NK1-R antagonist. As expected, blocking NK1-R resulted in decreased expression of NK1-R in VAT (Fig. 4, A: OVA-obese *versus* OVA-obese + CJ, $p = 0.0385$; OVA-lean *versus* OVA-lean + CJ, $p = 0.022$), SAT (Fig. 5, A: OVA-obese *versus* OVA-obese + CJ, $p < 0.001$; OVA-lean *versus* OVA-lean + CJ, $p = 0.002$) and lung epithelium (Fig. 6, A: OVA-obese *versus* OVA-obese + CJ, $p = 0.013$; OVA-lean *versus* OVA-lean + CJ, $p = 0.002$) of OVA-obese or OVA-lean treated mice. Remarkably, in contrast to VAT and SAT, the use of NK1-R antagonist did not decrease NK1-R expression in lung epithelium to normal (non-sensitized) levels either in lean or in obese mice (Fig. 6, A).

Expression of NK1-R was different among the groups tested (VAT: $p < 0.0001$, $R^2 = 0.427$; SAT: $p < 0.0001$, $R^2 = 0.699$; lungs: $p < 0.0001$, $R^2 = 0.475$).

Role of NK1-R signaling in lymphangiogenesis

When NK1-R was abrogated using NK1-R antagonist, a significant decrease in LYVE-1+ lymphatics was observed in VAT from both OVA-obese and OVA-lean treated mice to levels identical to the ones observed in untreated mice (Fig. 1B2 and B4; Fig. 1A: OVA-obese *versus* OVA-obese + CJ, $p = 0.026$; OVA-lean *versus* OVA-lean + CJ, $p = 0.017$). In SAT, treated mice showed increased number of LYVE-1+ lymphatics to normal (non-sensitized) levels (Fig. 2B2 and B4; Fig. 2A: OVA-obese *versus* OVA-obese + CJ, $p < 0.001$; OVA-lean *versus* OVA-lean + CJ, $p = 0.015$). Identical findings were observed

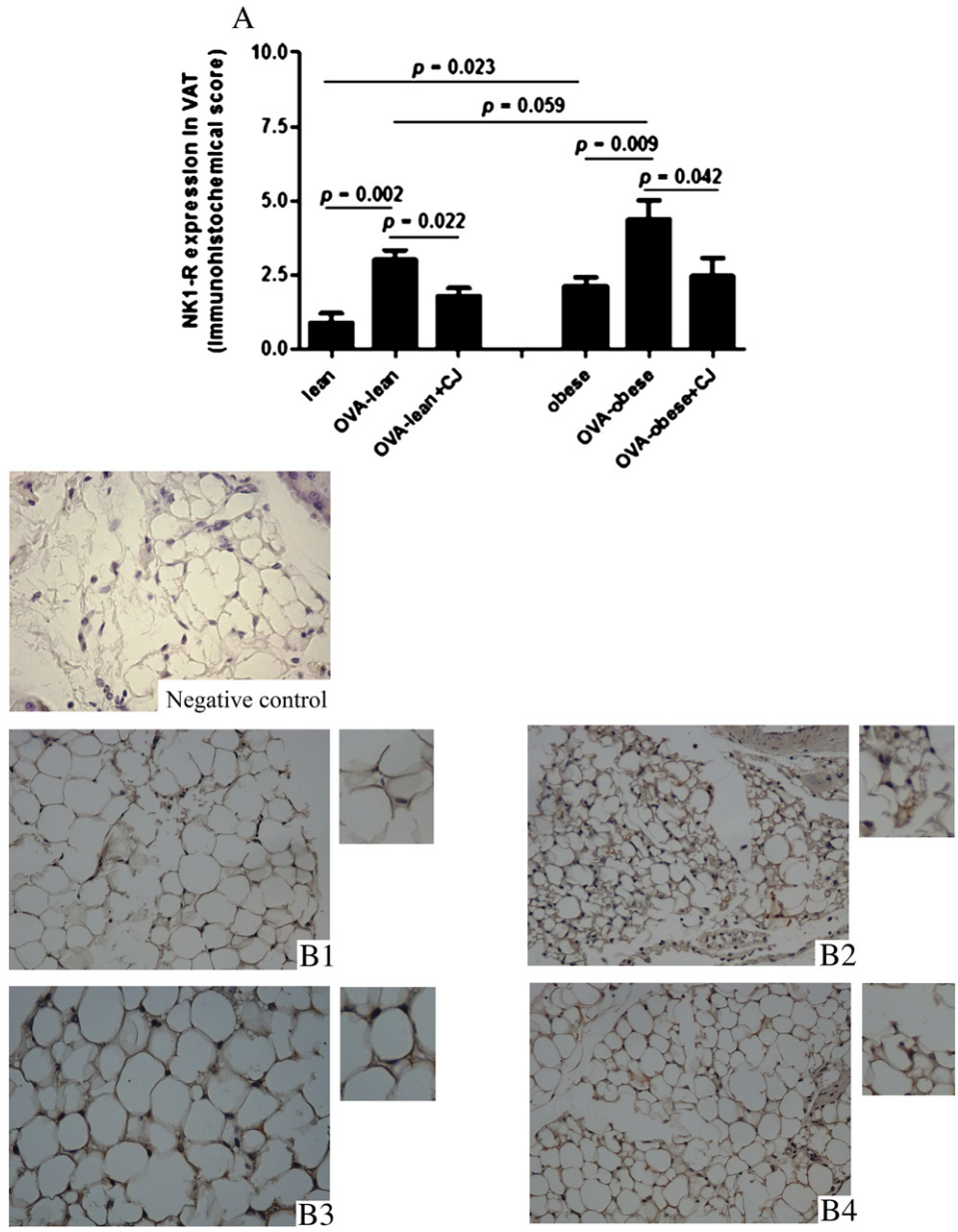

Fig. 4. NK1-R expression on VAT. A — Immunohistochemical score for NK1-R on VAT; animals *per group* 6–8; CJ indicates groups treated with NK1-R antagonist. B — Immunohistochemistry for NK1-R on VAT (NK1-R+, 200×); B1, lean; B2, OVA-lean; B3, obese; B4, OVA-obese; photomicrographs presented are representative of the respective group. Magnifications are shown in right side insights.

in the lungs, where NK1-R antagonist also reversed the number of LYVE-1 + lymphatics towards non-sensitized levels (Fig. 3B2 and B4; Fig. 3A: OVA-lean *versus* OVA-lean + CJ, $p = 0.0036$; OVA-obese *versus* OVA-obese + CJ, $p = 0.0447$). These findings imply that lymphangiogenesis depends on SP signaling pathway.

Discussion

To our knowledge, this is the first study to demonstrate that lymphangiogenesis is effectively imbalanced in visceral and subcutaneous adipose tissue depositions, as well as in lungs of obese-allergen challenged mice in comparison to lean nonsensitized ones. We were further able to show that this lymphangiogenesis imbalance depends on the expression of SP specific receptor, NK1-R.

As the remaining members of this tachykinin family of neuropeptides (neurokinin A — NKA, and neurokinin B — NKB), SP exerts its biological effects by activating specific receptors (Datar et al., 2004). NK1-R exhibits higher affinity for SP than any other tachykinin, being thus considered a selective receptor for SP (Datar et al., 2004; Douglas and Leeman, 2011). NK1-R was found in several structures of central nervous system, in peripheral nervous system (urinary bladder, salivary glands, small and large bowels), peripheral tissues (vascular endothelium, epithelium of bronchi and bronchioles and endothelium of bronchial microvessels, smooth muscle layers, submucosa and intestinal epithelium) and immune cells (lymphocytes, macrophages) (Quartara and Maggi, 1998). This widespread distribution of NK1-R reflects the variety of SP biological functions (Douglas and Leeman, 2011; Quartara and Maggi, 1998). Along with the recognition of its role in central nervous system as a pain neurotransmitter, SP has also been implicated in respiratory diseases, gut inflammation, obesity and diabetes (Chu et al., 2000; Datar et al., 2004; Karagiannides et al., 2011; Karagiannides et al., 2008). We have previously demonstrated that obesity and allergen challenge independently increased serum SP, and the presence of both obesity and asthma

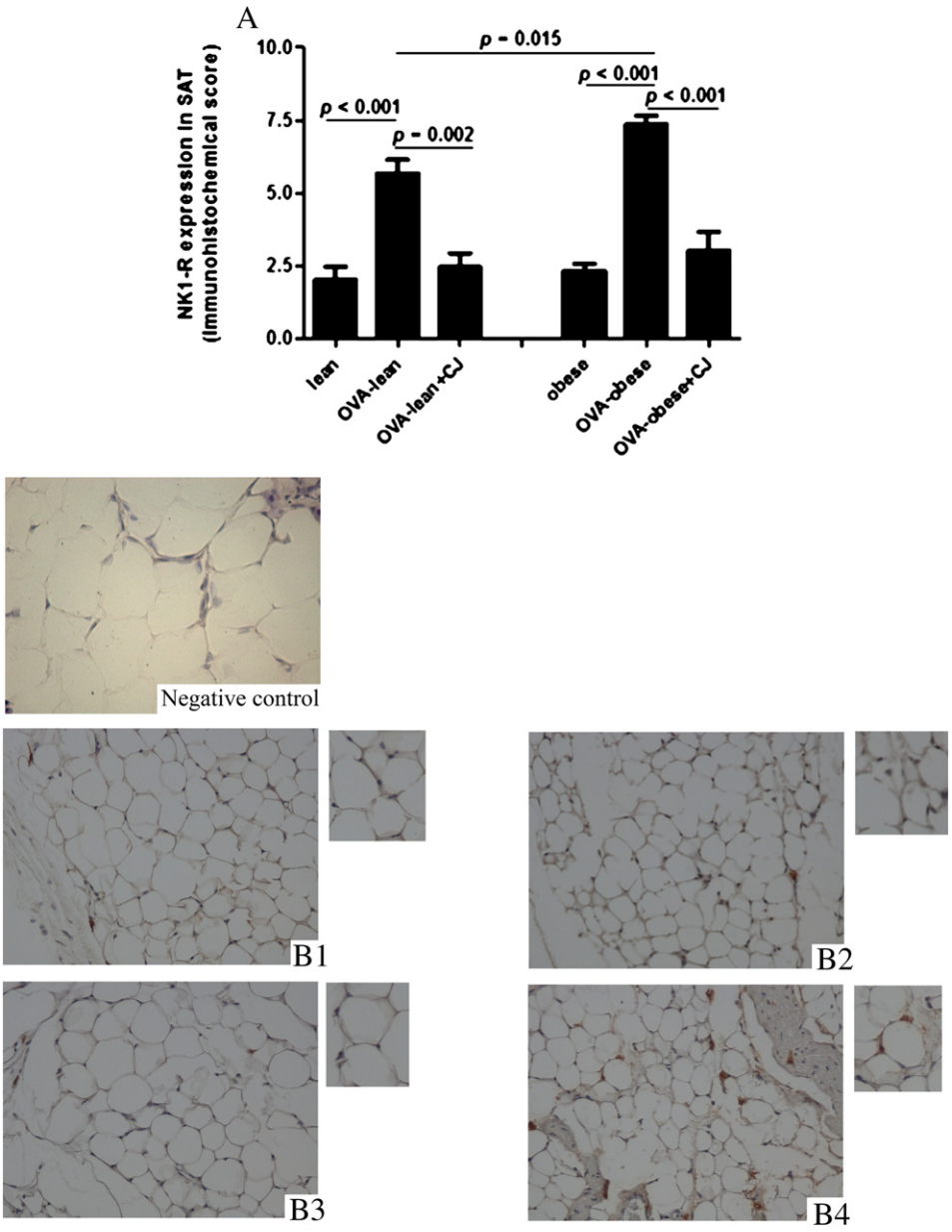

Fig. 5. NK1-R expression on SAT. A — Immunohistochemical score for NK1-R on SAT; animals *per group* 6–8; CJ indicates groups treated with NK1-R antagonist. B — Immunohistochemistry for NK1-R on SAT (NK1-R+, 200×); B1, lean; B2, OVA-lean; B3, obese; B4, OVA-obese; photomicrographs presented are representative of the respective group. Magnifications are shown in right side insights.

further increased these levels (Ramalho et al., 2012). Further, we observed a positive effect of a selective NK1-R antagonist on metabolic and allergen inflammatory parameters on the obese-asthma phenotype in mice (Ramalho et al., 2013). In the current study, we showed that NK1-R is expressed throughout VAT, in close vicinity to blood vessels in SAT and in lung epithelium, and this expression is increased in our mice model of obesity and asthma. The interaction between SP and NK1-R leads to the activation of NF-κB that enhances proinflammatory cytokines release, such as IL-1, IL-6, TNF-α, MIP-1β and IFN-γ, which are closely related to inflammatory conditions like obesity and asthma (Quartara and Maggi, 1997). This low grade inflammation is particularly associated with VAT rather than SAT, which explains the different NK1-R expressions observed between the two adipose tissue depots.

Another striking finding is the fact that lymphangiogenesis was increased in obesity and allergen challenge in VAT, but reduced in SAT. These findings were further exacerbated when obesity and allergen-sensitization and challenge were considered together, both in VAT and in SAT. Lymphatic vessels are physically associated with adipose tissue. VAT surrounds the collecting lymphatic vessels of mesentery and of intra-abdominal lymph nodes (Chakraborty et al., 2010; Harvey, 2008). Conversely, SAT is more closely associated to dermal lymphatic vasculature (Harvey, 2008). It has been previously reported that inflammation produced in the obese may promote lymphangiogenesis within VAT (Harvey, 2008; Schneider et al., 2005). Neolymphangiogenesis and leakage of lymphatic fluid from ruptured lymphatic vessels accompanies adipose tissue expansion and contributes to extension of inflammation (Chakraborty et al., 2010). These findings corroborate the different roles of these two adipose tissue depots. Furthermore, the use of NK1-R antagonist reversed lymphangiogenic effects in VAT, in SAT, and in lungs, implying a significant role of SP specific receptor in lymphangiogenesis. In agreement with these

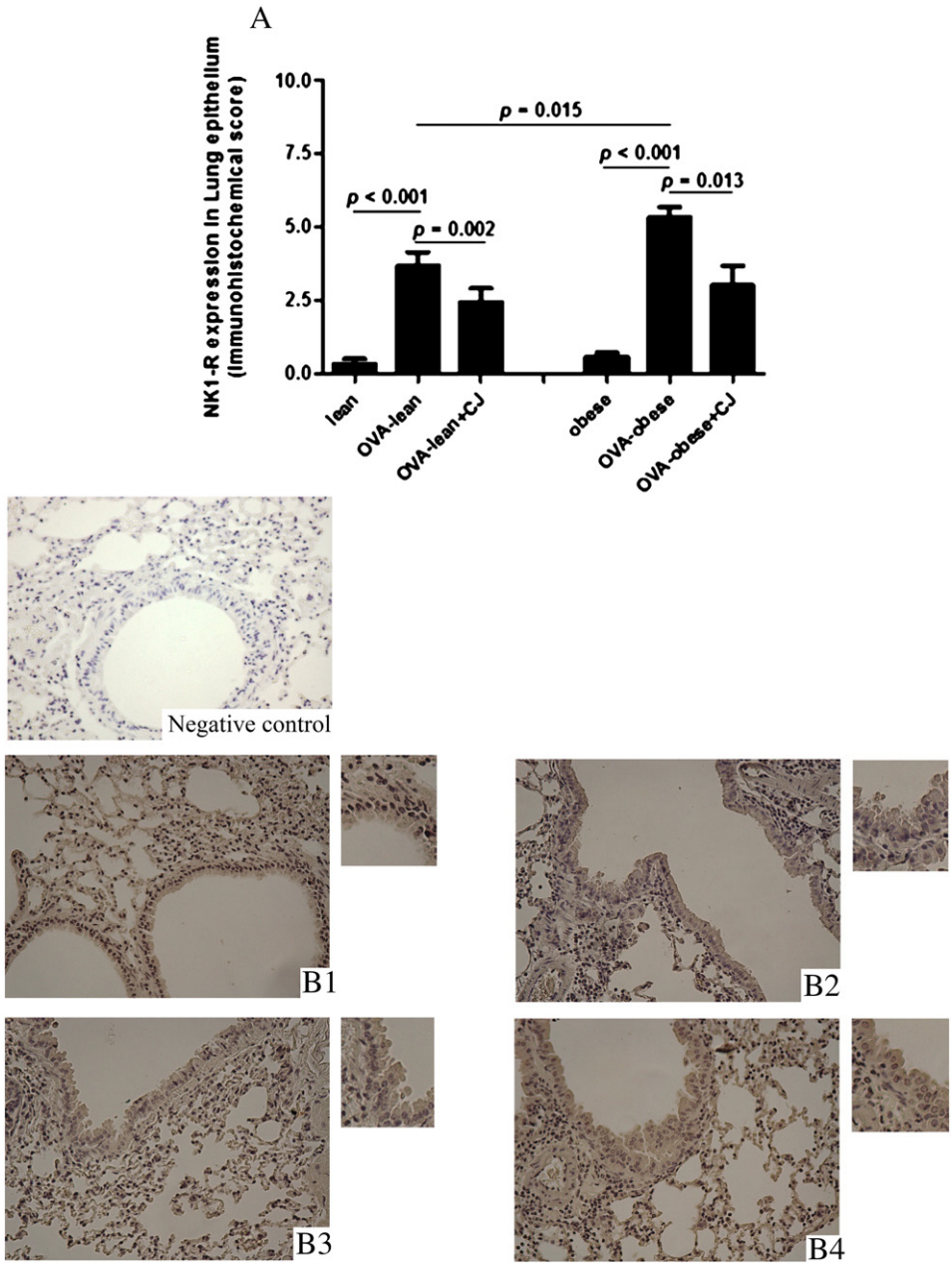

Fig. 6. NK1-R expression on lungs. A — Immunohistochemical score for NK1-R on lungs; animals *per group* 6–8; CJ indicates groups treated with NK1-R antagonist. B — Immunohistochemistry for NK1-R on lungs (NK1-R+, 200×); B1, lean; B2, OVA-lean; B3, obese; B4, OVA-obese; photomicrographs presented are representative of the respective group. Magnifications are shown in right side insights.

findings, previous studies from the literature reveal that SP modulates lymphatic contractility and contributes to chronic inflammation (Chakraborty et al., 2010; Kurowska-Stolarska et al., 2011).

In the lung, lymphatic system enables the clearance of edematous fluid (El-Chemaly et al., 2008). Impairment of lymphangiogenesis has also been considered to be implicated in asthma pathogenesis. In fact, recent data points to the lymphatic system as an important player in decreasing airway wall remodeling in asthma (Detoraki et al., 2010). Also, distribution of airway lymphatics was reported to be decreased in fatal asthma, and disruption of lymphatics with accelerated mucosal edema was also observed (Ebina, 2008). In our study the obese-asthma phenotype increased lymphangiogenesis in VAT, and decreased it in SAT and in lungs.

It is known that dysfunctional lymphatic vessels can cause pathology as well as normal lymphatics can exacerbate pathology (Jones and Min, 2011). In this scenario, different roles played by lymphatics in different tissues can be explained by their tissue-specific functionality; and their role in pathology may be explained by their degree of dysfunction. Increased number of lymphatics in VAT of obese-asthma mice model can be explained by increased lymphangiogenesis due to inflammation, a process in which VAT plays a major role. Also, the relevance of lymphatics in fat deposition recently studied by Harvey et al., suggests that lymph leakage may increase lipid storage in adipocytes causing hypertrophy (Harvey, 2008). Additionally, lymphatic vasculature has been recently reported to be involved in the reverse cholesterol (RCT) in mice (Martel et al., 2013). In their study, Martel C et al., demonstrated that the magnitude of inhibited RCT seems to be correlated with the extent to which lymphatic vessels are absent in skin surrounding the site were cholesterol-loaded macrophages are injected, and that clearance of RCT from the arterial intima relies

on lymphatic vessels. This may explain why number of lymphatics in SAT was reduced in these mice as compared to controls in our study (Martel, Li, 2013).

Our results may somehow reflect the increased severity of the obese-asthma phenotype, as a chronic pro-inflammatory condition during obesity may result in neo-lymphangiogenesis in VAT; on the other hand, airway inflammation may induce impaired lymphangiogenesis, reduced number of lymphatics, accumulation of extravasated fluid, and airway remodeling with persistent airflow obstruction.

Although immunohistochemistry enables the correct localization of LECs and NK-1R expression, it is limited for quantification purposes; however our results were strengthened by the use of a semi-quantification methodology that is reliable and broadly used in the majority of publications. This analysis is based on the intensity of staining and the number of cells stained; and the investigator responsible for the semi-quantification was always blinded to the study.

Conclusions

Altogether, our findings support the hypothesis that acting upon its specific receptor, NK1-R, in adipocytes or pulmonary epithelial cells, SP is able to influence lymphangiogenesis, which plays a crucial role in the obese-asthma phenotype, a disorder with increasing prevalence and incidence worldwide. Elucidating the molecular mechanisms triggered by NK-1R-SP signaling is mandatory in order to evaluate its putative effect as a novel therapeutic approach targeting this obese-asthma phenotype.

Conflict of interest statement

There is no conflict of interest.

Acknowledgments

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