Intratracheal administration of bone marrow c-kit^{+} cells offered hopes in ameliorating asthmatic pathologies via the control of miRNA-133 and -126

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Research

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Abstract

Background

There are still challenges regarding c-kit$^+$ cells therapeutic outcome in the clinical setting. Here, we examined of c-kit$^+$ cells effect on the alleviation of asthma by modulating miRNAs expression.

Methods

To induce asthma, male rats were exposed to ovalbumin. Bone marrow-derived c-kit$^+$ cells were enriched by MACS. Animals were classified into four groups (each in 6 rats). Control rats received PBS intratracheally; Ovalbumin-sensitized rats received PBS intratracheally; Ovalbumin-sensitized rats received PBS intratracheally containing 3×10^5 c-kit$^+$ and c-kit$^-$ cells. Cells were stained with Dil fluorescent dye to track *in vivo* condition. Pathological changes were monitored in asthmatic rats after transplantation of c-kit$^+$ and c-kit$^-$ cells. Serum levels of IL-4 and INF-γ were measured by ELISA. Transcription of miRNAs (-126 and 133) were assessed by real-time PCR analysis.

Results

Pathological examination, Th1 and Th2 associated cytokines fluctuation confirmed the occurrence of asthma in rats indicated by chronic changes and prominent inflammation compared to the control group ($p<0.05$). Both c-kit$^+$ and c-kit$^-$ cells were verified in pulmonary niche. Administration of c-kit positive cells had potential to changes INF-γ/IL-4 ratio and closed to the normal values compared to matched-control asthmatic rats ($p<0.05$). We also found that c-kit$^+$ cells regulated the expression of miRNA-126 and -133, indicated by increase of miRNA-133 and decrease of miRNA-126 compared to cell-free sensitized groups ($p<0.05$). c-kit$^-$ cells were unable to promote any therapeutic outcomes in asthmatic milieu.

Conclusions

In overall, c-kit$^+$ cells had potential to diminish asthma-related pathologies presumably by controlling the transcription of miRNA-126 and -133.

Background

Despite advantages in human medicine, there are some pathological conditions without definite evidence of therapeutic approach. Among them, asthma is highly prevalent in all parts of the world, ranging from 0.2 to 21.0%, with considerable healthcare costs [1, 2]. The current strategies on asthmatic subjects rely on the modulation of inflammatory responses by the application of pharmacological agents. However, these agents are not applicable for a long period and conceived as a symptomatic treatment to alleviate or suppress asthmatic attacks due to commonly reported side effects and inefficiency to alleviate an aberrant remodeling [2, 3]. Therefore, researches targeting pathogenesis and treatment protocols have been a hot topic in the context of asthmatic milieu. Most of the asthmatic signs are originated from

unwanted and recurrent inflammatory response toward exogenous allergens during a long period of exposure[4]. Evidence suggests that conversion of Th1/2 ratio to a Th2 type and production of Th2 cytokines in response to inhaled allergens plays a pivotal role in the pathogenesis of asthma [5]. Therefore, novel approaches should meet modalities with ability to changes of Th2/Th1 ratio in favor of Th1 [6]. Despite the promising effects of multiple stem cell types in the healing and restoration of injured tissues, there are long ways to decipher optimal cell types with high efficient outcomes [7, 8]. This issue is extremely highlighted when some experiments showed poor stem cell viability, and inappropriate engraftment [7]. Therefore, the priority issue is to find proof-of-concept observation directs the application of suitable stem cell types in different injuries. In addition, the discovery of underlying mechanisms governed by stem cells helps us to apply these results from animals studies to human medicine [9]. Recently, a fraction of bone marrow stem cells, namely c-Kit (CD177) positive cells, attracts public attention to be used for the alleviation of different pathologies [8, 10, 11]. The term c-Kit stands for a kind of tyrosine kinase receptor that is located on the cell surface and usually used for the characterization of candidate cells. Due to the existence of heterogeneity in differentiation and therapeutic effects of bone marrow resident c-kit cells, it is logical to hypothesize that diverse therapeutic outcomes will be achieved after administration of these cells into the target sites[8].MiRNAs, small, short (with 19 to 25 nucleotides) noncoding RNAs, exert inhibitory effects at transcriptional and post-transcriptional levels after attachment to the mRNAs 3'UTRs.These regulatory agents participate in different cell bioactivities[12, 13]. It has been revealed that the expression of miRNAs is prominently altered in the pulmonary system after the onset of inflammatory cytokines synthesis. However, the critical role of miRNAs has not been neglected in relation to tissue regeneration. According to previous experiments, these genetic elements could be used as biomarkers for the detection different pathologies [14, 15]. Even though, the modulating effect of miRNA should be determined to control immune-mediated *inflammatory responses* [2, 13, 15]. Previously, the critical role of both miRNA-126 and miRNA-133 has been diagnosed in animal asthma models. For instance, the modulation of miRNA-126 and miRNA-133 was shown to reduce asthmatic remodeling via the inhibition of TH2 inflammatory bioactivity [15, 16].To our knowledge, no documents exist regarding the modulatory effects of c-kit^{+} cells on the expression of miRNA-126 and miRNA-133 in asthmatic rats. Therefore, we proposed that the administration of c-kit^{+} cells could inhibit/reduce asthmatic changes in the rat model via the regulation of miRNA-126 and miRNA-133.

Methods

Experimental animals and group assignment

The present study was done by enrolling 20male Wistar rats (8–10 weeks old, weighing 200–250 g). Animals were kept at 22°C ± 2°C temperature with 12 h light/dark cycle. All rats were allowed to access to water and rodent pellets. After a two-week inhabitation period, 10 rats were blindly selected for isolation of bone marrow content c-Kit^{+} cells. Animals were randomly allocated into four groups (each in 6 rats) as follows: Healthy rats only received 50 µl normal saline intratracheally (group C); sensitized rats received 50 µl normal saline intratracheally (group A); Sensitized rats received 50 µl PBS intratracheally containing

3×10^5 c-kit$^-$ cells (group A+ c-kit$^-$); and Sensitized rats received 50 µl normal saline intratracheally containing 3×10^5 c-kit$^+$ cells (group A+ c-kit$^+$) (**Fig. 1a, b**).

Animals sensitization protocol

We used a protocol a period of 32 ± 1 days for induction of asthmatic changes according to our previous experiment [2]. For this propose, rats were received 1 ml sterile normal saline containing 1 mg ovalbumin (Sigma -Aldrich, USA) and 200mg aluminum hydroxide intraperitoneally on days 1and 8. Six days post intraperitoneal injection; the animals were exposed to aerosolized ovalbumin (4% w/v) from day 14 to 32 ± 1 for 5 min daily using an ultrasonic nebulizer (CX3, Omron Co., Netherland) connected to a Plexiglas chamber (30 cm × 20cm × 20 cm). The control rats were treated with saline instead of OVA. After completion of OVA treatment, all rats were anesthetized on day 33, dissected via the ventral neck and received PBS, c-kit$^+$ and c-kit$^-$ cells according to group allocation [8, 17]. Animals were kept for the next 14 days [17].

Isolation of c-Kit$^+$ cells by Magnetic Activated Cell Sorting (MACS)

After cervical dislocation, upper and lower extremities of femurs were cut by sterile scissors. Medullary contents were washed by pushing PBS containing 2% fetal bovine serum (FBS; Gibco, USA) using a syringe connected to 18-gauge needle. Mononuclear cells (MNCs) were collected by gradient centrifugation using Ficoll-Hypaque® solution (Sigma-Aldrich, USA). To this end, cells were centrifuged at 400*g* for 20 min and monolayer cell located interphase gently collected. Following twice wash with PBS, MNCs were incubated in PBS containing 1% FBS and incubated at 4˚C for 30 min. Then, cells were incubated with mouse-anti human c-Kit microbead (*Miltenyi Biotech*, Germany) according to manufacturer's instructions. By passing cells through the LS columns (Miltenyi Biotec, Germany) and c-Kit$^+$ and c-Kit$^-$ cells were isolated and used for different analyses[18].

Immunophenotyping of c-kit$^+$ cells by flow cytometry

The multipotentiality of isolated cells was studied after MACS by flow cytometry analysis [18].In short, cells both groups were incubated with FITC-conjugated mouse-anti human CD117 (c-kit$^+$) at 4˚C for 30 min. The samples were analyzed by BD FACS Calibur and raw data processed using FlowJo software (Ver. 7.6.1).

Cell labeling

Cells were labeled by using Cell Tracker™ CM-DiI as previously described [9]. Cells were re-suspended in 20 µM Cell Tracker™ CM-DiI solution and kept at 37˚C for 30-40 min. Thereafter, cells were washed with PBS three times (each for 5 min). 50 µl PBS aliquots containing 3×10^5 c-Kit$^+$ and/or c-Kit$^-$ cells were prepared.

Serum levels of IL-4 and IFN-γ

Fourteen days after completion of asthma induction, animals were euthanized by the overdose of Ketamine and Xylazine. Blood samples were collected via the inferior vena cava, allowed to clot at RT condition and serum harvested by centrifugation at 3000 rpm at 4°C for 10 min. The serum levels of cytokines IL-4 and IFN-γ were measured using rat ELISA kits (Sigma-Aldrich, USA) according to the manufacturer's instructions [19].

Real-time PCR analysis

Transcription of miRNA-126 and miRNA-133 was quantitatively measured by conventional real-time PCR assay[9].the Total RNA content was extracted from left lungs using a total RNA extraction mini kit (Yekta Tajhiz, Iran) and quantified using a Nano Drop ND-1000 spectrophotometer (Thermo Scientific, Wilmington, DE, 19810 USA). Total RNA was transcribed into cDNA using cDNA synthesis kit (Yekta Tajhiz, Iran). Real-time PCR reaction *was* done on a Corbett *Rotor-Gene 3000 instrument* (Corbett Life Science, Australia) using SYBR Green master mix (Yekta Tajhiz, Iran). In the current experiment, miRNA-191 was used to normalize miRNA-126 and miRNA-133 values. The data were analyzed in accordance with $2^{-\Delta\Delta Ct}$method.Primers used in this study are listed in **Table 1**.

Histopathological examination

Right pulmonary lobes were fixed in 10% neutral buffered formalin solution. Paraffin-embedded samples were cut into the 4-μm thick longitudinal sections by using Leica microtome. Hematoxylin-Eosin (H&E) and Periodic acid–Schiff (PAS) staining was used to show the pathological changes [2, 20]. The existence of chronic pathological features such as hyperemia, emphysema, interstitial pneumonitis and epithelial cells injury was scored by an independent pathologist. A four-point semi-quantitative score system ranging from 0 to 3 [0: absence, 1: mild injury, 2: moderate injury, and 3 severe injuries was used to report the extent of pathological changes.

Data analysis

All quantitative results are presented as mean ± SEM and analyzed using a One-Way ANOVA and Tukey–Kramer post hoc test. Pathological scores were evaluated using the Kruskal-Wallis test followed by post-hoc Mann-Whitney analysis. Statistical significance was set at $p<0.05$.

Results

Confirmation of c-Kit$^+$ cell phenotype by flow cytometry

The percent of c-kit$^+$ cells was calculated after MACS enrichment by flow cytometry method. Flow cytometry analysis showed the existence of 95 ± 4.9% c-Kit$^+$ cells after enrichment, indicating suitable purification and enrichment of the desired population prior to transplantation (**Fig. 2**).

Homing of transplanted cells into the pulmonary niche

Immunofluorescence staining showed the presence of red-colored (DiI^+) cells in the pulmonary tissue, showing the ability of both negative and positive c-kit cells to reach pulmonary tissue via the intra-tracheal route (**Fig. 3**).

c-kit$^+$ cells showed the ability to change the systemic levels of IL-4 and IFN-γ in asthmatic rats

Serum levels of IL-4, IFN-γ, and IFN-γ/IL-4 ratio were studied in asthmatic rats after transplantation of c-kit$^+$ and c-kit$^-$ cells. ELISA assay showed a prominent difference in the levels of these cytokines in OVA-sensitized rats compared to the control rats. These data confirmed the pro-inflammatory status in asthmatic rats induced by OVA challenge. Compared to healthy control rats, we found significantly higher levels of IL-4 and reduction of INF-γ and IFN-γ/IL-4 ratio in asthmatic rats (p<0.001 to p<0.01; ***Fig. 4a***, ***b***, **and *c***). Interestingly, a significant decrease in level IL-4 coincided with an increased INF-γ level and IFN-γ/IL-4ratio was notified in asthmatic rats received c-kit$^+$ cells compared to other sensitized rats (p<0.001 to p<0.01; ***Fig. 4a***, ***b***, **and *c***). No significant differences were found in the serum levels of IL-4, IFN-γ and IFN-γ/IL-4 ratio in rats from A and A+ c-kit$^-$ groups.

Local administration of c-kit$^+$ cells returned the expression of miRNA-126 and miRNA-133 in asthmatic rats to the normal levels

Real-time PCR analysis showed that miRNA-133 was down-regulated significantly in all sensitized rats in comparison with the control group (p<0.001, **Fig. 5A**). There was a significant increase in the expression of miRNA-133 in rats from A+ c-kit$^+$ group as compared to other sensitized rats (p<0.05 **Fig. 5a**). However, non-significant differences were found in the values of miRNA-133 from A and A+ c-kit$^-$ groups (**Fig. 5a**). Contrast to miRNA-133 levels, the expression of miRNA-126 was significantly increased after asthmatic induction as compared with control rats (p<0.001 to p<0.01 **Fig. 5a**). Transplantation of c-kit$^+$ cells was shown to prominently decrease the expression of miRNA-126 in A+ c-kit$^+$ group in comparison with other sensitized rats (p<0.01 **Fig. 5b**). No statistically significant differences were found in the expression of miRNA-126 between A and A+ c-kit$^-$ groups (**Fig. 5b**). These data demonstrated that the transplantation of c-kit$^+$ cells, but not c-kit$^-$cells, is potential to decrease asthmatic changes by the modulation of miRNA-126 and miRNA-133.

Transplanted c-Kit$^+$ cells alleviated the progression of asthmatic pathologies

The pattern of chronic pathological changes confirmed the efficiency of our protocol in induction of asthma in the rat model (Fig 6, Table 2). Pathological injuries in the lung tissues of all sensitized groups were significantly higher than C group (p<0.001 to p<0.05). Transplantation of c-kit$^+$ cells caused decrease in all pathological indices compared to A and A + c-kit$^-$ groups (p<0.001 to p<0.01). The score of pathological features in A + c-kit$^-$ group was similar to scores obtained from A group **(Fig. 6a, Table 2).** PAS staining revealed the existence of goblet cells proliferation filled with the glycosylated protein inside cytoplasm coincided with the detachment of bronchial epithelial cell layer. We also found the infiltration of inflammatory cells and isolated epithelial cells inside the bronchiolar conduits. Enhanced

polysaccharide content was also detected in the context of pulmonary parenchyma which could be related to the existence of recruited immune cells as well as pneumonocytes' proliferation. In group received C-kit positive cells, but not C-kit negative cells, we found a prominent decrease in the PAS-stained intensity and decrease of goblet cells in epithelial layer **(Fig. 6b,).**

Discussion

Careful insight into patients undergone cell therapy and trials revealed the necessity for discovery of reliable cell source with regenerative potential that could circumvent the pitfalls and incomplete regeneration of injured tissues to inspire deep and secure sense in the target population[21]. Although the most of experiment showed the therapeutic effects of stem cells but mechanisms beyond regeneration have been tremendously neglected yet[9]. To our best of knowledge, the critical role of genetic players needs to be elucidate in relation to paracrine activity of transplant cells peculiarly stem cells. Of most notably, c-kit$^+$ cells belonging to stem cells have been shown to possess unique regenerative capacity and immuno-modulatory effects in animal cardiac tissues [22-25]. However, experiments and therapies based on c-kit$^+$ cells for airway inflammation are still in its infancy[26]. The current study targets to reveal the correlation between anti-inflammatory effects of c-kit positive cells and distinct miRNAs contributing to therapeutic paracrine outcome. The study and monitoring of miRNA expression pattern is a sophisticated movement to dictate specific cell behavior or the ultimate regenerative potential in asthmatic lungs[16].

Considering unique anatomical property and microstructural feature of pulmonary tissue, target cells could be administrated either via intratracheal or systemic routes[17]. To ascertain an efficient cell homing to the pulmonary niche, we selected local intratracheal administration of bone marrow c-kit$^+$ cells in the asthmatic rats[17].

As expected, we successfully induced the asthma in rats evident by pathological features and changes in the systemic levels of IL-4 and INF-γ[27]. By transplanting c-kit$^+$ cells, the intensity of pathological features and production of above-mentioned cytokines were changed and reached near-to-control levels. Based on the previous data, the promotion of Th1/2 imbalance with enhanced Th2 activity exacerbated allergic pulmonary responses by releasing multiple cytokines IL-4, -5, and -13 [28]. Therefore, regulation of Th2 activity could be touted as strategic approach in alleviation of asthmatic complications. Considering the close correlation of asthmatic changes with Th1/2 imbalance, it is logical to mention that the reduction of pathological changes in c-kit$^+$ cells group correlates with clonal expansion and activity of specific Th subpopulation. Consistent with our results, Spaziano and co-workers the intratracheal administration of 5×10^4 murine pulmonary c-kit cells had potential to suppress the production of cytokines IL-4, -5, and -13 with polarization of macrophages to M2-like phenotype orchestrated possibly in paracrine and/or juxtacrine manner. In addition, the local content of IL-10 was also increased post-c-kit transplantation[26] . The advantage of the current experiment is to investigate the potent anti-asthmatic activity of bone marrow c-kit cells (3×10^5 cell) compared to local c-kit lineage and to highlight the underlying role of distinct mi-RNAs in pulmonary tissue. As the number of pulmonary -specific progenitor

cells is trivial in lungs compared to bone marrow microenvironment and there are serious ethical issues regarding isolation of these cells from pulmonary tissue, therefore, the use of bone marrow cells are more applicable in human medicine compared to c-kit from other sources[29].

miRNAs are currently accepted as potent biomarkers in the diagnosis and treatment and follow-up of asthmatic subjects [16]. As such, miRNA-126 and -133 play critical role in the pathogenesis and dynamics of asthma[16]. Based on our data, the injection of c-kit^{+} cells adjusted the transcription of these miRNAs to the normal levels. These changes were in accordance with the decrease of Th2 subtype. The correlation of miRNA-126 and -133 with production of pro-inflammatory cytokines was previously determined in asthmatic subjects[30, 31]. Previous data showed that the decrease of miRNA-126 per se diminished the recruitment of eosinophils to asthmatic niche[30]. The decrease of miRNA-133 stimulates bronchiolar smooth muscle cells relation via engaging Rho signaling pathway[31]. Changes in the clonal activity of distinct T helper type and regulation of miRNA-126 and -133 expressions in rats received c-kit cells showed the potency of c-kit marker harboring cells in the control of asthma pathogenesis via miRNAs element. It seems logical to mention that cells belonging to c-kit negative lineages are oriented to functional maturation with directed immune reaction activities. Therefore, these cells could possibly, but not completely, loss regenerative properties in response to different stimuli compared to the c-kit positive cells. The introduction of c-kit negative cells with limited regenerative capacity not only alleviate the asthmatic changes but also could exacerbate immunological responses in the inflamed tissues in response to multiple arrays of cytokines. There are some limitations related to this study. We suggest that monitoring the activity of miRNA-126 and -133 target genes could give us helpful information about mechanisms beyond c-kit application in the asthmatic rats.

Conclusions

In overall, the local administration of c-kit^{+} cells could alleviate the asthmatic pathology possibly by the control of miRNA-126 and -133. No obvious immunomodulatory effects were found in rats received c-kit^{-} cells.

Declarations

Ethics approval All phases of this study were fulfilled in a guideline with of “The Care and Use of Laboratory Animals (NIH Publication No. 85- 23, revised 1996) and were reviewed and approved by Animal Research Ethics Board of Tabriz University of Medical Sciences (IR.TBZMED.VCR.REC.1398.090).

Competing interests The authors declare that they have no conflict of interest.

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Authors' contributions R.K., F.M., and H. H. performed rat model of asthma and sampling, H.S.B., S.S., A.R., and H. R., performed ELISA and real-time PCR analysis; R.R., A.D., and Y. B. did the pathological

examination and IF imaging; M. A. supervised the study. All authors approved the manuscript.

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Availability of data and materials The data to support the findings of this study are available from the corresponding author upon request.

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Tables

Table 1. Primer set list used for miRNAs

Gene name	Gene bank Accession No.	Target sequence[a]
miR-126	MIMAT0002957	UCGUACCGUGAGUAAUAAUGC
miR-133	MIMAT0017124	AGCUGGUAAAAUGGAACCAAAU
miR-191	MIMAT0000866	CAACGGAAUCCCAAAAGCAGCUG

[a]Sequences were derived from miRBase (http://www.mirbase.org).

Table 2. Pathological scores in the lungs of control group (C), sensitized group (A), sensitized animals received c-kit$^-$ cells (A + c-kit$^-$ group), sensitized animals received c-kit$^+$ cells (A + c-kit$^+$ group) (for each

group, n=6). Statistical differences between control and different groups: +; p<0.05, ++; p<0.01 and +++; p<0.001. Statistical differences between A+ c-kit^{+} and A+ c-kit^{-} vs A group: *; p<0.05 and **; p<0. 01. Statistical differences between A+ c-kit^{+} and A+ c-kit^{-} groups: $; p<0.05, $$; p<0. 01. The *lowest*–highest pathological *values* in each group were showed between the parentheses.

Pathological findings	**Scores in groups(for each group, n = 6)** (Minimum-Maximum)			
	C	A	A + c-kit^{-}	A + c-kit^{+}
Hyperemia	(1-0)	(1-3) +++	(2-3) +++	(1-2) + * #
Interstitial pneumonitis	(0-0)	(2-3) +++	(2-3) +++	(1-2) + ** ##
Emphysema	(0-0)	(2-3) +++	(1-3) +++	(1-2) ++ ** #
Epithelial cells injury	(0-0)	(2-3) +++	(1-3) ++	(0-2) + **

Figures

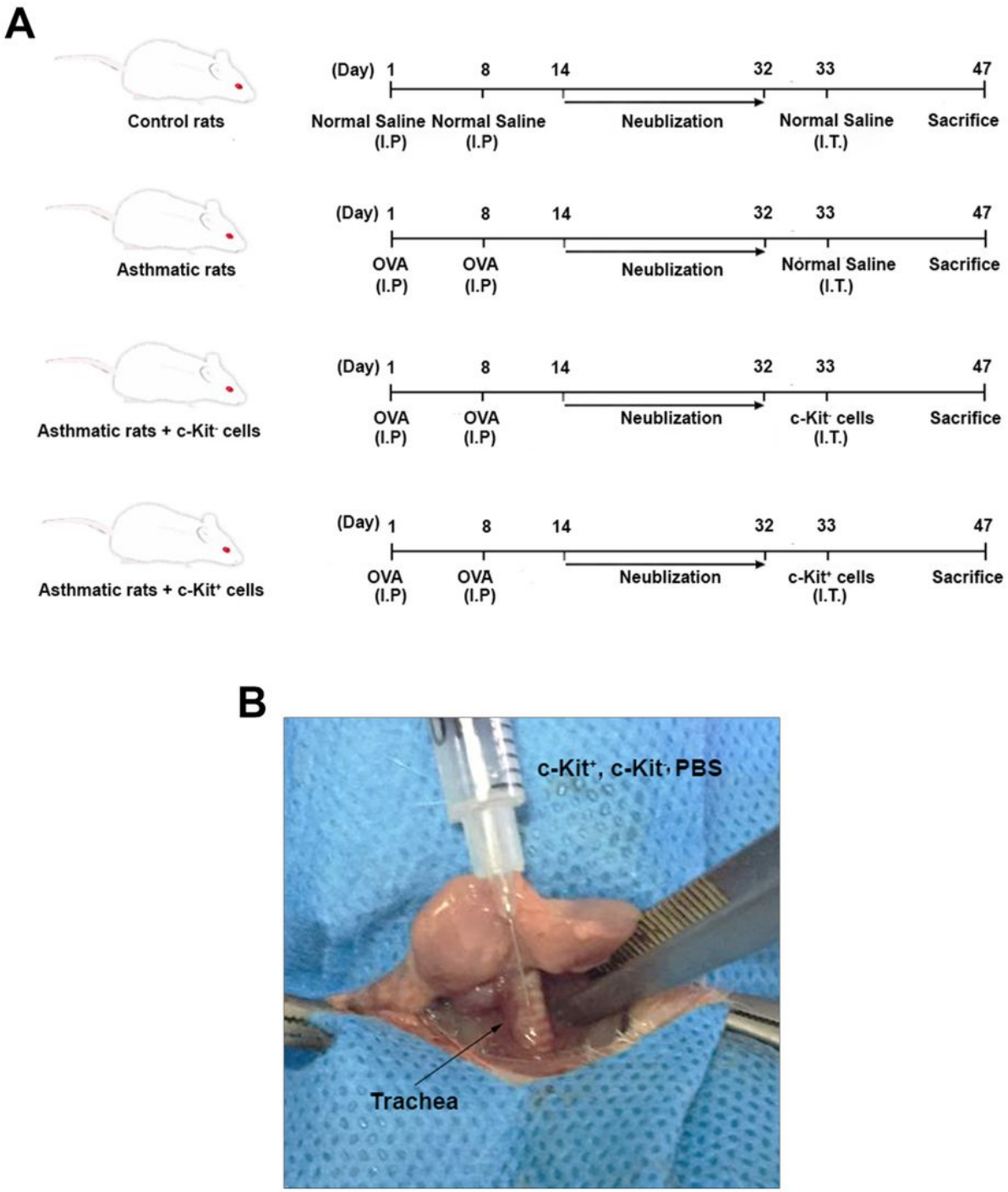

Figure 1

Total scheme of experimental design in this study (A). Intratracheal administration of c-Kit positive and negative cells (B). A total volume of 50 µl samples containing 3 × 105 cells was injected.

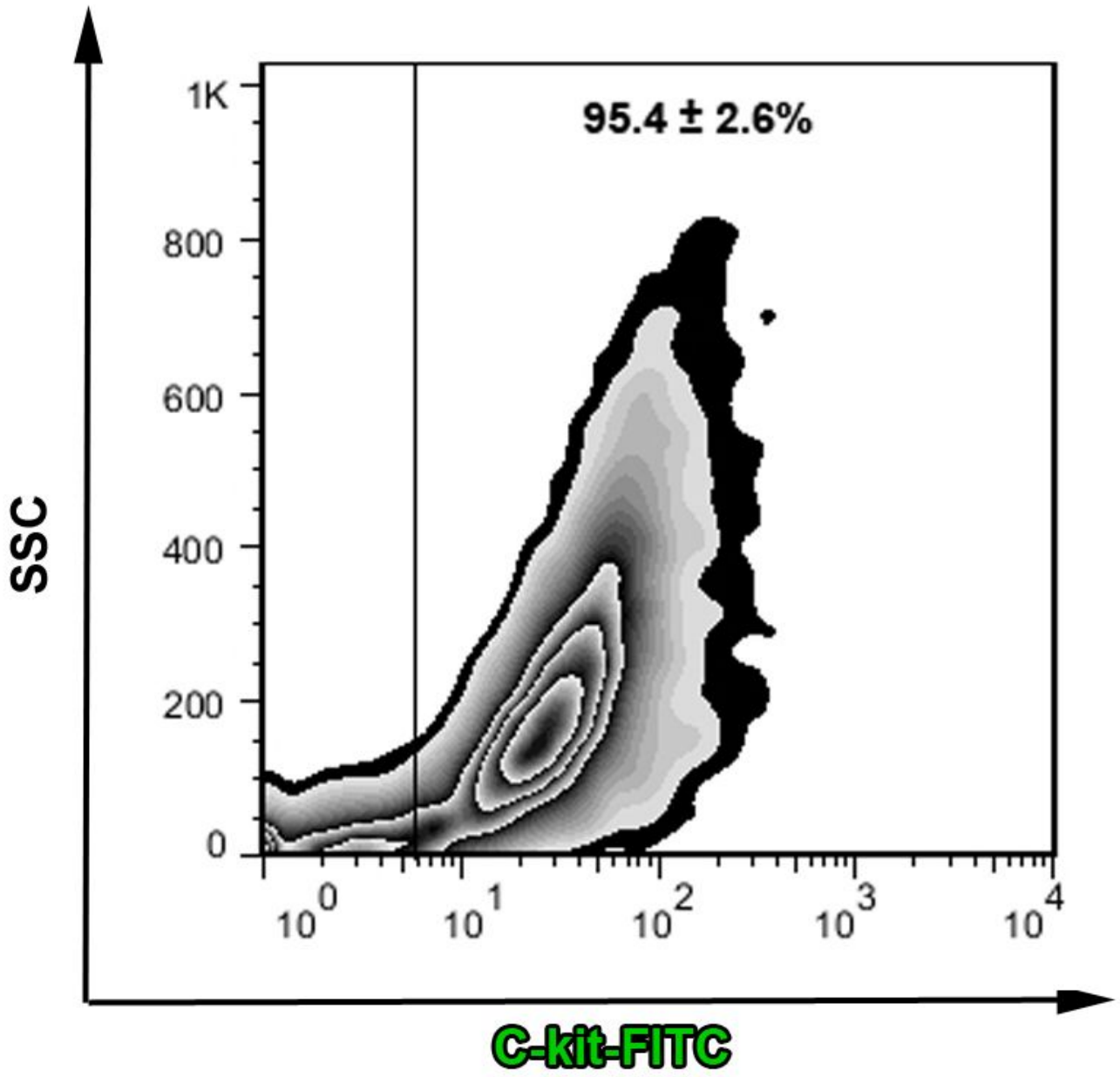

Figure 2

Confirmation of enriched c-kit+ phenotype after MACS procedure. A high rate of pure cells (over 90% desired cell phenotype) was observed after flow cytometry analysis (n=3).

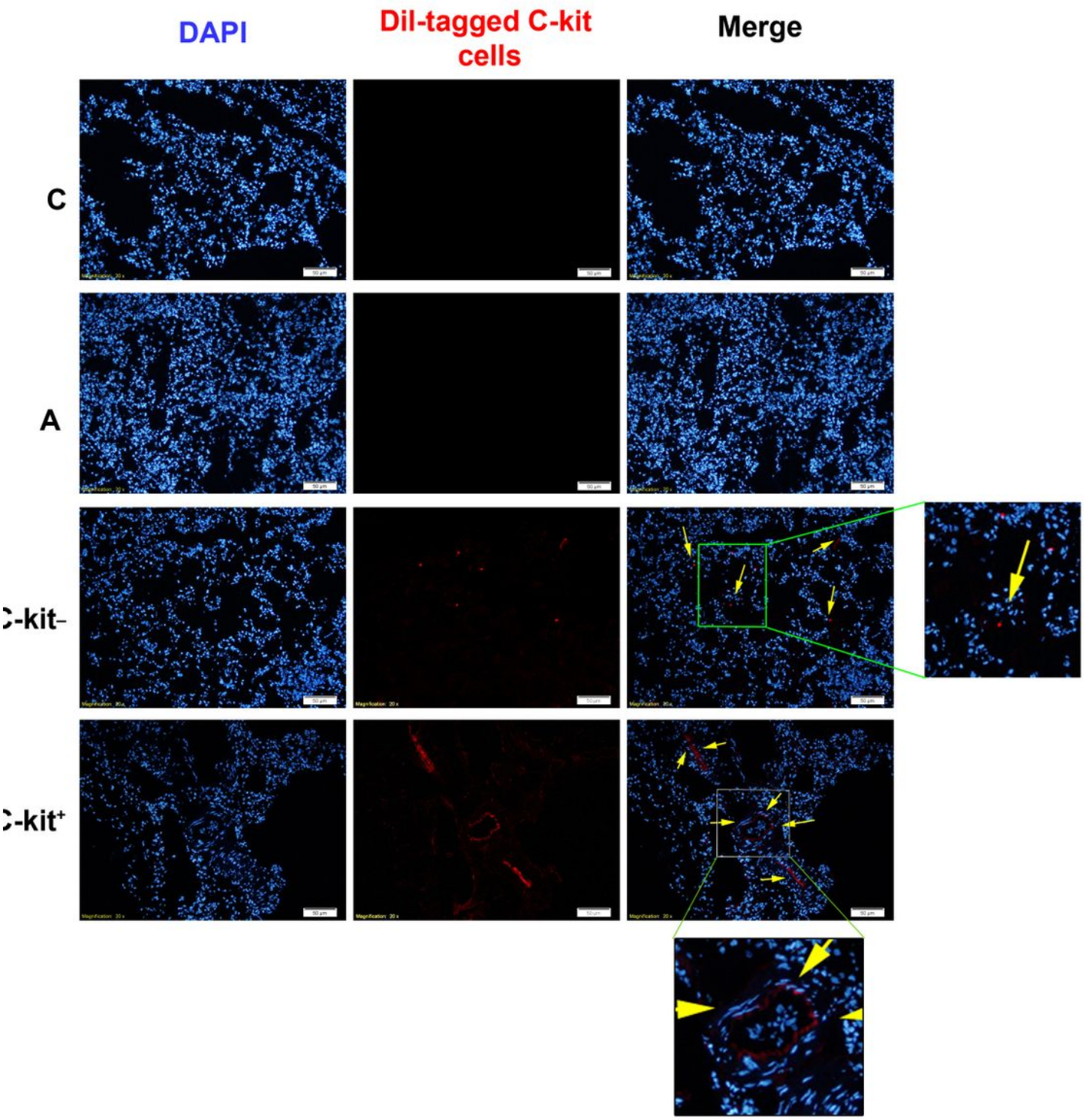

Figure 3

Monitoring the existence of Dil-labelled c-kit+ and c-kit- in pulmonary sections. IF staining revealed the presence of transplanted cells in pulmonary niche indicated by red-colored appearance. To stain nucleuses, we used DAPI stain. Control group (C), sensitized group (A), sensitized animals received c-kit- cells (A + c-kit- group), sensitized animals received c-kit+ cells (A + c-kit+ group) (for each group, n=6).

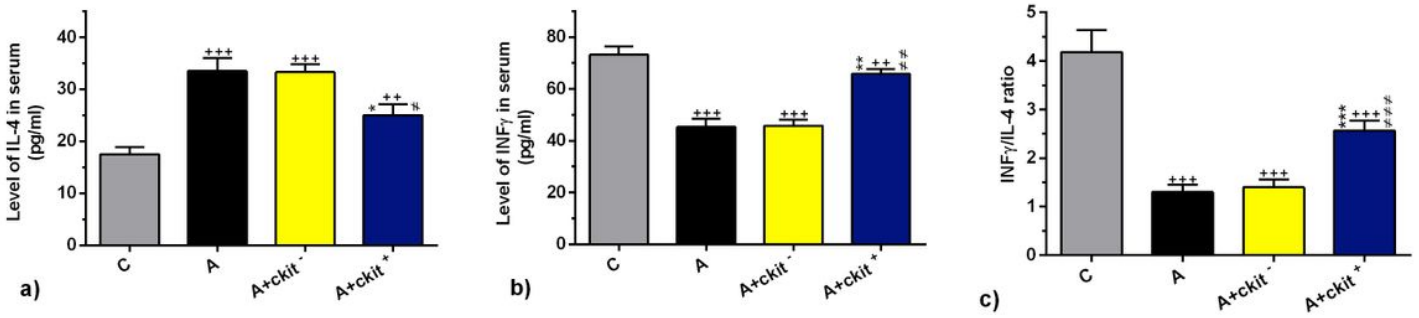

Figure 4

Serum levels of IL-4 (a), IFN-γ (b) and ratio of IFN-γ to IL-4 (c) in control group (C), sensitized group (A), sensitized animals received c-kit- cells (A + c-kit- group), sensitized animals received c-kit+ cells (A + c-kit+ group) (for each group, n=6). Bars represent the mean ± SEM. Statistical differences between control and different groups: ++; p<0.01 and +++; p<0.001. Statistical differences between A+ c-kit+ and A+ c-kit- vs A group: *; p<0.05, **; p<0.01and ***; p<0. 001.Statistical differences between A+ c-kit+ and A+ c-kit- groups: #; p<0.05, ##; p<0.01and ### p<0.001.

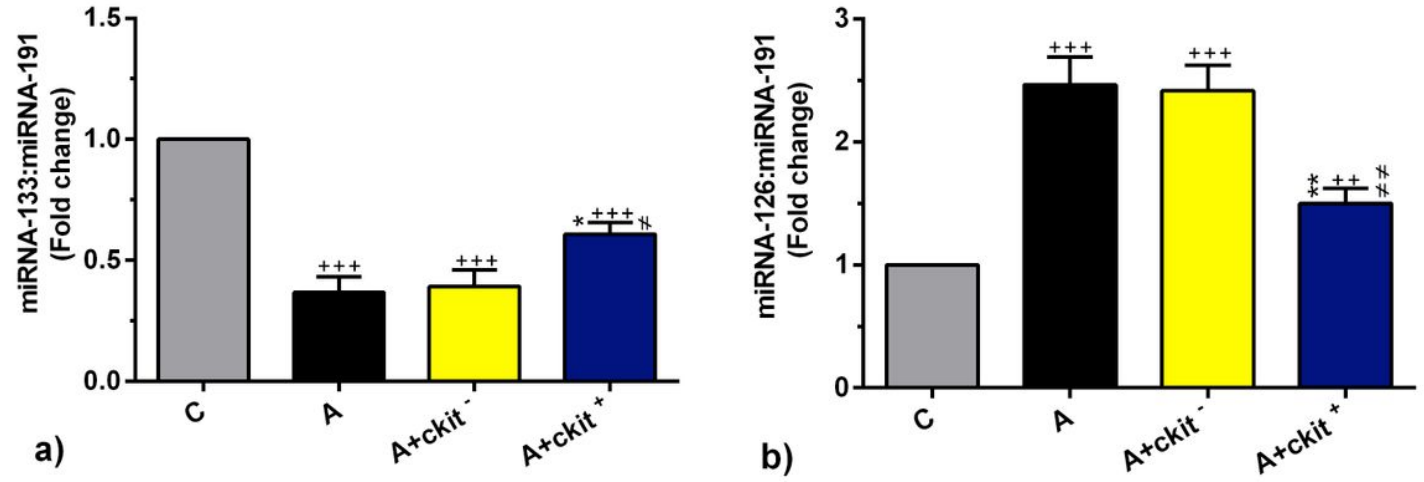

Figure 5

Real-time analysis of miRNA-126(a) and miRNA-133(b) expressions in the lungs of control group (C), sensitized group (A), sensitized animals received c-kit- cells (A + c-kit- group), sensitized animals received c-kit+ cells (A + c-kit+ group) (for each group, n=6). Bars represent the mean ± SEM. Statistical differences between control and different groups: ++; p<0.01 and +++; p<0.001. Statistical differences between A+ c-kit+ and A+ c-kit- vs A group: *; p<0.05 and **; p<0. 01. Statistical differences between A+ c-kit+ and A+ c-kit- groups: #; p<0.05, ##; p<0. 01.

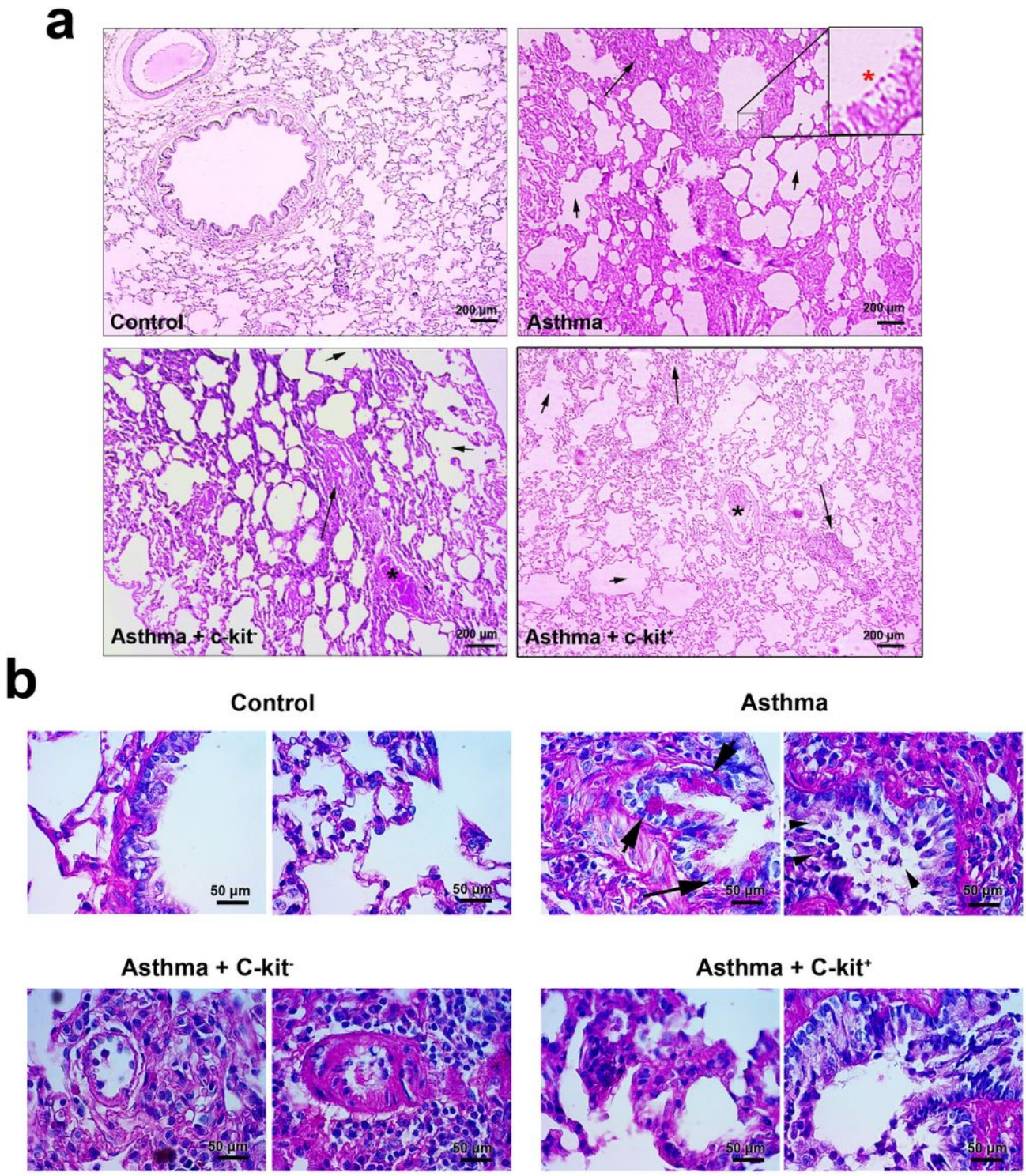

Figure 6

Bright-field images from pulmonary tissue sections stained with H&E (a). Normal lung tissue (group C). hyperemia, immune cells infiltration, emphysema and interstitial pneumonitis were seen in rats from groups A and A + c-kit-. In contrast, the transplantation of c-kit+ cells in asthmatic rats caused slight decrease in immune cells infiltration and interstitial pneumonitis. The extent and intensity of other pathological features were also improved in sensitized rats received c-kit+ (A + c-kit+ group). Black

Asterisks: Hyperemia; Long arrows: Interstitial pneumonitis; Short arrows: Emphysema; Red asterisks: Epithelial cells injury. PAS solution (b). PAS staining revealed the accumulation of accumulation glycosylated component in the asthmatic niche compared to the control group. In A group, there are a lot of goblet cells in the bronchioles epithelial cell layer filled with the glycosylated proteins, showing an increased mucus production in A group (Arrows). High magnification imaging revealed the detachment of epithelial cell layer and infiltration of immune cells into pulmonary conduits (Arrow head). The transplantation of positive C-kit cells, but not negative C-kit cells, could decrease the accumulation of glycosylated components and immune cells recruitment to the asthmatic niche.