The Role of ICOSL Antibody Intervention in Mouse Model of Neutrophil Asthma

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Research

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Abstract

Background: To investigate the role of ICOSL (Inducible costimulatory molecular ligands) antibody in the mouse model of neutrophilic asthma, and to explore the role of ICOSL in the pathogenesis of Th1/Th2/Th17 imbalance.

Methods: 24 Balb/c mice were randomly divided into four groups: C group, N group, my group and G group. The peripheral blood, BALF and lung tissue were collected within 24 hours after the last challenge. Total count and classification of BALF (bronchoalveolar lavage fluid) cells; Detection cytokines of peripheral blood and BALF. Pulmonary tissue section for pathological observation.

Results: During seven stimulations,asthma performance in group N was the earliest and obvious and was improved in group I,there was no asthma behavior in group C.The total number of BALF in N group was the highest, in C group was the lowest,($P<0.05$).The concentrations of IL-6, IL-13 and IL-17 in peripheral blood and BALF of N group were the highest,($P<0.05$).The IFN-γ concentration of C group was higher than that of N and G groups,($P<0.05$).HE(hematoxylin-eosin) and PAS(periodic acid-schiff) staining showed that inflammatory infiltration in I group was relative improved than N and G groups.ICOSL immunohistochemical analysis,ICOSL positive cells of alveolar interstitial and airway of N and G groups were more than I group,($P<0.05$).

Conclusions: After the intervention of ICOSL antibody,the performance of asthma relieved,and airway inflammatory cell infiltration, mucus secretion were reduced.The levels of IL-6, IL-13 and IL-17 in peripheral blood and BALF were partly decreased, and the level of IFN-γwas increased;These results suggest that blocking the ICOS/ICOSL signaling pathway may partially block the development of neutrophilc asthma and provide a new target for the treatment of asthma.

Background

Bronchial asthma (asthma) is one of the most common chronic respiratory diseases in children,consuming huge medical and health resources[1].Asthma is a chronic inflammatory disease of the airways with various cell and cell components involved in airway inflammation and airway remodeling[2].The pathogenesis of asthma is complex, and the immunological mechanism plays an important role in the development of asthma.Previously, It has been considered that the classical pathogenesis of asthma is that the imbalance of Th1/Th2 leads to the increase of IgE production, stimulating the proliferation and activation of eosinophils, and then secreting a variety of inflammatory mediators to cause chronic airway inflammation[3–5].However, this imbalance does not explain all types of asthma. Some studies have found that there are no neutrophils in the airways of healthy people, while the number of neutrophils in sputum, BALF and airway tissues of asthmatic patients increased significantly,and some patients showed only neutrophils increased, but eosinophils not[6].

Costimulatory signals and costimulatory molecules were proposed and confirmed by Brestcher and Cohen in 1970 on the basis of the dual-signal theory of T lymphocyte activation[7].ICOSL molecule is a member of CD28/B7 superfamily[8],ICOSL binds to its receptor ICOS to form ICOS/ICOSL signaling pathway.Studies show that ICOS/ICOSL signaling pathway plays an important role in maintaining T-lymphocyte effect and activation of memory cells, T-cell proliferation, lymphokine secretion, immunoglobulin type conversion, and regulating the polarization of Th1, Th2 and Th17 cells[9–12].In vitro studies showed that ICOS co-stimulation increased the production of many cytokines such as IL-4, IL-6 and IL-17 in T cells[13–14].It is speculated that ICOS/ICOSL signaling pathway may play an important role in participating in and aggravating inflammation. In this study, OVA + LPS was used to induce neutrophilc asthma in mice, and ICOSL antibody was used to intervene during induction period to understand the immune mechanism of ICOSL in neutrophilc asthma, so as to find new ideas for the treatment of severe and refractory asthma with neutrophils infiltration.

Methods

2.1.Mouse model(□□□□□□□□?)

Twenty-four SPF grade 6–8 week old Balb/c female mice(Purchased from Animal Laboratory Center of Suzhou University) were randomly divided into four groups.In C group,PBS 0.2 ml was injected intraperitoneally on day 0, 7 and 14, and PBS was stimulated by ultrasonic atomization for seven consecutive days from day 22, 30 minutes a day.N group, I group, G group were intraperitoneally injected with OVA + LPS suspension 0.2 ml on day 0, 7 and 14.I group and G group were intraperitoneally injected with ICOSL monoclonal antibody 50 g and IgG homologous antibody 50 g on day 0, 3, 7, 10 and 14, respectively. From day 22, three groups were given 1% OVA solution ultrasound atomization 30 minutes a day for seven consecutive days.This study was performed after Ethics committee of Children's Hospital of Soochow University approval was obtained.

2.2.specimens

The mice in each group were anesthetized within 24 hours after the last stimulation (3% chloral hydrate, 0.30 ml/mouse, intraperitoneal injection). The eyeballs were taken for blood 1 ml and placed in EDTA for anticoagulation.The mice were necked off to death. The neck and chest were exposed in supine position and fixed on the operating table. Intravenous indwelling needle was used for tracheal intubation. Surgical suture was used to ligate the right lung at the right lung root. The left lung was lavaged with 0.5 ml PBS three times. About 1.5 ml of alveolar lavage fluid was recovered (the total recovery rate was not less than 80% as qualified). The whole blood was centrifuged at 4℃ for 5 min at 2500 r/min. The upper plasma was collected in EP tube. The lavage solution was centrifuged at 4 ℃ for 5 min at 1500 r/min, and then supernatant was taken into EP tube. The supernatants of upper plasma and lavage fluid collected after centrifugation were stored at − 80 ℃ for cytokine detection. Cell sediment was added to 100 UL PBS suspension precipitation for total cell count and classification count.

2.3.Data detection

The expressions of IL-6, IFN-γ, IL-13 and IL-17 in peripheral blood and BALF were detected by ELISA. The total number and classification of BALF cells were counted under the microscope.Inflammatory cell infiltration, airway and alveolar changes and ICOSL expression were detected by periodic acid-Schiff (PAS), hematoxylin-eosin (HE) and immunohistochemical (SABC) staining of lung tissue sections.Image-Pro Plus 6.0 system was used to analyze the results of immunohistochemistry. Ten different visual fields were randomly selected from each slice under 400-fold objective lens. The blank area was used as control. The average gray value of positive visual field was analyzed by computer. The larger of the relative gray value, the stronger of the dyeing strength.

2.4.Statistical analysis

SPSS18.0 software was used to analysis data, measurements were expressed by (x + SD), rank sum test was used for inter-group comparison, chi-square test was used for comparison of rates, $P < 0.05$ was the significant difference.

Results

3.1. Mouse behavioral observation

The behavioral changes of mice after atomization for seven consecutive days are not obvious in C group.In N group,after the first stimulation for about 5 minutes, the mice can observe sneezing, rapid breathing, irritability, frequent scratching of the neck and skin, and the above performance can occur after the fifth stimulation starts to atomize for a few seconds. And the reaction is more obvious, nodding, breathing and incontinence.The above performance was observed in the mice at the third challenge of I group.After the seventh stimulation, nod breathing was relatively insignificant, and the overall behavioral performance improved compared with the N group.The overall behavioral performance of group G was slightly improved compared with group N, which was more obvious than group I.

3.2. BALF cytometry total and cell classification

The BALF recovery rate of all mice was greater than 80%, and the average recovery was (87.40 ± 3.37)%. The total number of cells in the BALF of mice was the highest in N group, followed by the lowest in group I and group G, and the lowest in group (*P < 0.05*). The proportion of neutrophils in BALF was the lowest in group C, which was only 1.30 ± 0.25, and that in group I was 10.46 ± 2.14. The highest in group N and group G were 21.2 ± 4.21 and 20.68 ± 4.61(*P < 0.05*)(Table 1).

Table 1
Total number and classification of BALF cells in each group(x ± SD)

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Group	EOS(%)	NEU(%)	LYM(%)	MAC(%)	TOTAL(×104/ml)
C group	1.03 ± 0.72	1.30 ± 0.25	16.25 ± 3.04	78.86 ± 3.52	37.66 ± 2.39
N group	4.02 ± 1.06a	21.2 ± 4.21a	50.32 ± 16.14a	24.33 ± 9.8a	316.13 ± 20.78a
I group	4.08 ± 1.95a	10.46 ± 2.14ab	64.34 ± 7.1a	16.44 ± 4.01a	275.28 ± 16.72ab
G group	3.34 ± 1.75a	20.68 ± 4.61ac	52.76 ± 14.95a	25.66 ± 8.97a	271.61 ± 17.70ab
P	*0.009*	*0.001*	*< 0.001*	*0.001*	*0.001*

Note: a,compared with group C,P < 0.05; b,compared with group N,P < 0.05; c,compared with group I,P < 0.05.

3.3. Expression of cytokines in peripheral blood and BALF

3.3.1.Expression of various cytokines in peripheral blood

The concentration of IFN-γ in peripheral blood of group C was significantly higher than that of group N,group I and group G (*P < 0.05*). There was no significant difference between the N group, the I group and the G group. The IL-6 concentration in the peripheral blood of the I group was the lowest, the second was G group and C group, the N group was the highest (P < 0.05).The concentration of IL-13 was significantly lower than that in N group, I group and G group (*P < 0.05*). The concentration of IL-17 of N group was significantly higher than that in group C, I and G (*P < 0.05*).(Table 2).

Table 2
Expression of cytokines in peripheral blood

	IFN-γ (pg/ml)	IL-6 (pg/ml)	IL-13 (pg/ml)	IL-17 (pg/ml)
C group	432.93 ± 50.74	67.21 ± 4.27	386.32 ± 27.16	111.54 ± 14.52
N group	266.05 ± 23.11a	82.78 ± 13.37a	565.30 ± 70.77a	178.64 ± 11.82a
I group	256.42 ± 32.51a	44.10 ± 8.48ab	453.62 ± 57.88b	110.05 ± 11.89b
G group	272.14 ± 33.61a	56.60 ± 4.81abc	542.25 ± 76.41ac	118.54 ± 9.52b
P	*0.001*	*0.001*	*< 0.001*	*0.001*

Note: a,compared with group C,P < 0.05; b,compared with group N,P < 0.05; c,compared with group I,P < 0.05.

3.3.2.Expression of various cytokines in BALF

IFN-γ was highest in group C, higher than group N and G, and the difference was statistically significant(*P < 0.05*).The IL-6 level in group C was the lowest, and the difference was statistically significant (*P < 0.05*). The difference between group N and group G was not significant.IL-13 was the lowest in group C and the highest in group N. The group I and group G were lower than those in group N(*P < 0.05*). IL-17 was the highest in N,C, I and G groups were all lower than group N. The difference was statistically significant.IL-17 levels after ICOSL antibody intervention were lower than those without intervention, and the difference was statistically significant(Table 3).

Table 3
Expression of various cytokines in BALF

	IFN-γ (pg/ml)	IL-6 (pg/ml)	IL-13 (pg/ml)	IL-17 (pg/ml)
C group	211.02 ± 9.16	20.35 ± 1.26	127.02 ± 9.88	62.02 ± 11.16
N group	140.17 ± 28.86a	37.09 ± 2.19a	160.19 ± 17.63a	97.65 ± 5.91a
I group	183.89 ± 24.36b	30.17 ± 2.13ab	135.07 ± 5.29b	79.32 ± 7.13ab
G group	150.56 ± 15.29ac	33.09 ± 4.66a	139.62 ± 3.31b	93.22 ± 6.37ac
P	*< 0.001*	*0.001*	*0.001*	*0.001*
Note: a,compared with group C,P < 0.05; b,compared with group N,P < 0.05; c,compared with group I,P < 0.05.				

3.4. Histopathological analysis

3.4.1.HE staining pathology of lung tissue

Under HE staining, the tracheal structure, lung tissue and alveolar septum in group C were normal, no obvious inflammatory cell infiltration.The infiltration of neutrophils, lymphocytes and plasma cells was obvious intracheal stenosis, bronchial epithelial edema, shedding and degeneration, peri-tracheal lung tissue and alveolar septum of N and G group.Intravenous mucosal epithelial hyperplasia and infiltration of inflammatory cells around the trachea, lung tissue and alveolar space in group I were better than those in group N and G.(Figs. 1 ~ *8*).

3.4.2.PAS staining pathology of lung tissue

Mucus secretion was obvious in N and G groups, and no obvious mucus secretion was observed in group C. There was a certain degree of mucus secretion in group I but improved compared with group N and group G.(Figs. 9 ~ *16*).

3.4.3.ICOSL immunohistochemistry in lung tissue

After DAB color development, hematoxylin counterstaining, ICOSL positive expression cells were stained brownish yellow.The expression of ICOSL-positive cells in the alveolar interstitial and airway of N and G

groups were significantly higher than that of I group. There were no ICOSL-positive cells in the alveolar interstitial and airway of C group(*P < 0.05*).(Figs. 17 ~ *24*,Table 4).

Table 4
ICOSL expression in lung tissue (mean gray value, IOD)

group	ICOSL(%)
C group	6.77 ± 2.75
N group	75.62 ± 10.52a
I group	48.71 ± 13.32ab
G group	70.31 ± 13.65ac
P	*0.002*
Note: a,compared with group C,P < 0.05; b,compared with group N,P < 0.05; c,compared with group I,P < 0.05.	

Discussion

With intensive study on airway inflammation mechanism of bronchial asthma, it has been found in recent years that a large number of neutrophils exist in bronchial biopsy and induced sputum of some patients with severe asthma and acute exacerbation of asthma, but there is no typical eosinophil infiltration[15~17].Neutrophils are the last differentiated cells with the shortest life span in blood cells and the most important inflammatory cells in body. Normally, neutrophils start their spontaneous apoptotic process from the beginning of maturation.Active damage and repair mechanisms may exist in the respiratory tract during the onset of asthma, and many mediators produced by neutrophils play an important role in this process.Studies have shown that the levels of IL-6, IL-8 and IL-17 in peripheral blood or BALF are significantly increased during acute attack of asthma, and these cytokines can inhibit the apoptosis of neutrophils and promote their aggregation into inflammatory airways[18~20].ICOSL molecule is the main member of CD28/B7 superfamily [8]. Because of its importance in T lymphocyte activation, polarization of Th1/Th2 subgroup and immunoglobulin homology transformation, ICOSL molecule has attracted much attention and become a research hotspot in immunology.In order to observe the pathophysiological effects of ICOSL in mice with neutrophilic asthma, the model of neutrophilic asthma was established by OVA + LPS, and ICOSL antibody was added to the model during induction period. Through the study of behavioral manifestations, characteristic cytokine levels of Th1, Th2 and Th17 cells and pathological changes of lung tissue to understand the role of ICOSL antibody intervention in neutrophilic asthma, and to deduce the pathophysiological role of ICOSL in neutrophilic asthma.In this experiment, the mice with neutrophilic asthma showed obvious sneezing, accelerated breathing, restlessness and frequent scratching of the face and neck skin. Lung histopathology showed obvious infiltration of neutrophils in lung tissue, alveolar interstitium and around the official cavity, hypersecretion

of mucus in the official cavity, tracheal stenosis and so on, which indicated that the model of neutrophilic asthma in mice was successfully established.

During seven stimulations,asthma performance in group N was the earliest and obvious and was improved in group I,there was no asthma behavior in group C.In group N, the performance of asthma appeared earliest.The behavioral changes of mice after ICOSL intervention were delayed.,The overall clinical manifestations of group I were milder than those of group N.These results suggest that ICOSL antibody intervention before stimulation in mice with neutrophilic asthma can slow down the behavioral manifestation of asthma and alleviate the symptoms of asthma until the mice are tolerant to stimulation. It is inferred that ICOSL is involved in the occurrence, development and acute attack of neutrophilic asthma.

Current studies on ICOSL are mostly focused on inflammatory diseases, autoimmune diseases and tumors. ICOSL is highly expressed in inflammatory diseases and is closely related to the severity of inflammation[21].The role of ICOSL in asthma is relatively rare, and the conclusions are inconsistent.In Matsic's study, ICOS + T cells transfected with OVA-specific T cell receptor were transplanted into OVA-sensitized BALB/c mice,observed that lymphocytes, macrophages, neutrophils and eosinophils were significantly increased in bronchial lavage fluid[22].However, Akbari used allergen-induced asthma model in mice to study the role of ICOS/ICOSL signaling pathway. It was found that the co-stimulation of ICOS induced the production of regulatory T (Treg) cells. Treg cells depended on the high level of ICOSL expressed by pulmonary dendritic cells. Treg cells could inhibit the function of antigen-specific T cells and the formation of AHR[23~24].Lung histopathology showed that there were obvious infiltration of inflammatory cells and neutrophils and hypersecretion of airway mucus in group N and group G. After intervention with ICOSL antibody, the infiltration of inflammatory cells and mucus secretion in airway could be alleviated, indicating that ICOSL was involved in the pathological damage process of respiratory tract in neutrophilic asthma.Compared with group C, the percentage of total cells and neutrophils in BALF in group N was significantly higher than that in group C. The levels of cytokines IL-6, IL-13 and IL-17 in peripheral blood and BALF in group N were significantly higher than those in group C, and IFN-γwas decreased.The levels of IL-6, IL-13 and IL-17 decreased and IFN-γincreased after ICOSL antibody intervention compared with group N, but there were still differences compared with group C, suggesting that ICOSL antibody could partially alleviate the pathological and immune inflammation process of neutrophilic asthma.ICOSL antibodies were given to mice at 0, 3, 7, 10 and 14, respectively. The irritation time of mice was delayed compared with that of neutrophilic asthma group. No obvious nodding breathing was observed after continuous atomization for one week. The overall symptoms were improved, the infiltration of inflammatory cells in lung tissue and airway was alleviated, and the secretion of IL-6, IL-13 and IL-17 was also reduced. The aggregation of neutrophils in lung tissue was relatively reduced.All of these prove that ICOSL is a positive regulator, which can synergistically stimulate T cell proliferation and promote the secretion of various cytokines and chemokines.

Conclusions

To sum up, ICOSL antibody intervention before stimulation delayed the onset of asthma and alleviated the symptoms.ICOSL intervention can reduce inflammatory cell infiltration and mucus secretion in airway, partly reduce IL-6, IL-13, IL-17 levels in peripheral blood and BALF, and increase IFN-γlevels.These results suggest that blocking ICOS/ICOSL signaling pathway may partially block the development of neutrophilic asthma and may provide a new target for the treatment of asthma.

Abbreviations

ICOSL	inducible co-stimulator ligand
IFN-γ	interferon-gamma
IL-4	interleukin-4
IL-17	interleukin-17
IgE	immunoglobulin E
Th	T helper cell
Th1	T helper cell 1
Th2	T helper cell 2
Th17	T helper cell 17
Treg	regulatory T cell
BALF	bronchoalveolar lavage fluid
ELISA	enzyme-linked immuno sorbent assay
NE	neutrophil elastase NE
MMP9	matrix metalloprotein-9,MMP-9

Declarations

Ethics approval and consent to participate

This study was performed after Ethics committee of Children’s Hospital of Soochow University approval was obtained.

Consent for publication

Not Applicable.

Availability of data and materials

Data and material are available and stored in Children's Hospital of Soochow University.

Competing interests

The authors declare that they have no competing interests.

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Not applicable.

Authors' contributions

Wei Ji. [1]and Zhengrong Chen.[1] designed the study. Heting Dong.[1*] and Yinying Ren. [2*]performed all the experiments and wrote the manuscript.

Xuejun Shao. [3]provided the experiment equipment.Yongdong Yan[1],Li Huang[1],Meijuan Wang[1],Wenjing Gu[1],Xinxing Zhang[1],Wujun Jiang analyzed data.

All authors read and approved the final manuscript.

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Figures

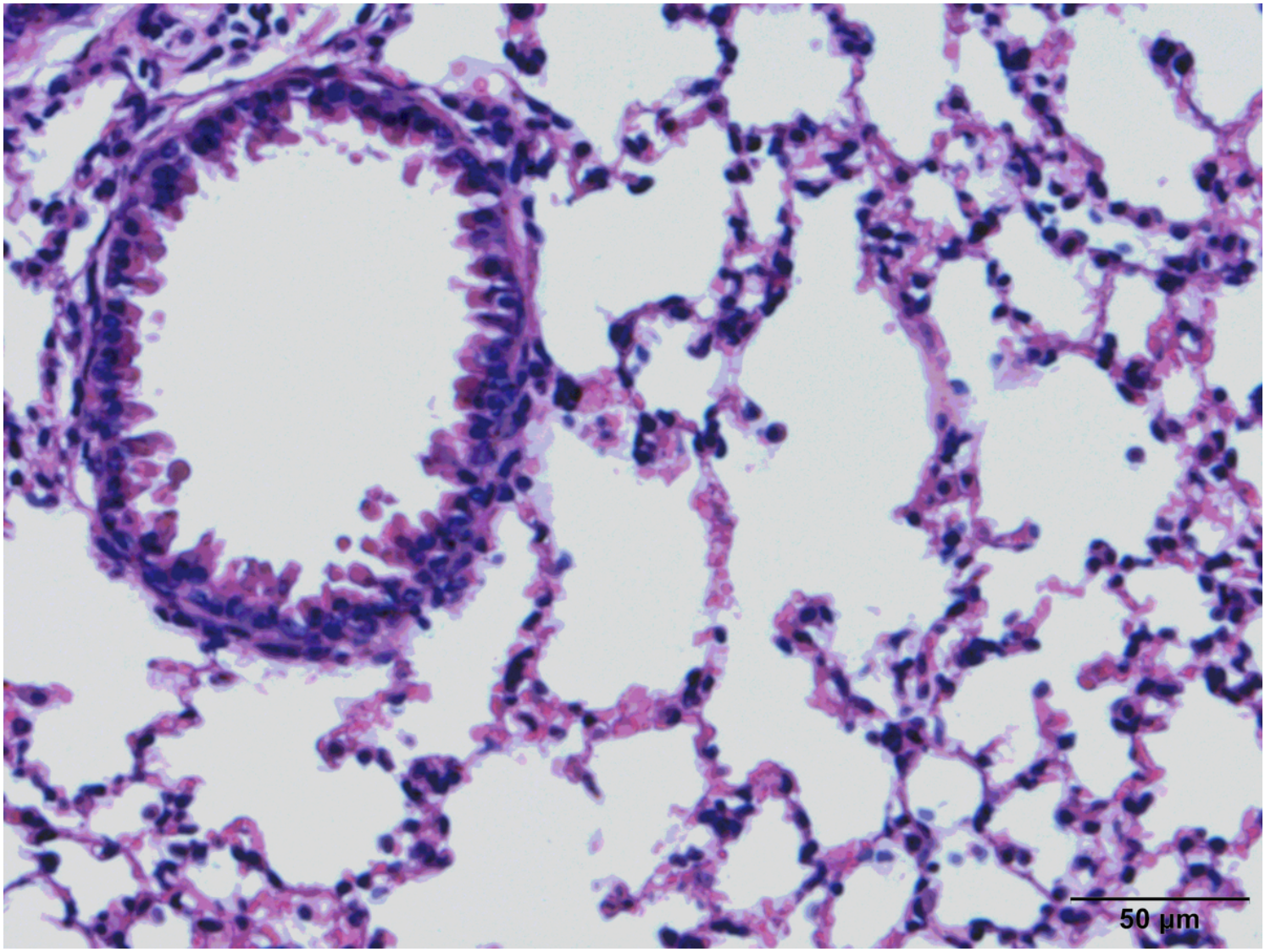

Figure 1

C group

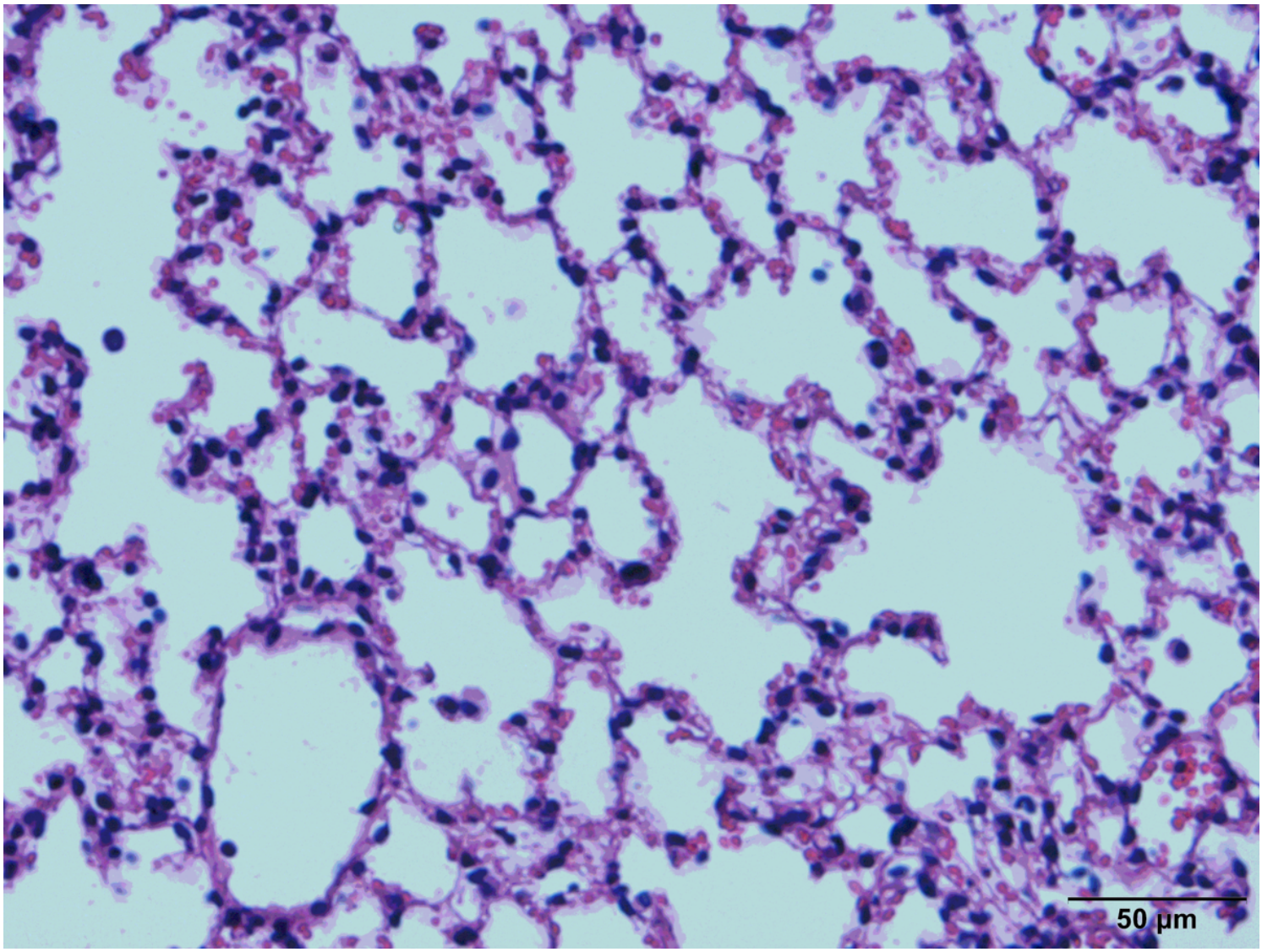

Figure 2

C group

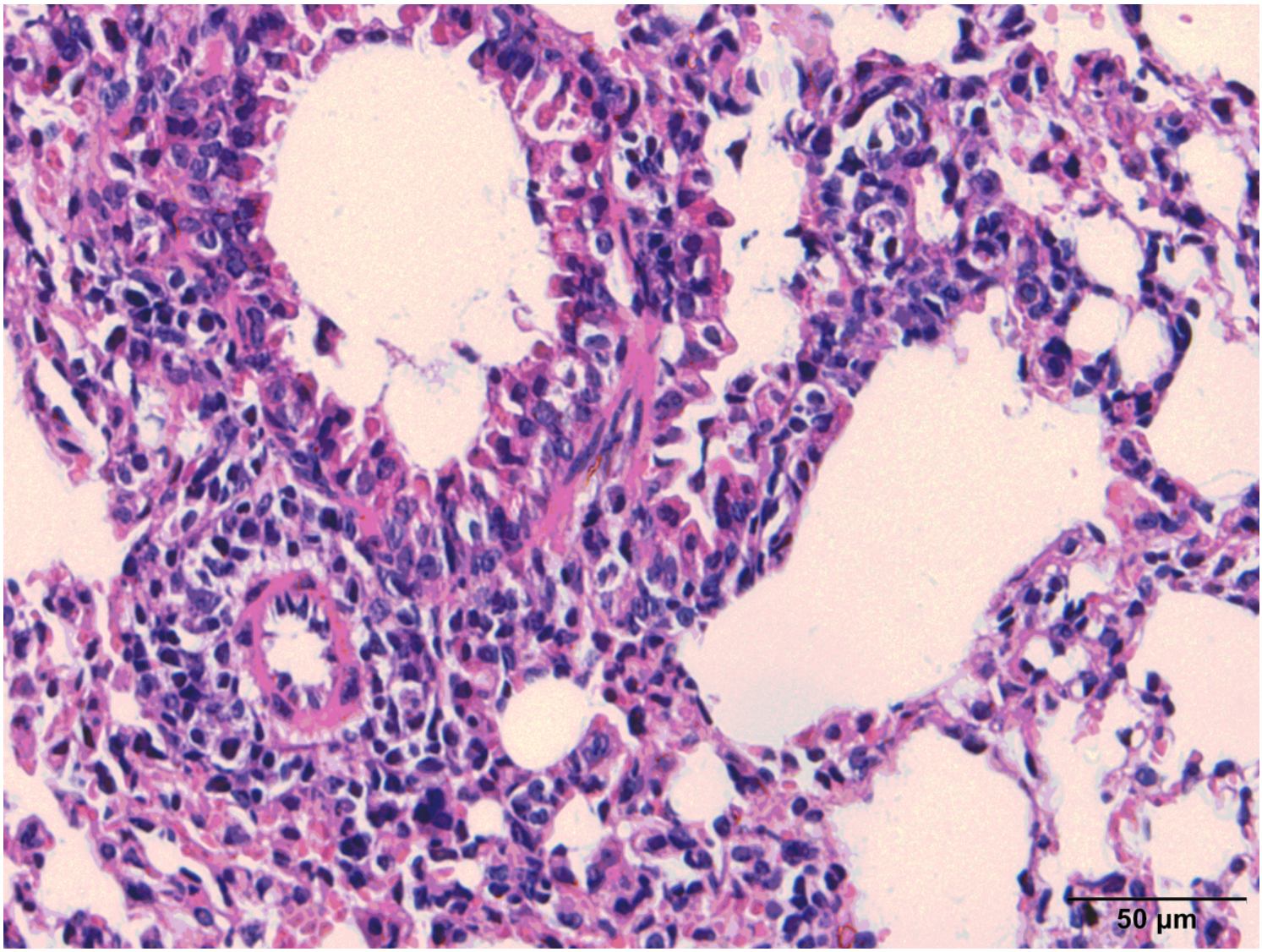

Figure 3

N group

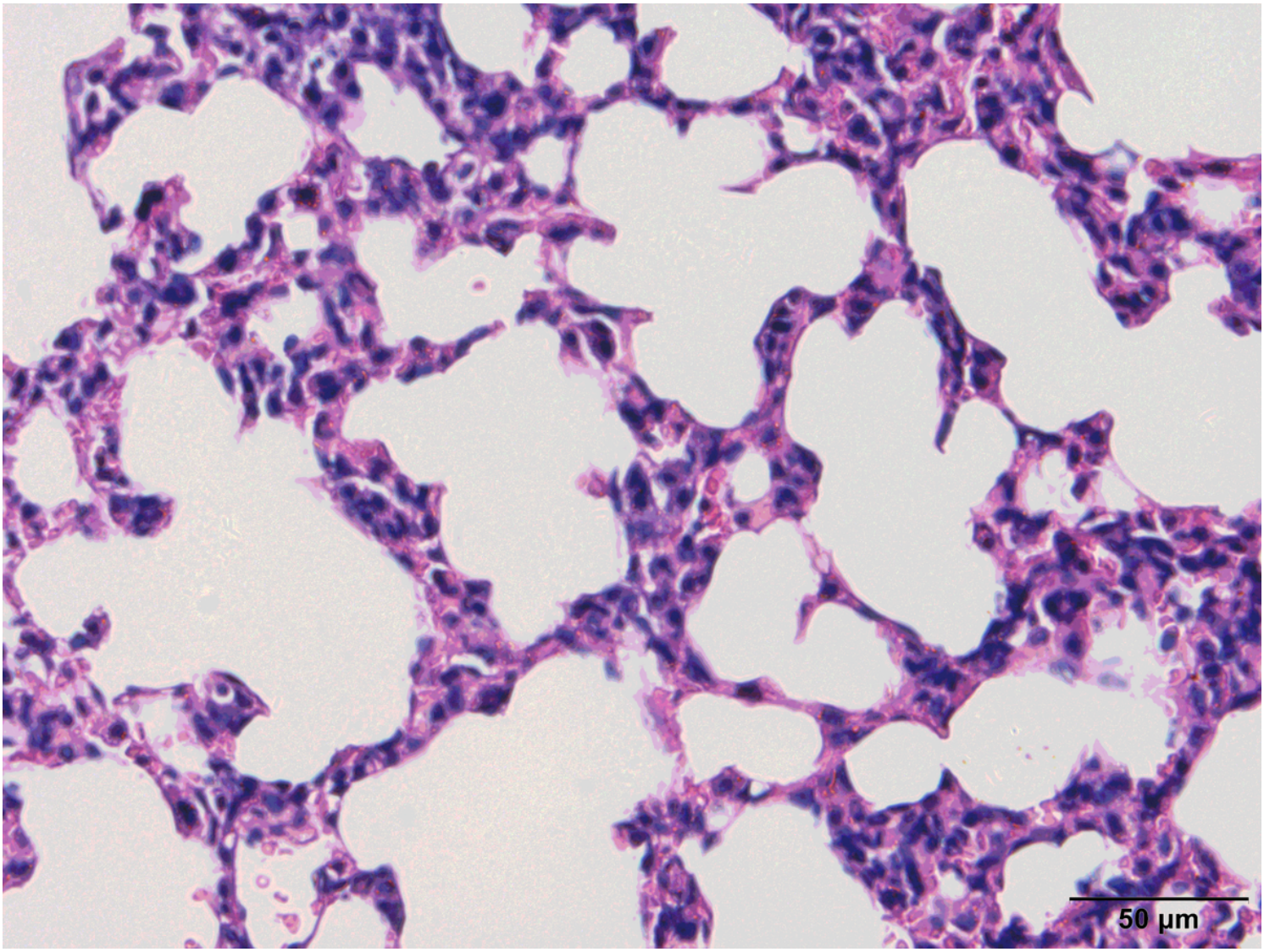

Figure 4

N group

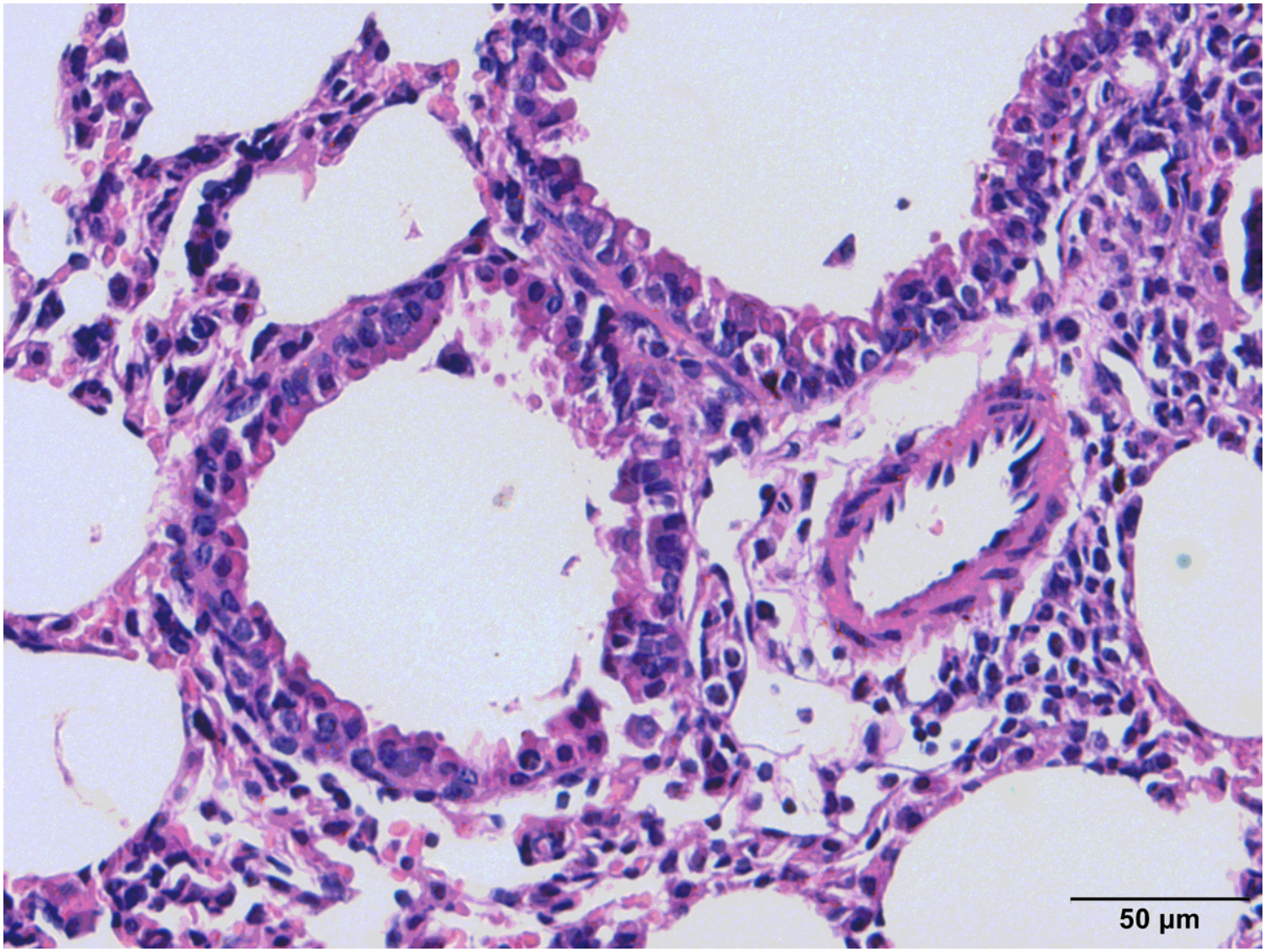

Figure 5

I group

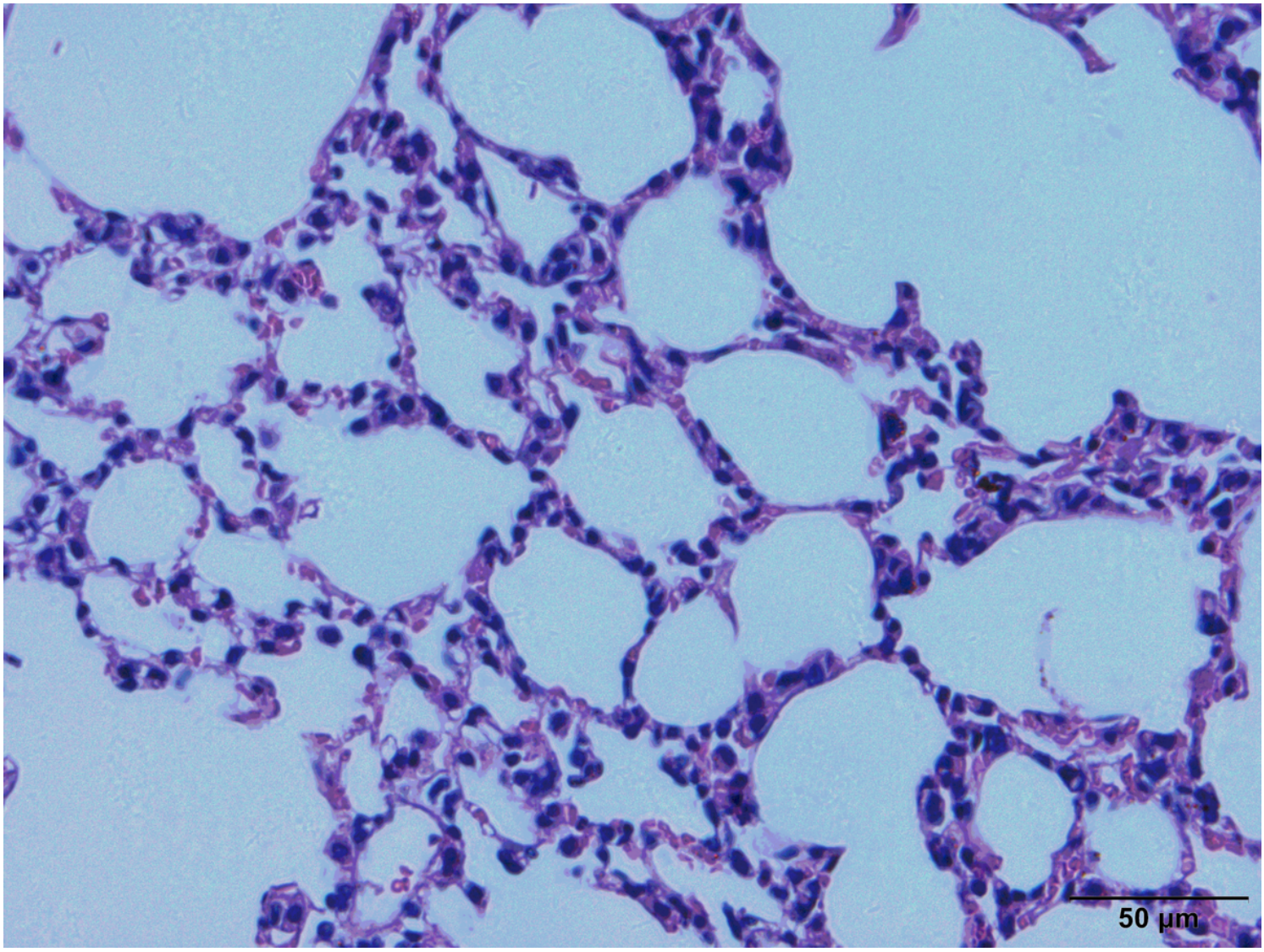

Figure 6

I group

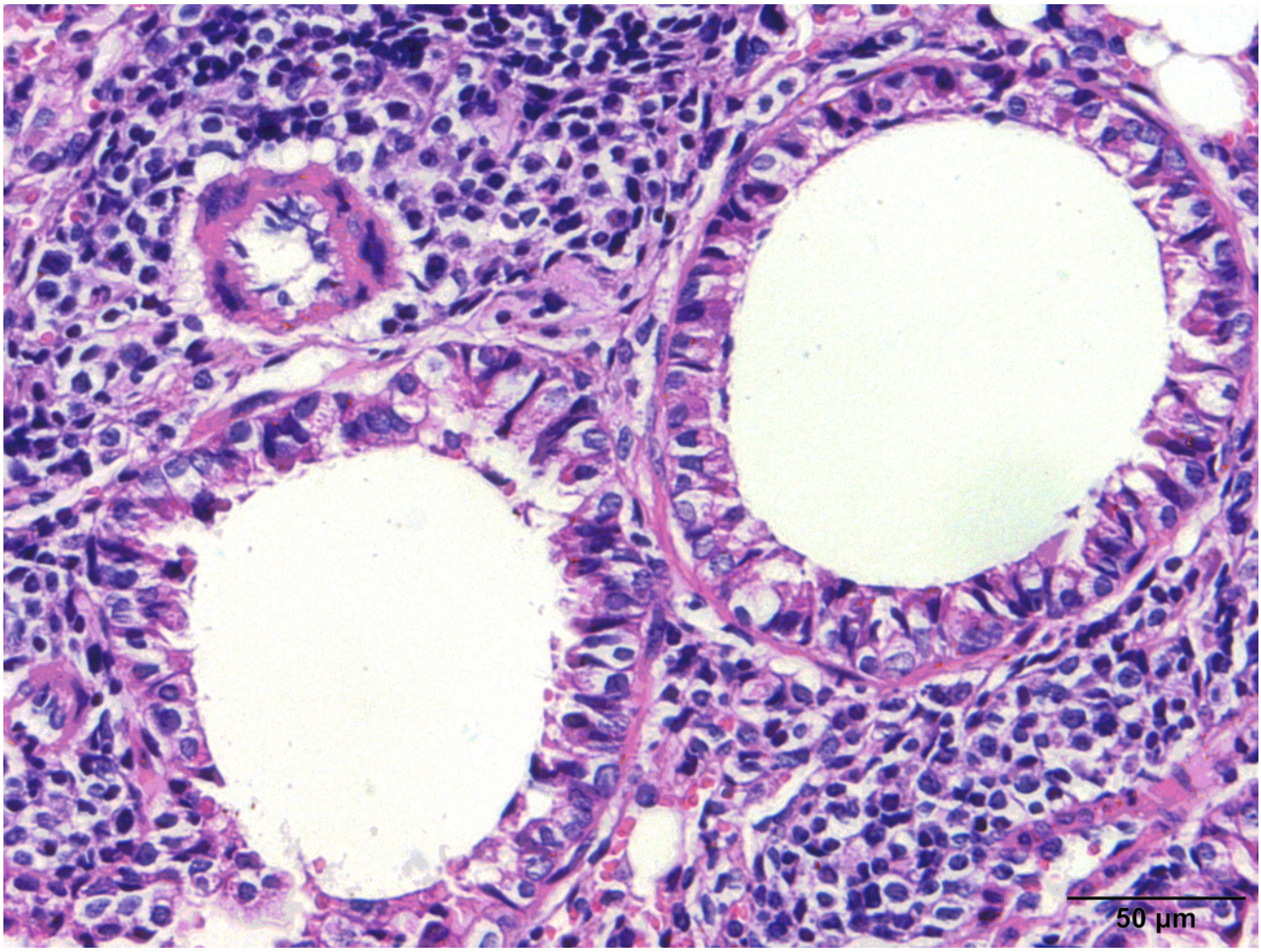

Figure 7

G group

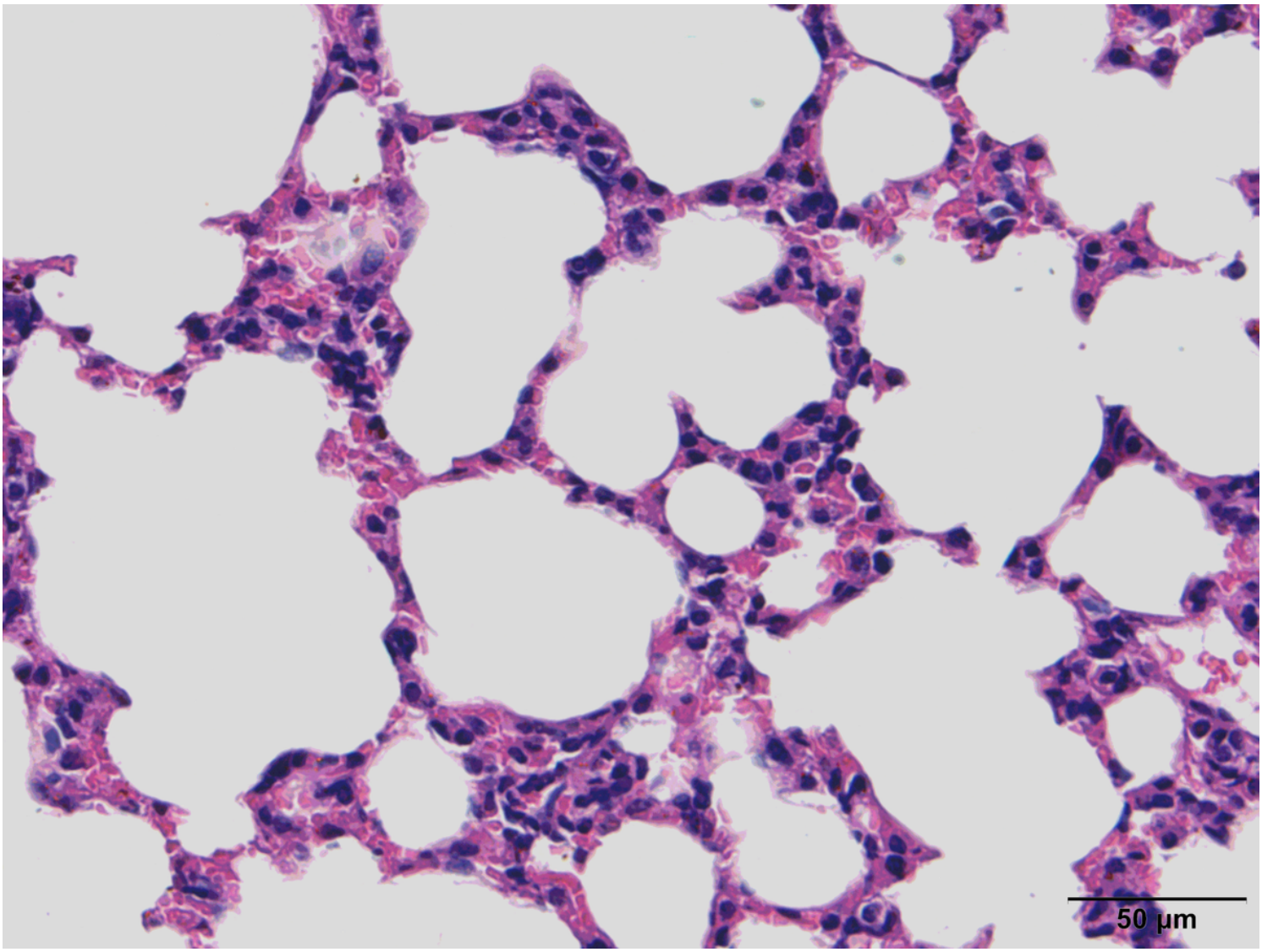

Figure 8

G group

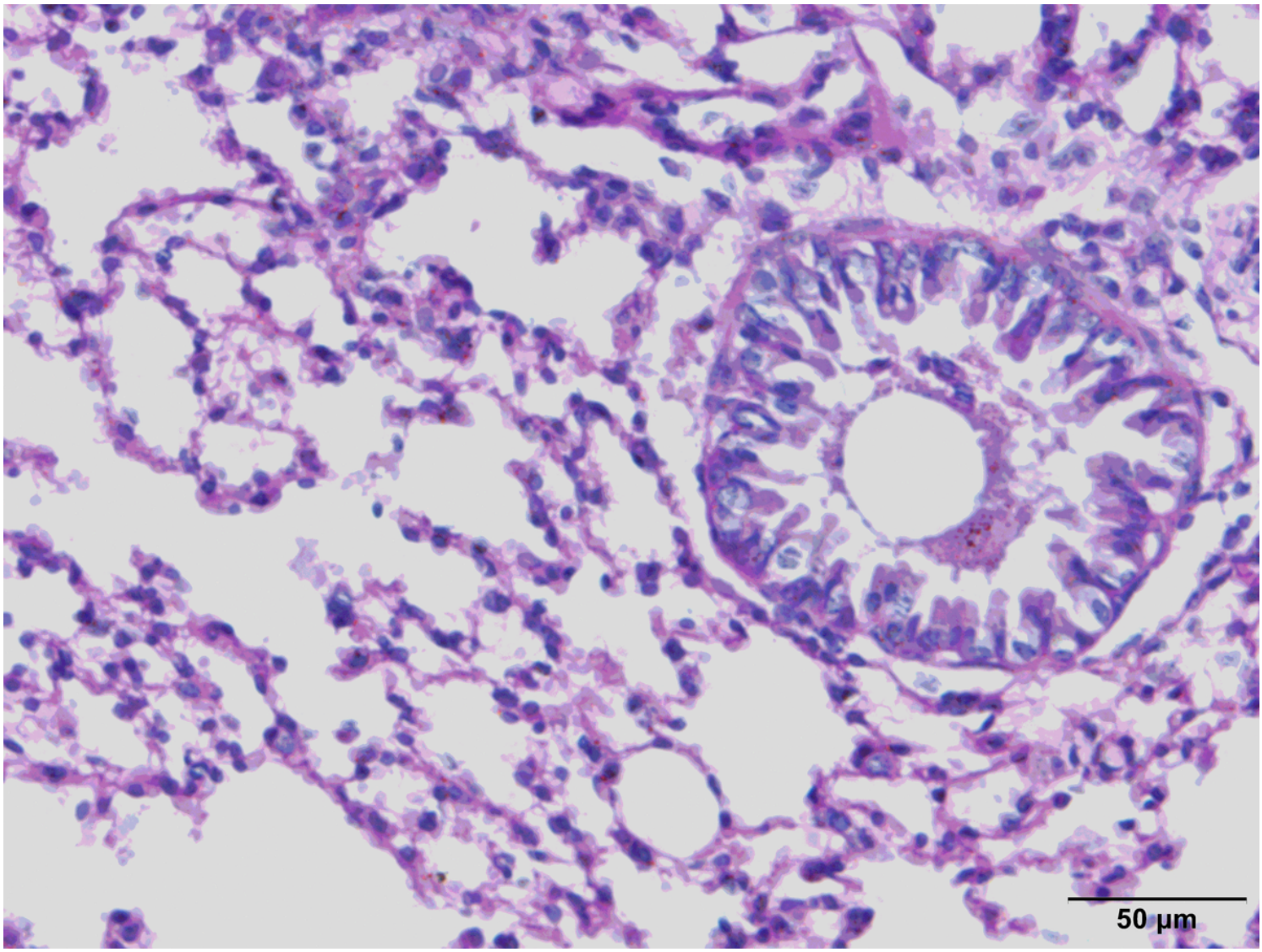

Figure 9

C group

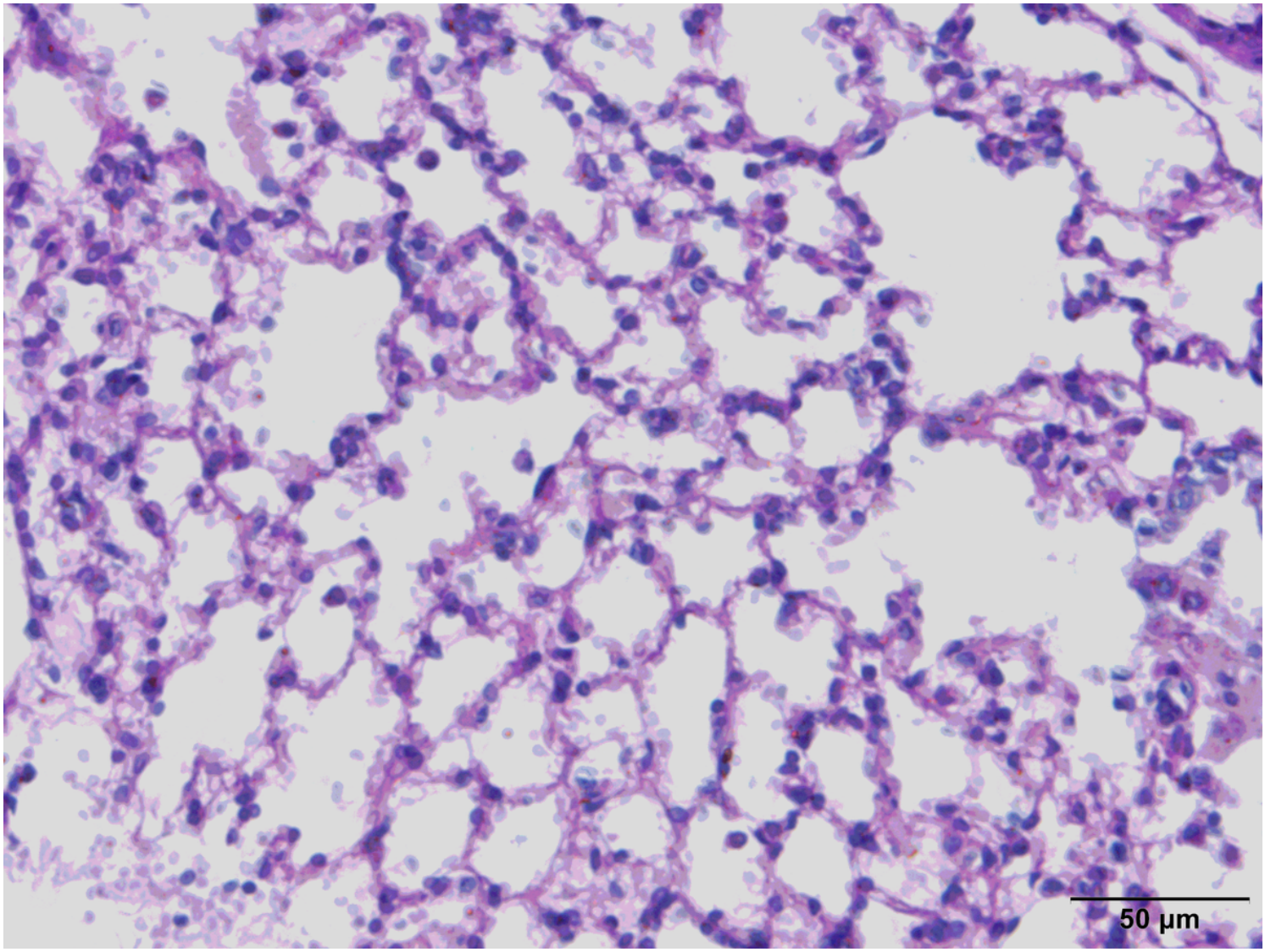

Figure 10

C group

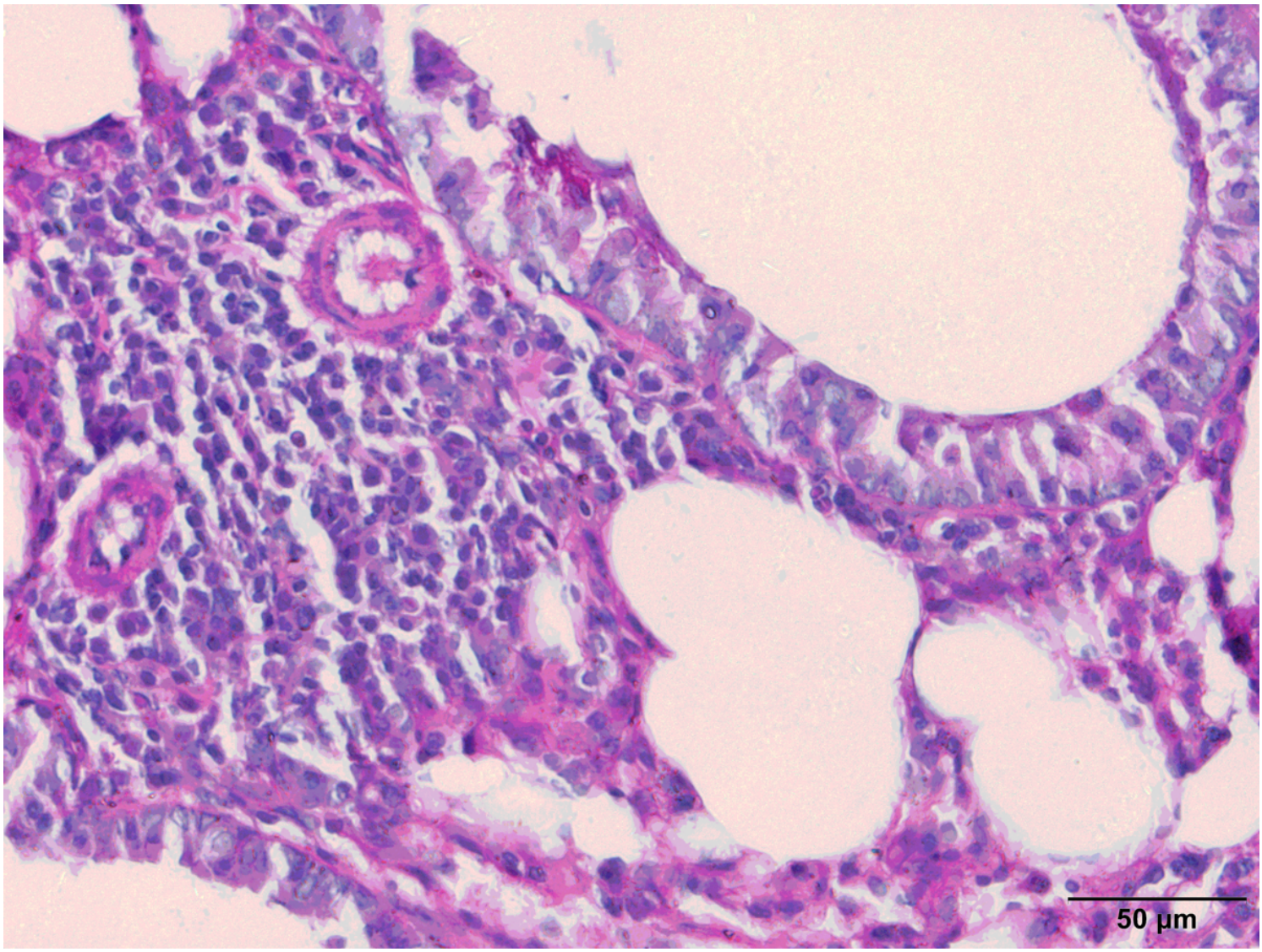

Figure 11

N group

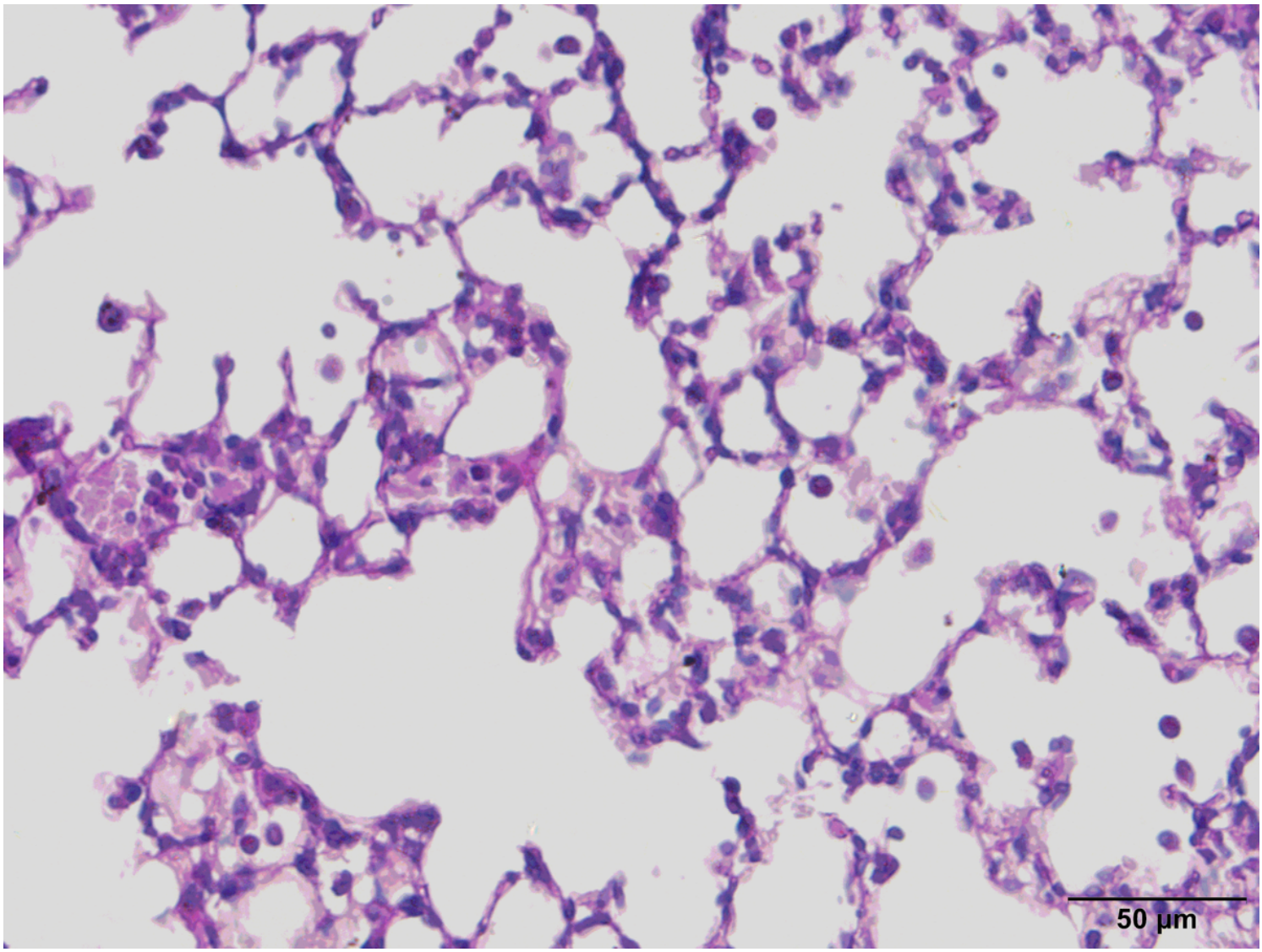

Figure 12

N group

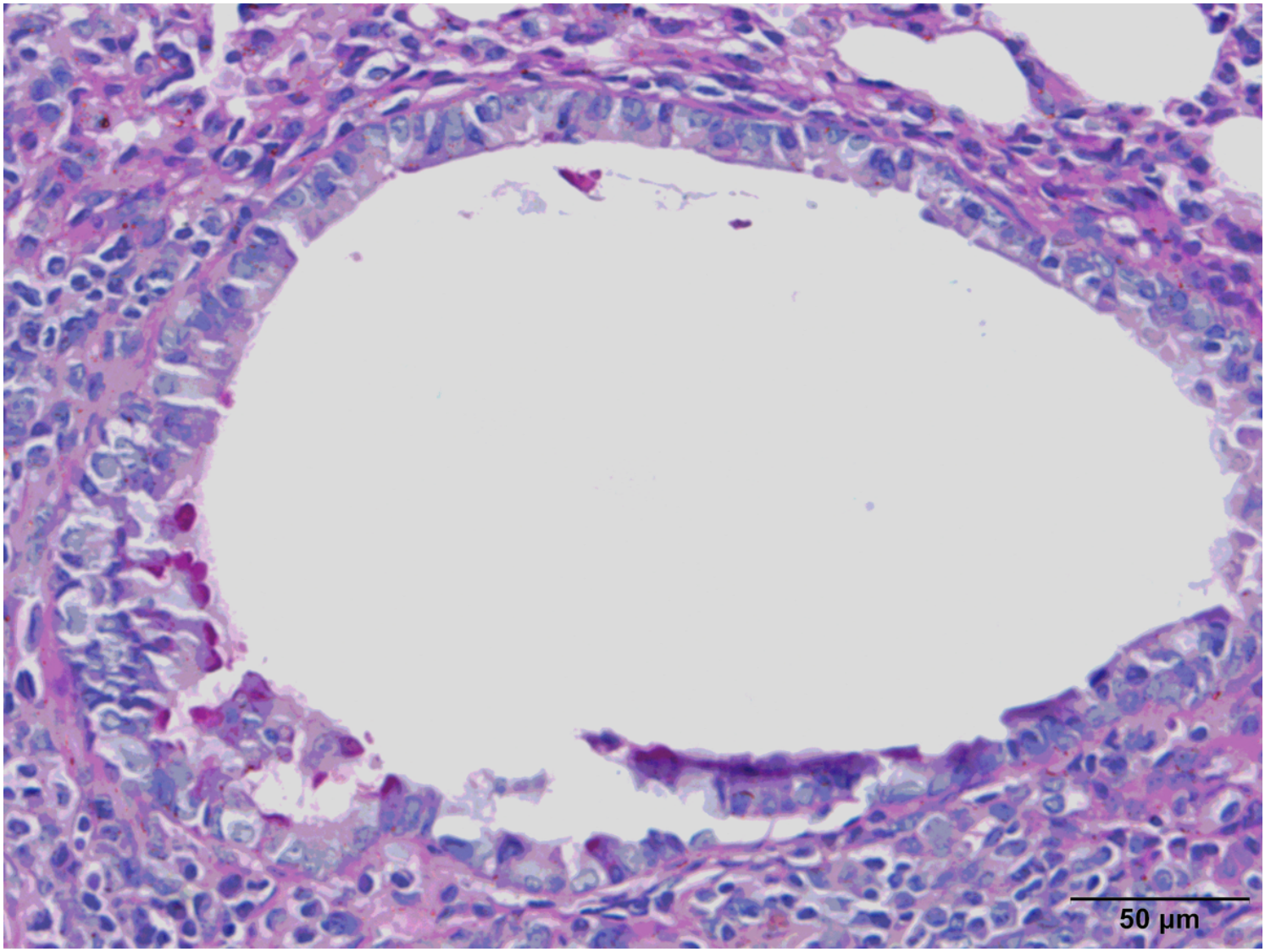

Figure 13

I group

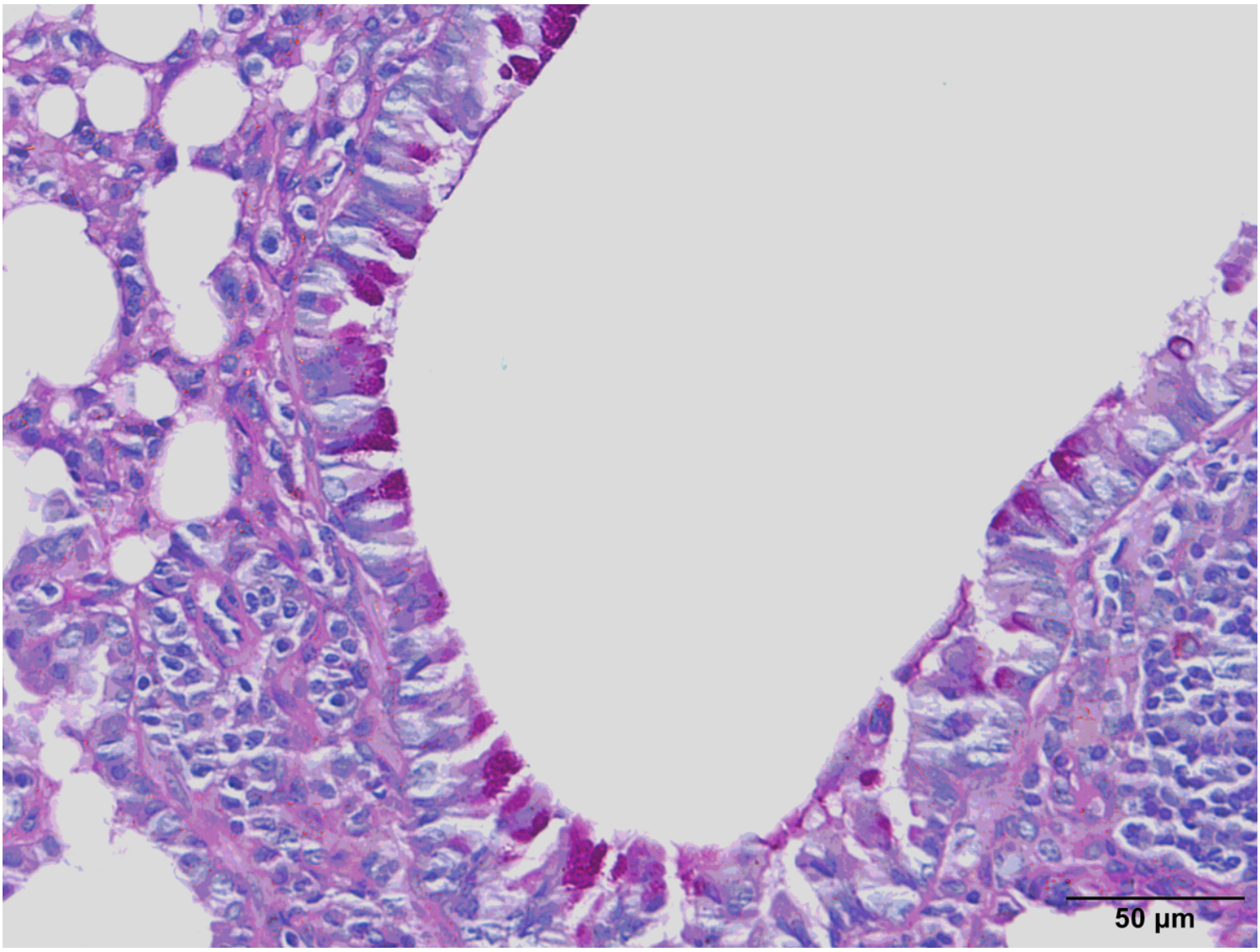

Figure 14

G group

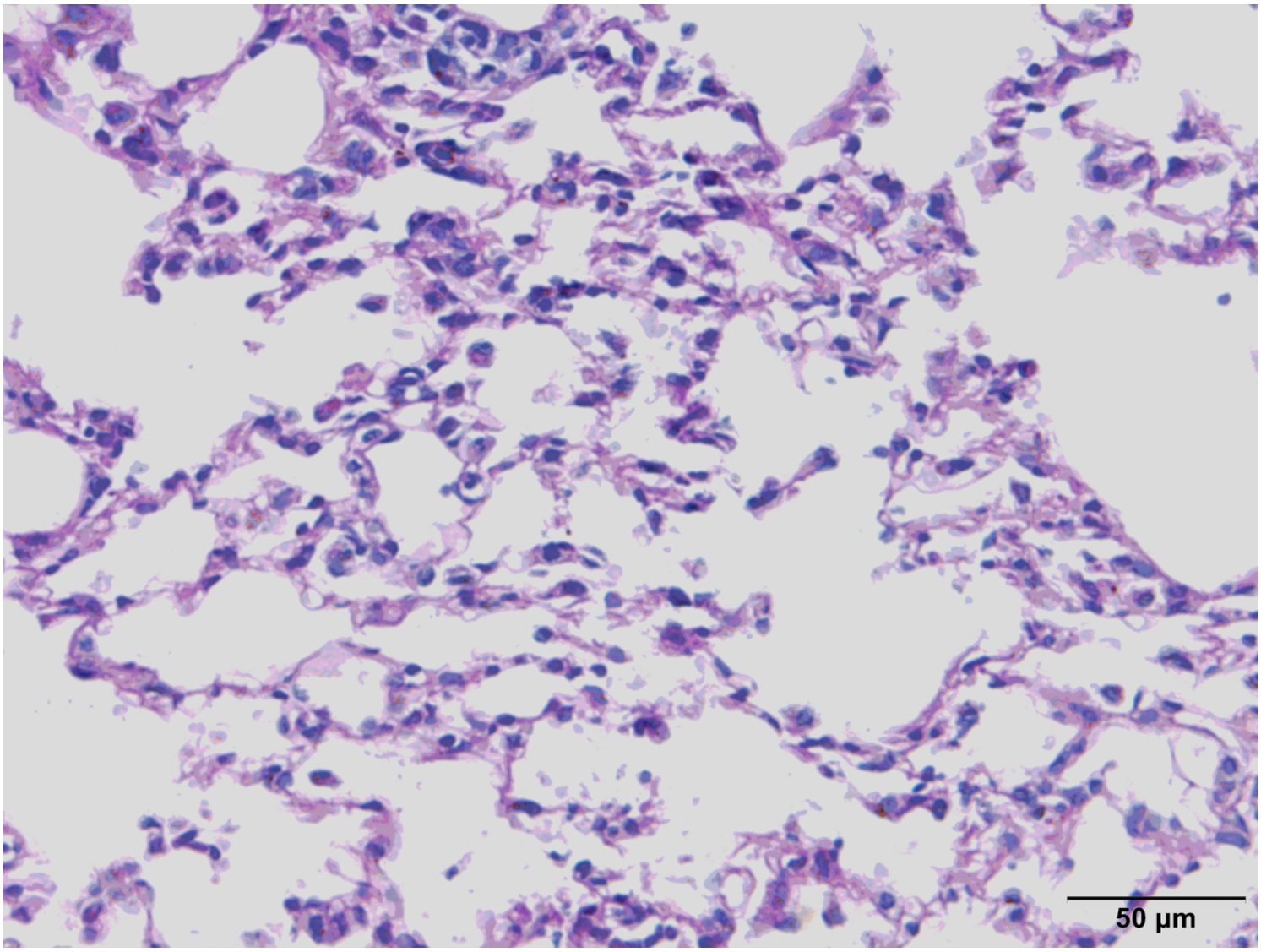

Figure 15

I group

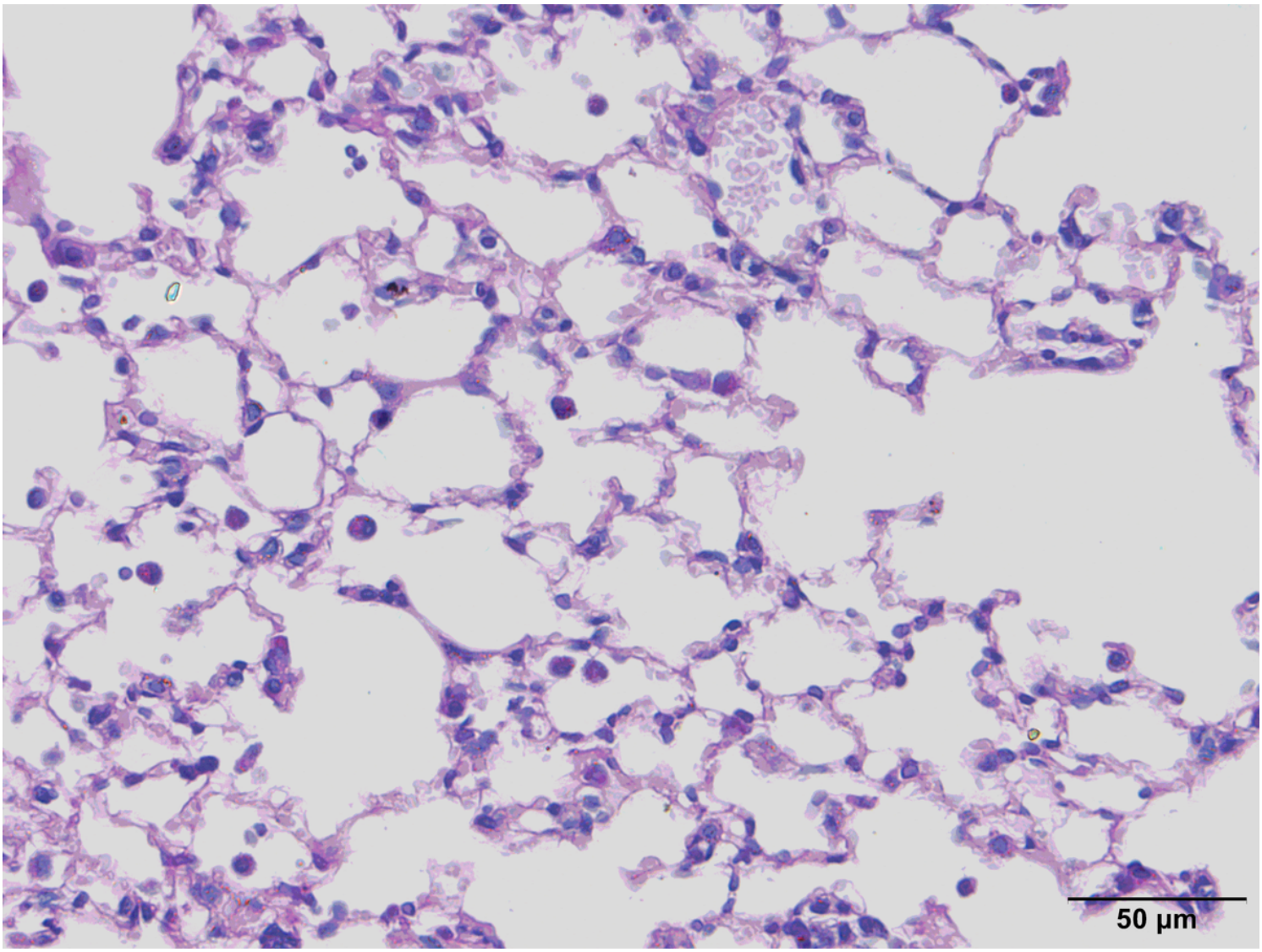

Figure 16

G group

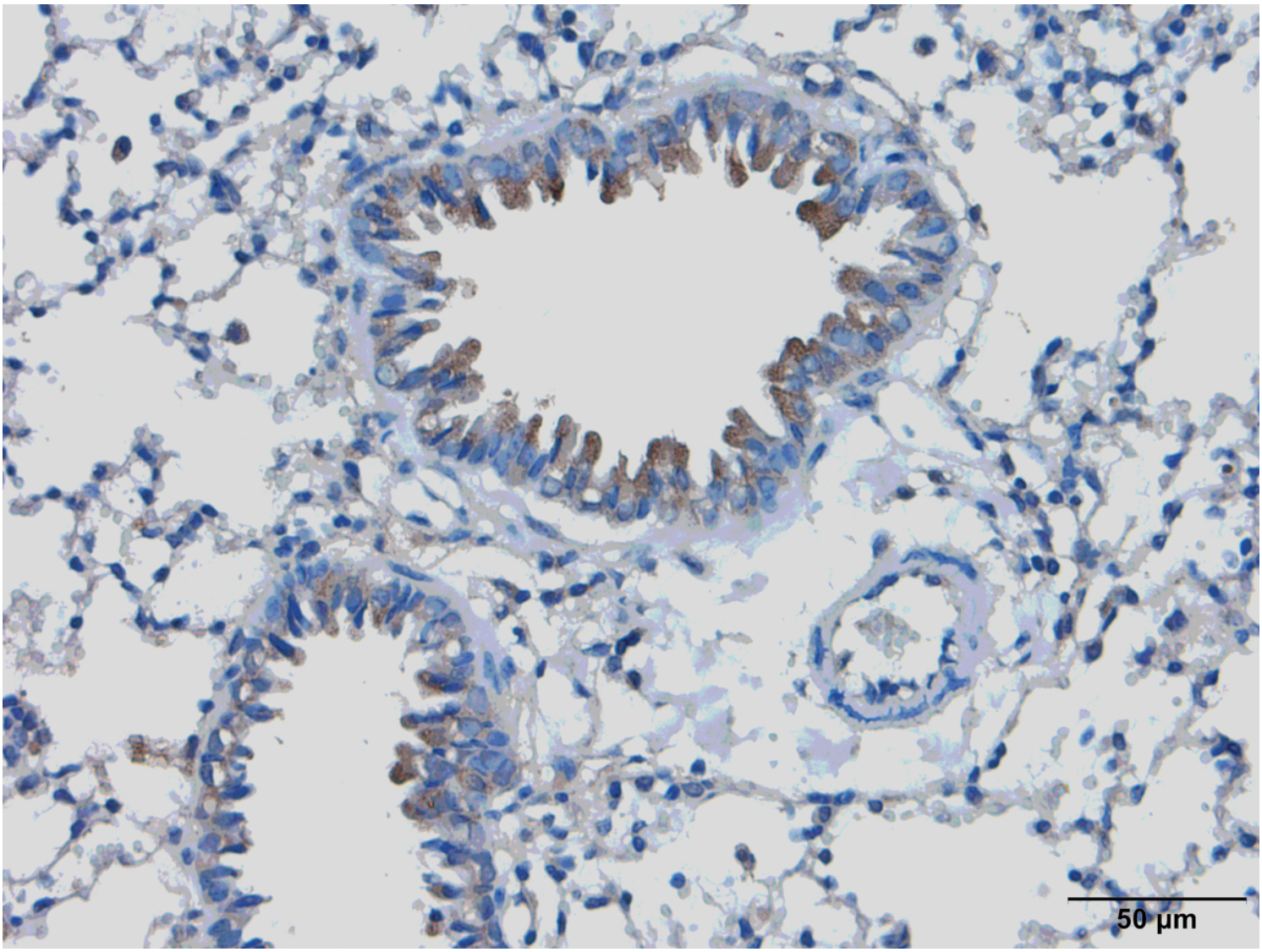

Figure 17

C group

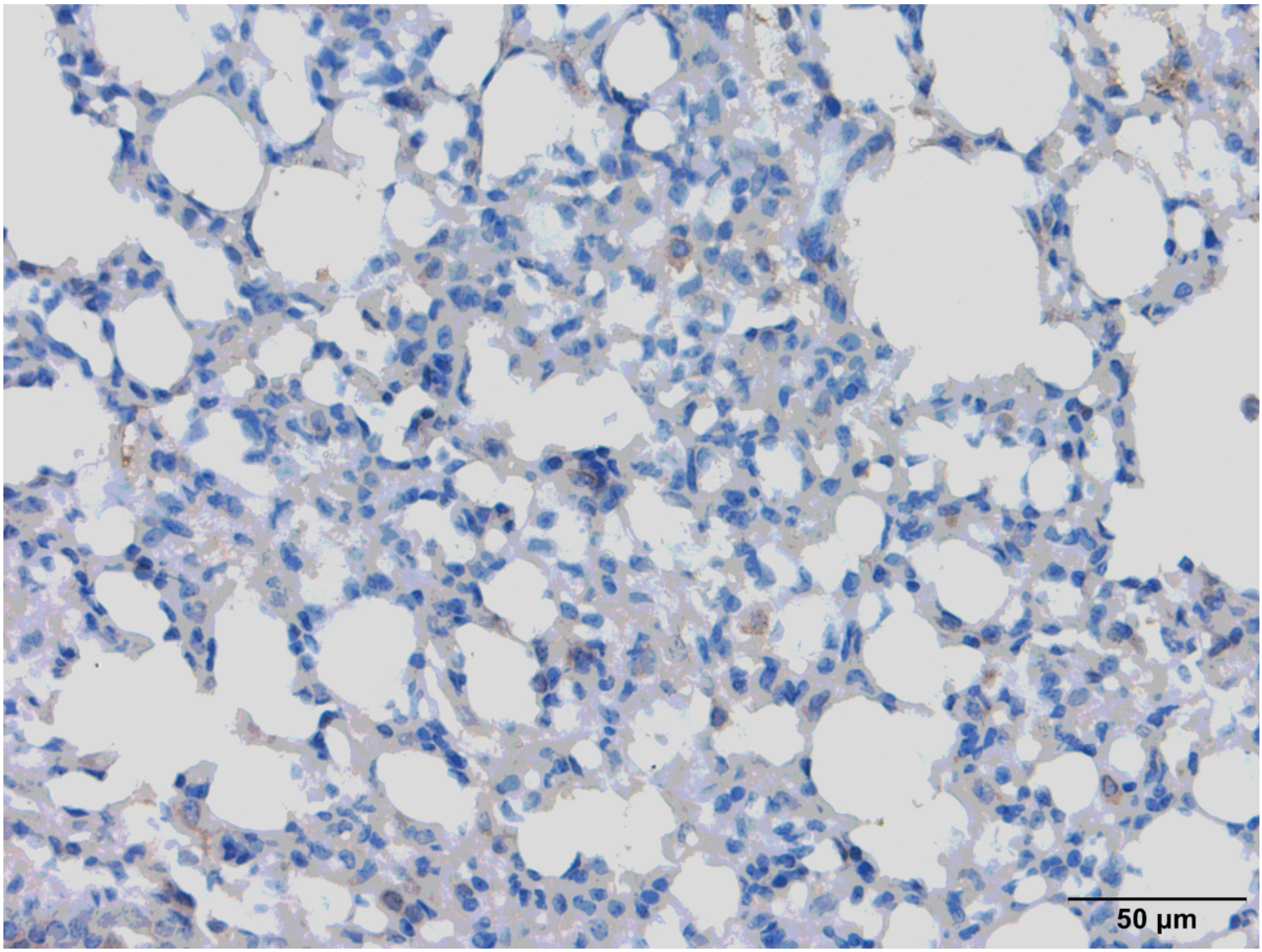

Figure 18

C group

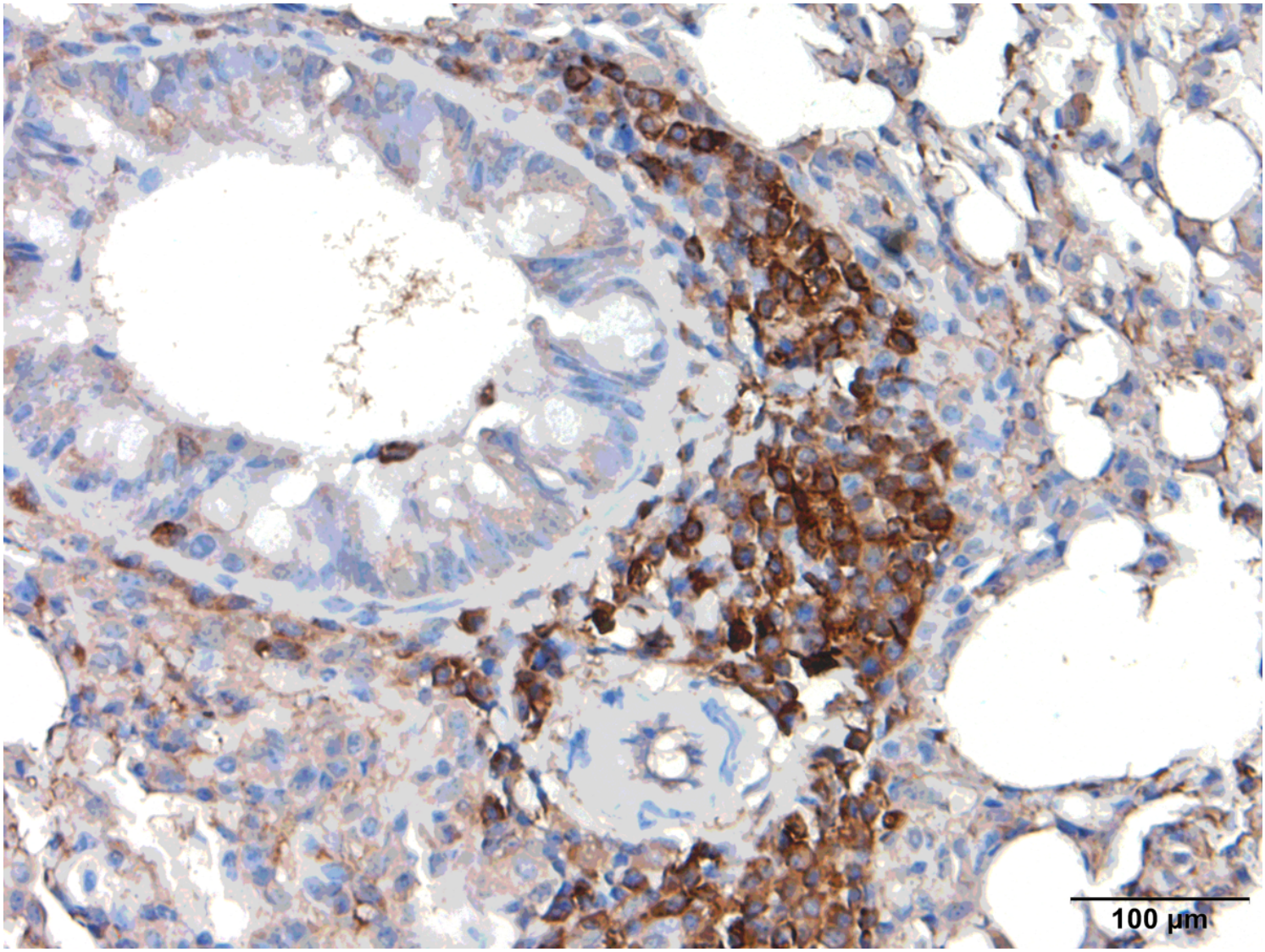

Figure 19

N group

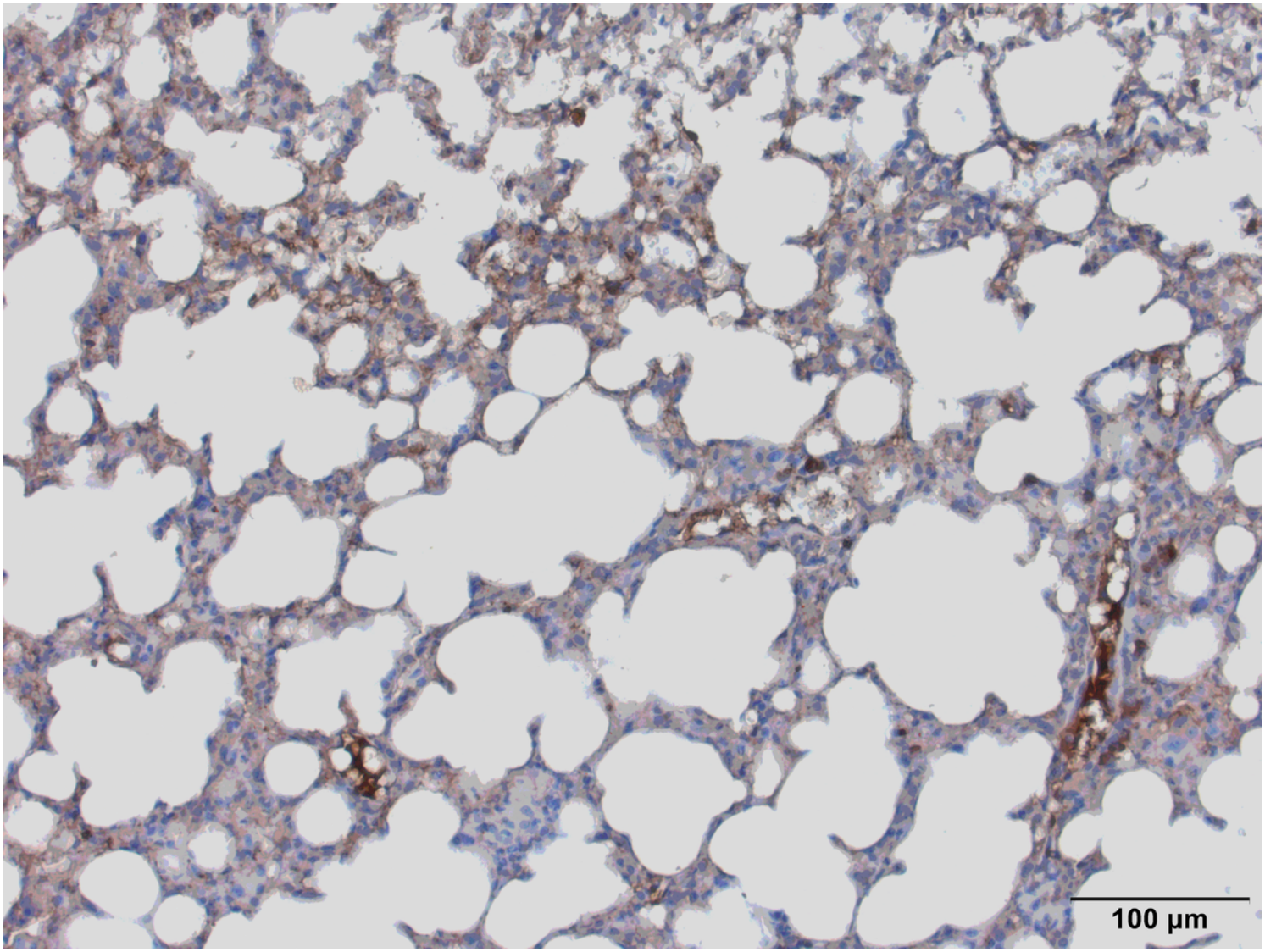

Figure 20

N group

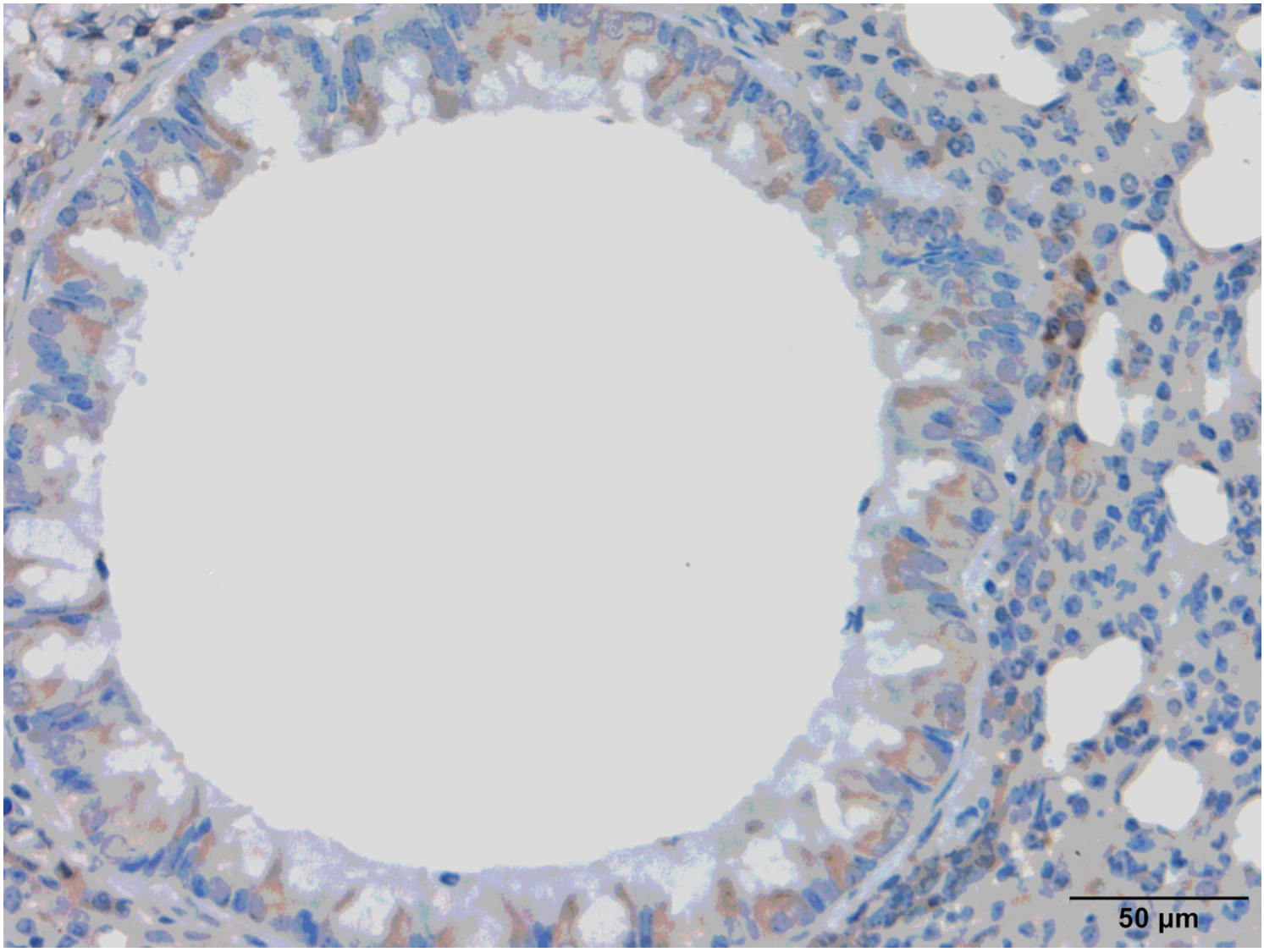

Figure 21

I group

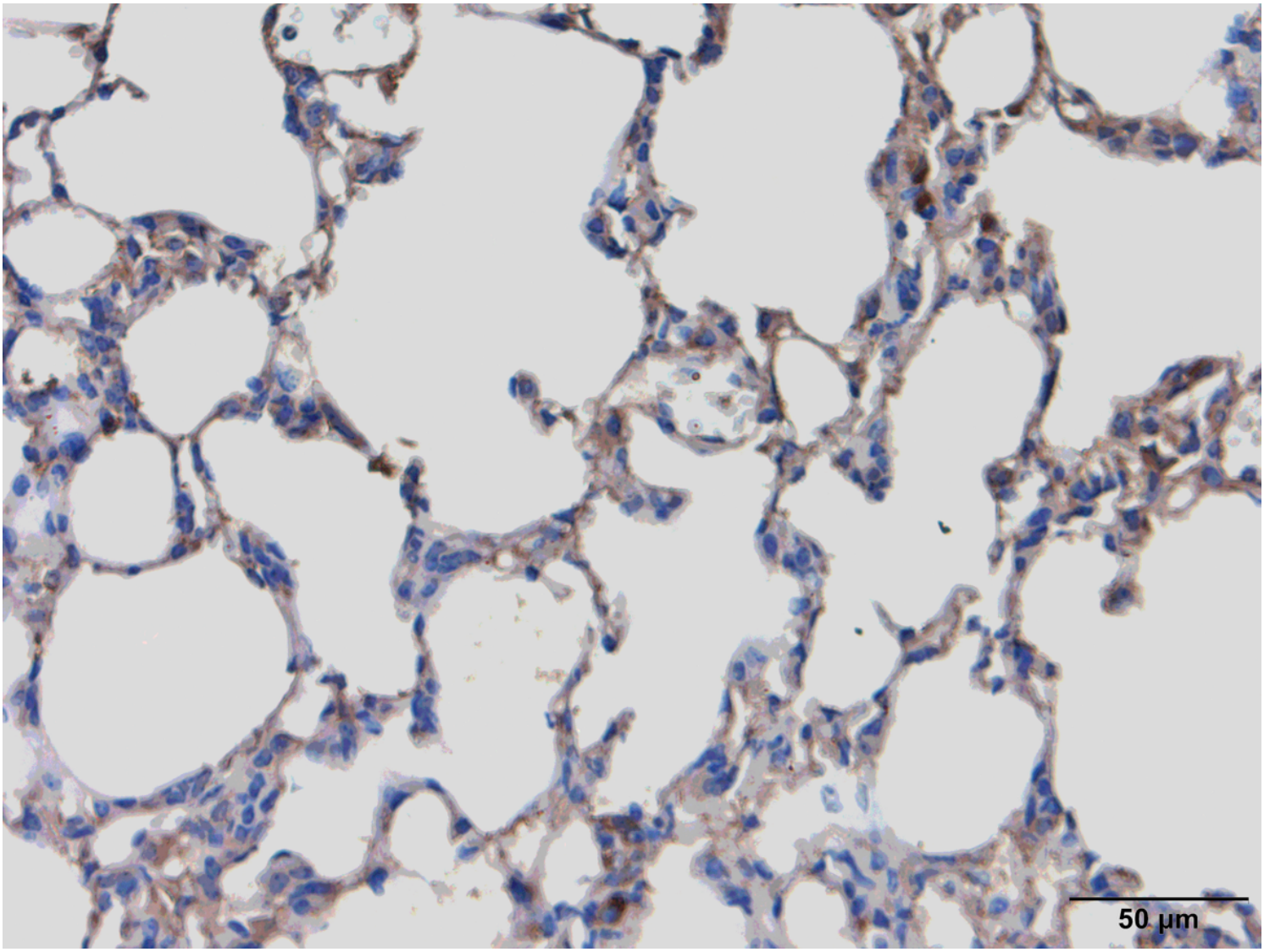

Figure 22

I group

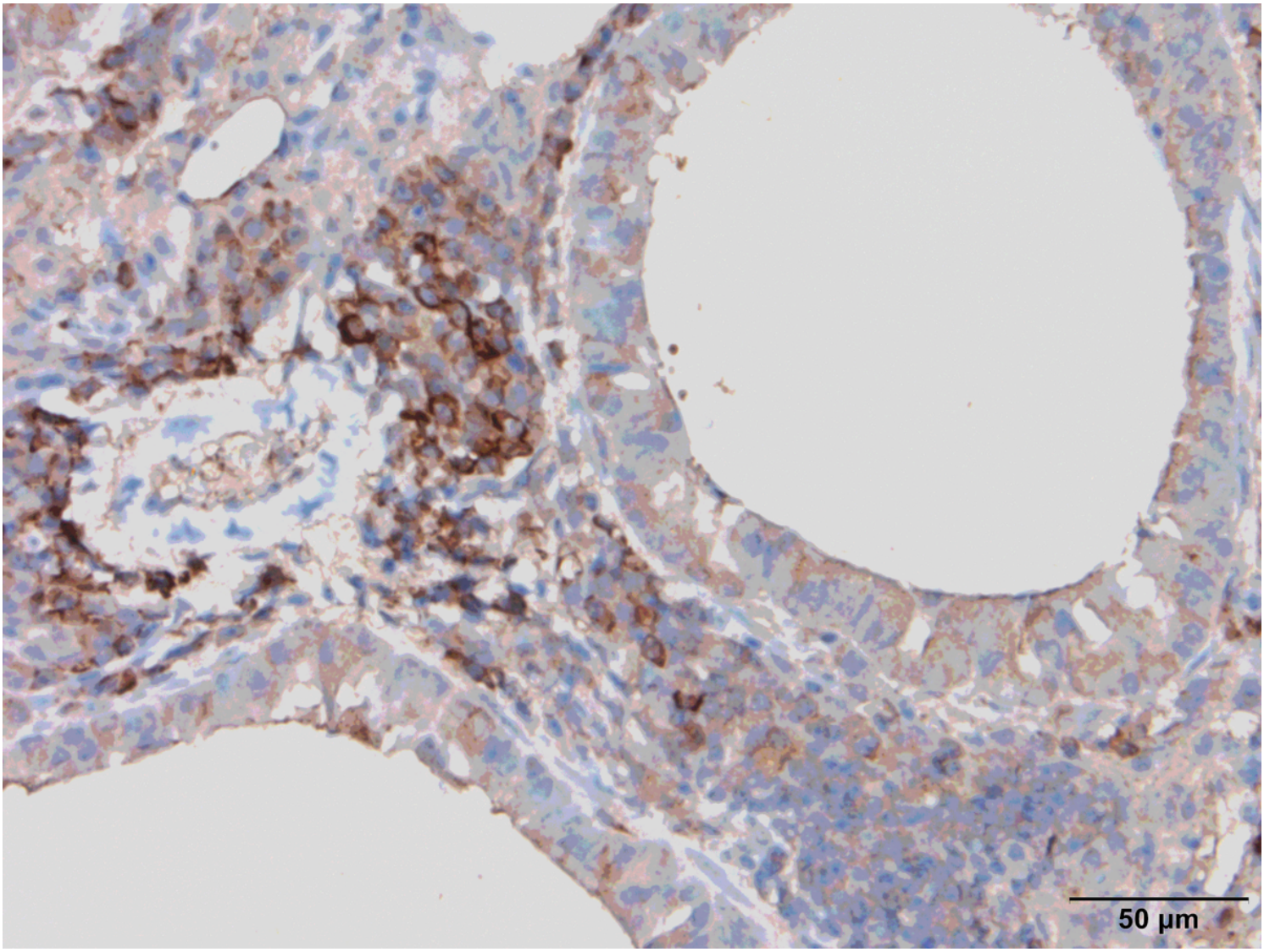

Figure 23

G group

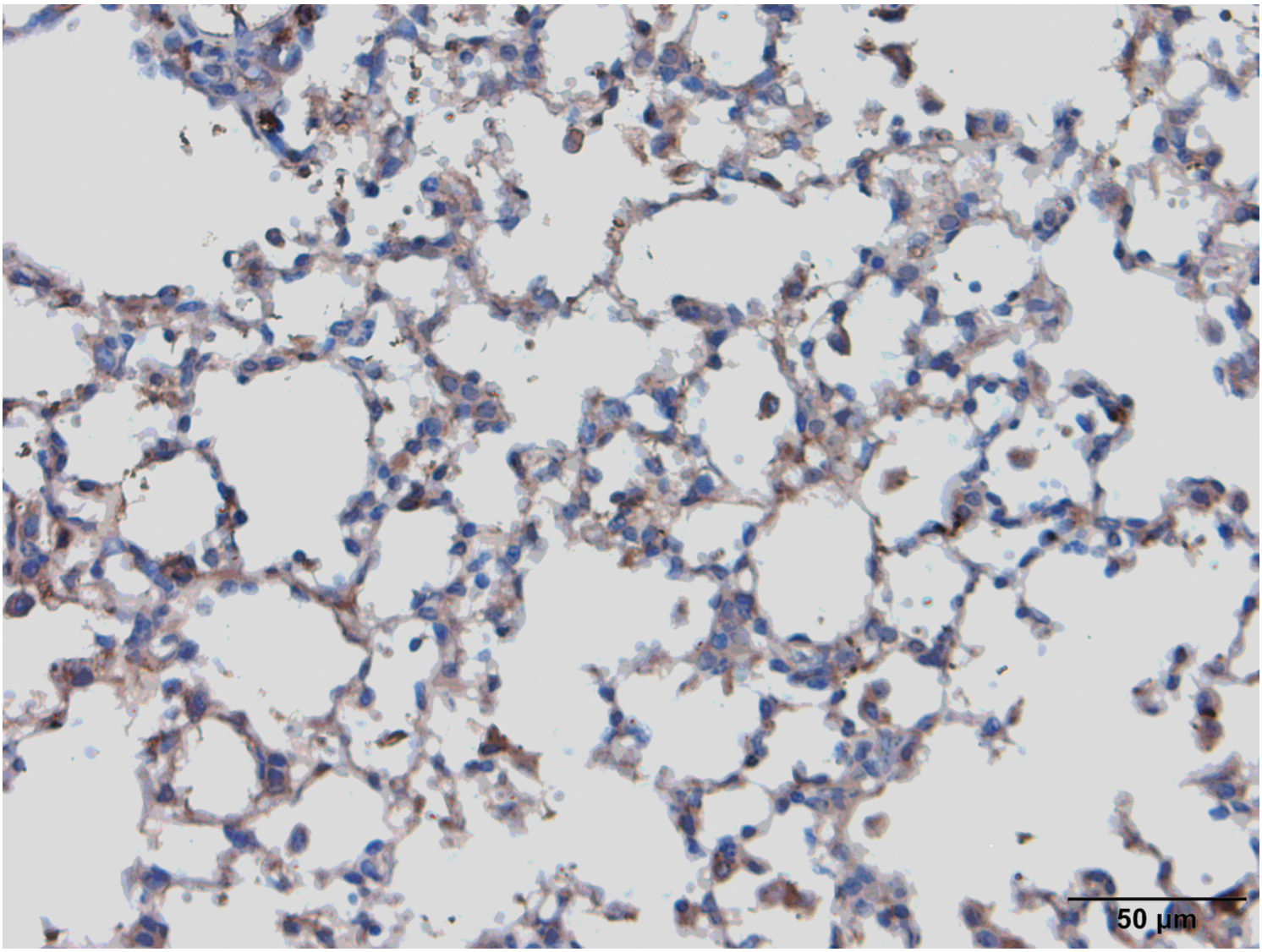

Figure 24

G group