学位論文

Pathological and clinical relevance of selective recruitment of Langerhans cells in the respiratory bronchioles of smokers

（喫煙者におけるランゲルハンス細胞の呼吸細気管支への選択的な集簇と病理学的及び臨床的関連性）

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Pathological and clinical relevance of selective recruitment of Langerhans cells in the respiratory bronchioles of smokers

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ABSTRACT

Background: Smoking causes an influx of inflammatory cells including Langerhans cells (LCs) into the airways and lung parenchyma, thus inducing histological changes, such as emphysema and fibrosis. We examined the distribution and quantity of Langerhans cells in relation to clinical and pathological findings and explored the association between smoking and accumulation of Langerhans cells in the respiratory bronchioles.

Methods: Fifty-three patients who underwent lung resection for primary diseases, including lung cancer, were recruited. Histological and immunohistochemistry analyses were utilized to identify CD1a-positive Langerhans cells in peripheral lung specimens separated from primary lesions. Clinical characteristics, pathological changes, and distribution of CD1a-positive Langerhans cells distribution were assessed.

Results: Of the 53 patients, 35 were smokers and 18 were non-smokers. The number of Langerhans cells in the respiratory bronchioles was significantly increased in smokers as compared to that in non-smokers ($p < 0.001$). The number of Langerhans cells in smokers was significantly higher in patients with mild emphysema than in those without emphysema ($p < 0.01$). The high-LC group showed more frequent smoking-related histological changes, such as respiratory bronchiolitis, parenchymal fibrosis, accumulation of macrophages, and smoking-related interstitial fibrosis, than the low-LC group. However, there were no differences in the smoking indices and pulmonary functions of the groups.

Abbreviations: BALF, Bronchoalveolar lavage fluid; BMI, Body mass index; COPD, Chronic obstructive pulmonary disease; CT, Computed tomography; DC, Dendritic cell; FEV1, Forced expiratory volume in one second; FVC, Forced vital capacity; H&E, Hematoxylin and eosin; IQR, Interquartile range; LC, Langerhans cell; PFT, Pulmonary function test; RB, Respiratory bronchiolitis; SRIF, Smoking-related interstitial fibrosis; UIP, Usual interstitial pneumonia; VC, Vital capacity.

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Conclusions: Selective accumulation of Langerhans cells in the respiratory bronchioles of smokers may lead to the development of smoking-related pathological changes.

1. Introduction

Smoking, a major cause of chronic obstructive pulmonary disease (COPD) and lung cancer [1–3], induces histological changes in lung parenchyma [4,5]. It causes interstitial lung disease [6–9] and is associated with specific histological changes including respiratory bronchiolitis (RB), mucostasis, peribronchiolar metaplasia, peribronchiolar fibrosis, emphysema, subpleural fibrosis, cysts, accumulation of macrophages, and smoking-related interstitial fibrosis (SRIF) [10–17].

Langerhans cells (LCs) are a well-characterized subset of dendritic cells (DCs) found in the skin [18], mainly the epithelium; they play an important role in activating cellular/cytotoxic immune responses. Demedts et al. reported selective Langerhans cell accumulation in the small airways of COPD patients, which initiated smoking-related airway inflammation and contributed to destructive COPD processes, even after smoking cessation [19,20]. The number of Langerhans cells were increased in the bronchoalveolar lavage fluid (BALF) of smokers [21–23]. In pulmonary Langerhans cell histiocytosis, Langerhans cells proliferating in the epithelia of airways cause cystic and fibrotic lesions [24]. However, the role of Langerhans cells in smoking-related lung pathology remains unclear.

Bronchioles bridge bronchi and alveoli. Respiratory bronchioles contain alveoli in their walls and combine air ducts with alveoli [10,25]. Reportedly, air remains longer in the respiratory bronchioles and alveolar ducts, as their diameters are larger than those of the upper airways [25]. Anthracosis initiates mainly in the respiratory bronchioles [26]. Therefore, the association between recruitment of Langerhans cells and development of RB, which leads to the pathogenesis of smoking-related lung diseases including COPD, requires verification.

This study hypothesized that Langerhans cells might infiltrate the respiratory bronchioles of smokers and cause histological changes leading to peripheral parenchymal lesions. Thus, we investigated the distribution of Langerhans cells, quantified Langerhans cells in 53 surgical specimens from smokers and non-smokers, and analyzed the relationship between the number of Langerhans cells and RB, in relation to clinical findings.

2. Patients and methods

2.1. Study population

We examined histological changes in the lungs of 61 consecutive patients (42 smokers and 19 non-smokers) who underwent lung resection for lung diseases (mainly lung cancer) between June and December 2014 at Kumamoto University Hospital, Japan. Among these, 53 patients were eligible, whereas seven patients exhibiting complications that influenced histological evaluation and one patient yielding inadequate specimens were excluded from the analysis. The study was approved by the Institutional Review Board of Kumamoto University Hospital (IRB No. 1831; July 7, 2014). As this was a retrospective analysis, informed consent was not obtained individually, but an opt-out method based on clinical practice guidelines in Japan was used.

2.2. Clinical data

Clinical characteristics, preoperative pulmonary function tests (PFTs), and chest computed tomography (CT) findings were retrospectively obtained from each patient's medical records. Non-smokers were defined as those who have never smoked. Those who had smoked within six months of surgery were defined as current smokers [11]. Both former and current smokers were included as smokers. Spirometry was performed according to the American Thoracic Society/European Respiratory Society consensus guidelines [27]. The Japanese Respiratory Society reference values for pulmonary function were used to evaluate the percentage of predicted (% predicted) values [28]. The severity of emphysema was visually assessed via chest CT by two independent chest physicians (HI and HK) using the modified Goddard scoring system [29]. Six images were analyzed via three slices (upper, middle, and lower lung fields) in both lungs and the average score of these six images was considered as representative of the severity of emphysema in each patient. Each image was classified as normal (score 0), $\leq$5% affected (score 0.5), $\leq$25% affected (score 1), $\leq$50% affected (score 2), $\leq$75% affected (score 3), or > 75% affected (score 4), with a minimum score of 0 and maximum of 4. Based on the average scores, all patients were classified into four groups; (1) no emphysema, (2) mild emphysema (average score < 1), (3) moderate emphysema (1 $\leq$ average score < 2.5), and (4) severe emphysema (average score $\geq$ 2.5).

2.3. Histological evaluation

All lungs were intra-bronchially infused with 10% buffered formalin for 12–24 h. Following resection, paraffin-embedded sections were prepared. Histological specimens including membranous bronchioles, respiratory bronchioles, and lung parenchyma were obtained from peripheral lung tissue away from the primary lesion. Sections of 4 μm thickness were cut, deparaffinized in xylene, and rehydrated via an ethanol series gradient. For histological evaluation, the lung specimens were stained with hematoxylin and eosin (H&E). Immunohistochemical analysis of Langerhans cells was performed using an automated immunostainer (Ventana EX, Roche, Basel, Switzerland). Antibodies against CD1a (1:25, DAKO, Santa Clara, CA, USA) were used as a Langerhans cell marker [22,30].

Smoking-related histological changes, such as RB, mucostasis in the respiratory bronchioles, peribronchiolar metaplasia, peribronchiolar fibrosis, emphysema, parenchymal fibrosis, subpleural collapse of the lung parenchyma, subpleural fibrosis, subpleural cystic lesions, accumulation of macrophages, and SRIF, were analyzed and evaluated by two pathologists (AM and TT). We detected the fibrosis around enlarged airspaces of emphysema as SRIF, according to the study by Katzenstein et al. [14], and usual interstitial pneumonia (UIP) pattern fibrosis was not included.

An eyepiece micrometer (OLYMPUS, Tokyo, Japan) was used to count Langerhans cell numbers (CD1a-positive DCs) per mm^2 in the respiratory bronchioles. We also counted the number of Langerhans cells in the membranous bronchioles.

2.4. *Statistical analysis*

Data are expressed as the median (interquartile range [IQR], 25–75%) or the number of patients (%). Differences between the two groups were calculated using the Mann-Whitney *U* test. Categorical variables were assessed using Fisher's exact test. Correlation between the number of Langerhans cells in the respiratory bronchioles and other variables, including smoking indexes and PFTs, was evaluated using Spearman's rank correlation coefficient. Statistical analyses were performed using the Statistical Package for Social Sciences Statistics (SPSS; IBM, Armonk, NY, USA). Statistical significance was set at $p < 0.05$.

3. Results

3.1. *Baseline characteristics*

Of the 53 patients, 18 were non-smokers and 35 were former or current smokers. Baseline characteristics of all patients are summarized in Table 1. The median age in both groups was 67. There were significant differences between non-smokers and smokers in sex proportion ($p < 0.001$). Among smokers, the median smoking index was 46 (IQR, 23–72) pack-years, ranging from 4 to 198. The most predominant underlying disease justifying lung resection was lung cancer. The median forced expiratory volume in 1 s/forced vital capacity ratio (FEV_1/FVC) and % FEV_1 were significantly lower in the smoker group than in the non-smoker group ($p = 0.048$ and $p = 0.004$, respectively). Chest CT revealed that emphysema was more frequent in smokers than in non-smokers ($p < 0.001$). Emphysema was observed in 27 out of 35 smokers. They were divided according to the severity of emphysema into different groups comprising 16 cases of mild emphysema, eight of moderate emphysema, and three of severe emphysema. Interstitial pneumonia was observed in seven (20%) smokers. No emphysema and interstitial pneumonia were found in non-smokers.

3.2. *Histological examinations*

Specific histological findings in the lung parenchyma of non-smokers and smokers are shown in Table 2. RB was found in 19 of 35 (54%) smokers, but none of the non-smokers had RB.

Table 1 – Baseline characteristics of patients.

	Non-smokers ($n = 18$)	Smokers ($n = 35$)	p value
Age (years)	67.0 (61.0–76.0)	67.0 (64.0–73.0)	0.799
Sex (male, %)	2 (11.1)	23 (85.7)	< 0.001
BMI	22.3 (19.2–24.0)	22.3 (19.9–25.1)	0.481
Smoking status			
Never/former/current	18/0/0	0/20/15	N.T.
Smoking index (Pack-year)	0 (0–0)	46 (23–72)	N.T.
Underlying diseases			0.369
Lung cancer	15	29	
Metastatic lung tumor	1	5	
Others	2	1	
Pulmonary function tests			
%VC	96.7 (83.2–107.3)	90.8 (83.7–101.7)	0.366
FEV1/FVC (%)	76.3 (72.0–85.2)	72.7 (65.3–78.3)	0.048
%FEV1	97.6 (83.9–110.6)	83.0 (77.3–90.2)	0.004
CT findings			
Emphysema	0	27 (77)	< 0.001
Interstitial pneumonias	0	7 (20)	0.081

Data are expressed as group median (interquartile range) or number of patients (%). The p values refer to comparisons between the non-smoker and smoker groups.
Abbreviations: BMI, Body mass index; VC, Vital capacity; FEV_1, Forced expiratory volume in 1 s; FVC, Forced vital capacity; CT, Computed tomography; N.T., not tested.

Mucostasis in the respiratory bronchioles, peribronchiolar metaplasia, peribronchiolar fibrosis, emphysema, parenchymal fibrosis, subpleural alveolar fibrosis, and accumulation of macrophages were observed significantly more frequently in smokers. SRIF was observed in 9 (26%) smokers

Table 2 – Histological findings in the lung parenchyma of non-smokers and smokers.

	Non-smokers ($n = 18$)	Smokers ($n = 35$)	p value
Respiratory bronchiolitis	0 (0)	19 (54)	< 0.001
Mucostasis in the respiratory bronchioles	6 (33)	25 (71)	0.017
Peribronchiolar metaplasia	2 (11)	17 (49)	0.008
Peribronchiolar fibrosis	5 (28)	27 (77)	< 0.001
Emphysema	4 (22)	28 (80)	< 0.001
Parenchymal fibrosis	2 (11)	19 (54)	0.003
Subpleural collapse of the lung parenchyma	11 (61)	25 (71)	0.539
Subpleural alveolar fibrosis	7 (39)	25 (71)	0.037
Subpleural cystic lesions	5 (28)	20 (57)	0.080
Accumulation of macrophages	1 (6)	20 (57)	< 0.001
SRIF	0 (0)	9 (26)	0.021
Pleural thickness and fibrosis	14 (78)	29 (83)	0.719
Anthracosis	17 (94)	31 (89)	0.651

Data are expressed as number of patients (%). The p values refer to comparisons between the non-smoker and smoker groups.
Abbreviations: SRIF, smoking-related interstitial fibrosis.

and this histological change, as with RB, was specific to smokers. Representative histological changes are shown in Supplemental Fig. S1.

3.3. *Quantification of Langerhans cells in the respiratory bronchioles*

Langerhans cells in the respiratory bronchioles were quantified and representative specimens indicating CD1a-positive Langerhans cells in the respiratory bronchioles are shown in Fig. 1. These Langerhans cells were mainly located in the respiratory bronchioles of smokers and to a lesser extent in the respiratory bronchioles of non-smokers. The median number of CD1a-positive Langerhans cells in the respiratory bronchioles was significantly increased ($p < 0.001$) in smokers (7.0 cells/mm^2) compared to that in non-smokers (2.8 cells/mm^2) (Fig. 2). By contrast, there was no significant difference between the number of Langerhans cells in the membranous bronchioles, which are present in the proximal portion of the respiratory bronchioles, of smokers and non-smokers ($p = 0.992$; Figs. 3 and 4).

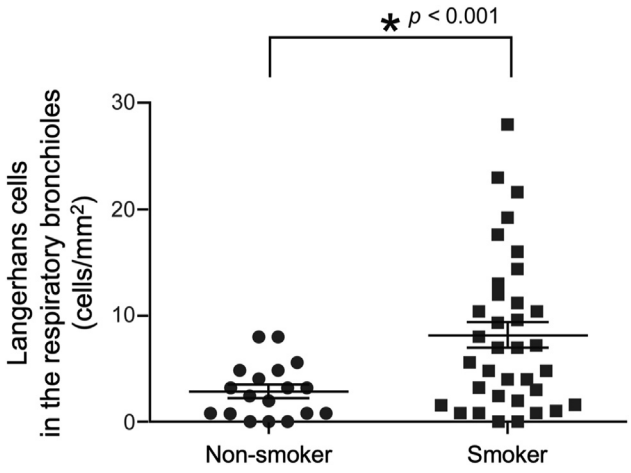

Fig. 2 – The number of CD1a-positive Langerhans cells in the respiratory bronchioles. The number of Langerhans cells in the respiratory bronchioles of smokers (7.0 cells/mm^2, 2.0–12.0 cells/mm^2) is significantly higher than that of non-smokers (2.8 cells/mm^2, 0.8–4.8 cells/mm^2, $p < 0.001$).

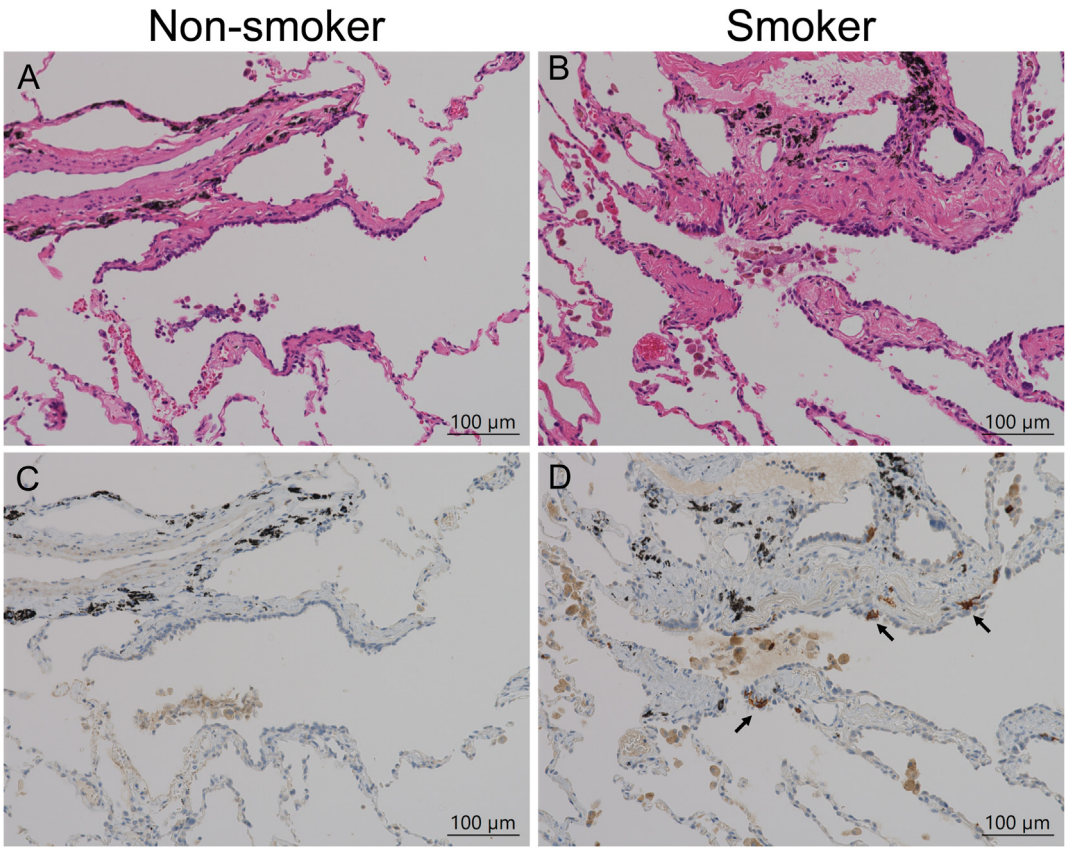

Fig. 1 – The distribution and quantification of CD1a-positive Langerhans cells in the respiratory bronchioles of a non-smoker and a smoker. Representative hematoxylin and eosin (H&E)-stained sections of the respiratory bronchioles in (A) a non-smoker and (B) a smoker (magnification, 200X). Respiratory bronchioles, connecting to alveoli, are present. Several macrophages are accumulated in the respiratory bronchioles. Mild fibrosis of the bronchiolar wall is observed in the smoker. Representative sections demonstrate immunostaining in Langerhans cells. Dark brown staining indicates membranes positive for CD1a. (C) CD1a-positive Langerhans cells are not observed around the respiratory bronchioles of the non-smoker. (D) CD1a-positive Langerhans cells are present in the respiratory bronchioles (arrow) of the smoker. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

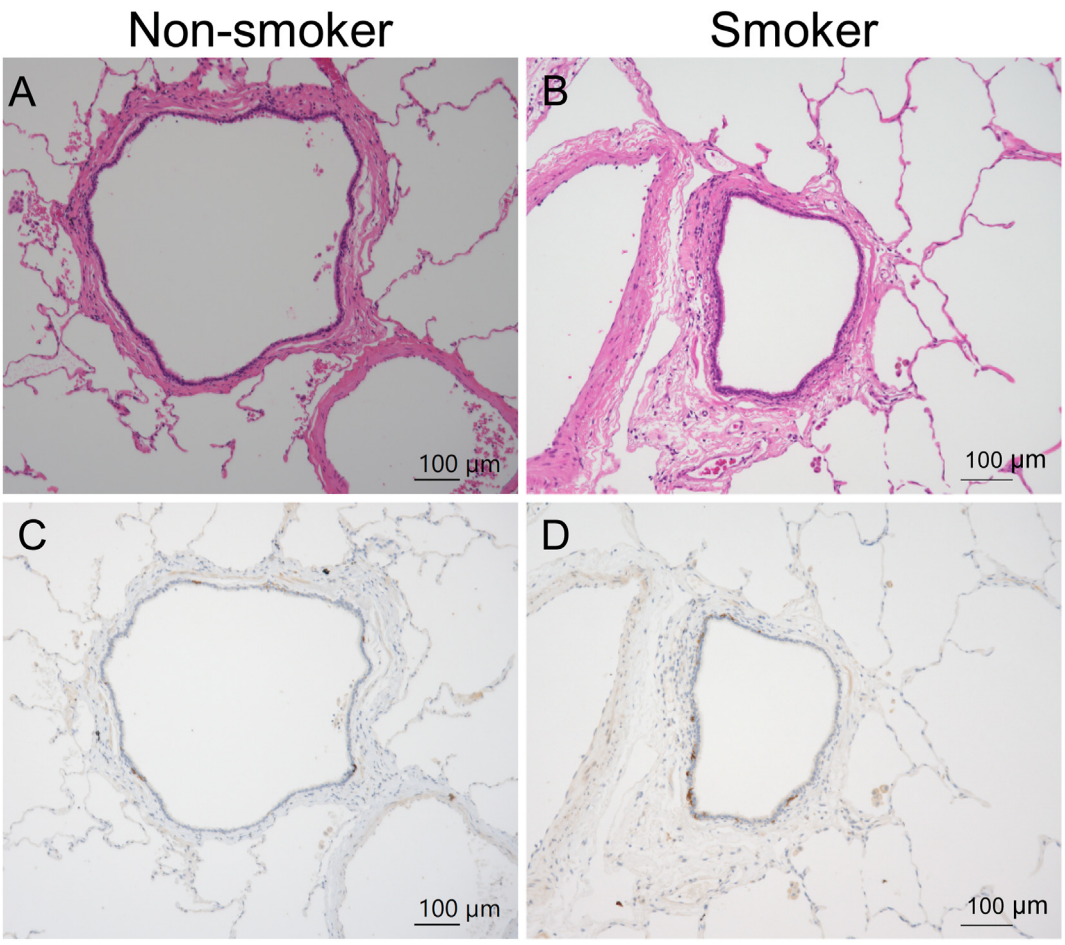

Fig. 3 – The distribution of CD1a-positive Langerhans cells in the membranous bronchioles of a non-smoker and a smoker. Representative H&E-stained sections of the membranous bronchioles in (A) a non-smoker and (B) a smoker (magnification, 100X). Membranous bronchioles with ciliated columnar epithelium are shown. CD1a-positive Langerhans cells infiltrate the epithelium of membranous bronchioles in (C) the non-smoker and (D) the smoker.

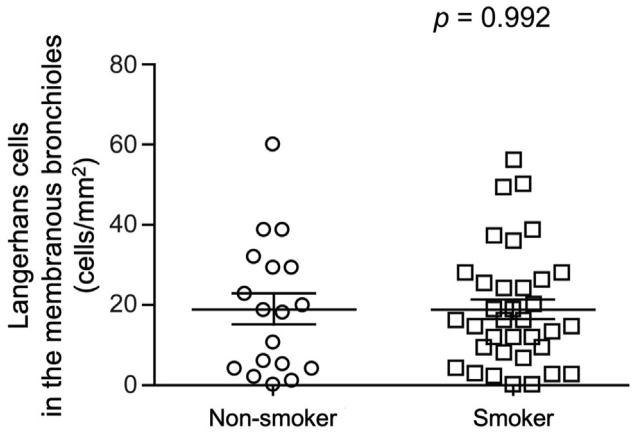

Fig. 4 – The number of CD1a-positive Langerhans cells in the membranous bronchioles. The number of CD1a-positive Langerhans cells is not significantly different between non-smokers (18.3 cells/mm^2, 4.0–29.3 cells/mm^2) and smokers (16.0 cells/mm^2, 8.0–26.0 cells/mm^2, $p = 0.992$).

The median number of Langerhans cells in the respiratory bronchioles was significantly increased ($p < 0.05$) in smokers with emphysema (9.3 cells/mm^2) compared to that in smokers without emphysema (3.2 cells/mm^2) (Supplemental Fig. S2). There was no significant difference between the number of Langerhans cells in the respiratory bronchioles of smokers with COPD and those without COPD ($p = 0.927$, data not shown).

3.4. *The relationship between the number of Langerhans cells in the respiratory bronchioles and clinical findings*

The number of Langerhans cells in the respiratory bronchioles of smokers was analyzed based on the severity of emphysema according to the modified Goddard's classification of chest CTs. The number of Langerhans cells was significantly higher in patients with mild emphysema than in patients without emphysema ($p = 0.006$; Fig. 5), and decreased with increasing severity of pulmonary emphysema. However, there was no significant correlation between the number of Langerhans cells and the smoking indices, % vital capacity (VC), FEV1/FVC (% predicted), and %FEV1.

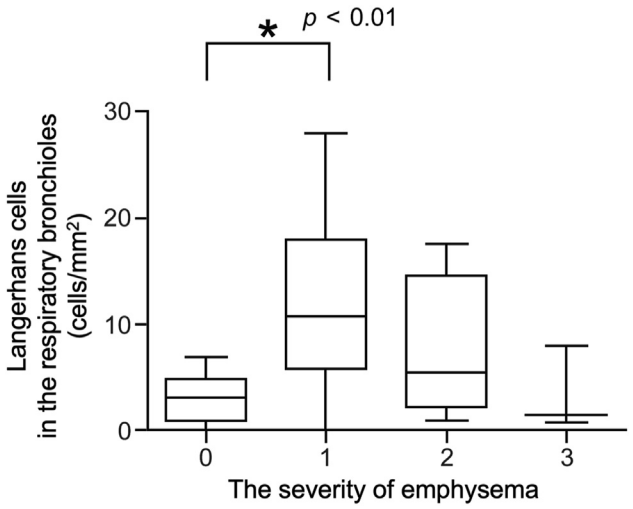

Fig. 5 – The relationship between the number of Langerhans cells in the respiratory bronchioles of smokers and the severity of emphysema in chest CT. The number of Langerhans cells in the respiratory bronchioles of patients with mild emphysema (10.8 cells/mm², 6.9–16.8 cells/mm²) is significantly higher than that in the respiratory bronchioles of those without emphysema (3.2 cells/mm², 1.2–4.8 cells/mm²) ($p < 0.01$).

Smokers were stratified as "low-LC group" and "high-LC group" according to the number of Langerhans cells, using the median number of Langerhans cells (7.0 cells/mm²) in the respiratory bronchioles. Emphysema was significantly more frequent ($p = 0.018$) in chest CTs of the high-LC group (94%) than in that of the low-LC group (59%) (Table 3). However, there were no differences between the smoking indices and pulmonary functions of the two groups. The incidence of histological findings was compared between the groups. The incidence of RB (78%), parenchymal fibrosis (72%), accumulation of macrophages (78%), and SRIF (50%) was significantly higher in the high-LC group than in the low-LC group ($p = 0.007$, $p = 0.044$, $p = 0.018$, and $p = 0.001$, respectively).

Table 3 – Comparison of the clinical factors and histological findings between low- and high-LC groups in the respiratory bronchioles of smokers.

	Low-LC group ($n = 17$)	High-LC group ($n = 18$)	p value
Smoking index (Pack-year)	45 (17.5–74.3)	48.8 (38.0–72.0)	0.443
Pulmonary function tests			
%VC	90.8 (83.2–109.9)	90.8 (83.6–97.6)	0.655
FEV1/FVC (%)	71.6 (61.3–78.4)	74.4 (67.6–79.8)	0.478
%FEV1	84.8 (72.0–103.5)	80.4 (77.3–87.4)	0.411
CT findings			
Emphysema	10 (59)	17 (94)	0.018
Interstitial pneumonias	1 (6)	6 (33)	0.088
Histological findings			
Respiratory bronchiolitis	5 (29)	14 (78)	0.007
Mucostasis in the respiratory bronchioles	11 (65)	14 (78)	0.471
Peribronchiolar metaplasia	6 (35)	11 (61)	0.181
Peribronchiolar fibrosis	12 (71)	15 (83)	0.443
Emphysema	12 (71)	16 (89)	0.229
Parenchymal fibrosis	6 (35)	13 (72)	0.044
Subpleural collapse of the lung parenchyma	11 (65)	14 (78)	0.471
Subpleural alveolar fibrosis	12 (71)	13 (72)	1.000
Subpleural cystic lesions	7 (41)	13 (72)	0.092
Accumulation of macrophages	6 (35)	14 (78)	0.018
SRIF	0 (0)	9 (50)	0.001
Pleural thickness and fibrosis	14 (82)	15 (83)	1.000
Anthracosis	16 (94)	15 (83)	0.603

Data are expressed as group median (interquartile range) or number of patients (%). The p values refer to comparisons between the low-LC group and high-LC group.
Abbreviations: LC, Langerhans cell; VC, Vital capacity; FEV_1, Forced expiratory volume in 1 s; FVC, Forced vital capacity; CT, Computed tomography; SRIF, smoking-related interstitial fibrosis.

4. Discussion

Although the inflammatory response induced by cigarette smoke in the respiratory system involves various cells, the exact role of these cells in the inflammation and remodeling of airways and lung parenchyma remains unclear. Moreover, the association between the accumulation of Langerhans cells due to smoking and the inflammation of airways and related emphysematous changes remains poorly understood.

To our knowledge, this is the first study that demonstrates the association between the number of Langerhans cells in the respiratory bronchioles of smokers and significant histological changes. The number of Langerhans cells in the respiratory bronchioles was significantly increased in smokers compared to that in non-smokers ($p < 0.001$). Additionally, the number of Langerhans cells was significantly increased in smokers with emphysematous changes on chest CT as compared to smokers without emphysema ($p < 0.05$). Chest CTs showed a higher number of Langerhans cells in the respiratory bronchioles of smokers with mild emphysema than in smokers with no, or more severe, emphysema. Furthermore, smoking-related histological changes, such as RB, parenchymal fibrosis, accumulation of macrophages, and SRIF, were more frequently observed in the high-LC group than in the low-LC group.

The results of studies that show the role of lung DCs, including Langerhans cells, in the pathogenesis of COPD due to cigarette smoking, are conflicting. In smokers, significantly increased numbers of Langerhans cells in BALF have been observed [21–23]. Stoll et al. reported that myeloid DCs of BALF, in current smokers with COPD, were characterized by the increased expression of CD1a-positive or langerin-positive DCs (believed to belong to Langerhans cells) [23]. Vassallo et al. showed that, in the total lung tissues of smokers with COPD, the number of Langerhans cells—as verified by the upregulation of langerin (*CD207*) mRNA—increased, while immunohistochemistry demonstrated an increase in CD1a-positive

Langerhans cells in the airway epithelium [31]. Though cigarette smoking reportedly increased the number of OKT6-positive Langerhans cells (compatible to CD1a-positive DC subsets) in alveolar parenchyma by 30-fold, it did not change the number of Langerhans cells in the bronchiolar epithelium [32]. Tsoumakidou et al. did not find significant differences between the number of CD1a-positive Langerhans cells in alveolar walls and small airways of non-smokers, ex-smokers, and COPD patients [33]. In our study, there was no difference between the number of Langerhans cells in the membranous bronchioles (anatomically located in the proximal area of respiratory bronchioles) of smokers and non-smokers. The number of Langerhans cells, distally located near the alveoli in respiratory bronchioles, was significantly higher in smokers than in non-smokers ($p < 0.001$). The diameters of respiratory bronchioles and alveolar ducts are larger than those of upper airways, allowing air to remain there for a longer time [25]. These anatomical structures may allow prolonged retainment of cigarette smoke in the respiratory bronchioles as compared to that in the membranous bronchioles, and induce the infiltration of Langerhans cells (a DC subset that acts as an antigen-presenting cell) into the respiratory bronchioles of smokers. However, results regarding the distribution and accumulation of Langerhans cells in the lungs and bronchioles obtained using different methodologies should be interpreted with caution. Smoking reportedly affected the maturation of airway myeloid DCs [34]. Thus, smoking status may lead to differences, not only in the distribution and accumulation, but also in the maturation of DCs/LCs in the lungs, thereby resulting in smoking-related histological changes.

In cigarette smoke-induced COPD animal models, inflammation of bronchi and bronchioles was associated with destructive changes adjacent to respiratory bronchioles [35]. In the current study, the number of Langerhans cells in the respiratory bronchioles of smokers was higher in those with emphysema on chest CT than in those without emphysema. Additionally, in the analysis of the severity of emphysema, the number of Langerhans cells in the respiratory bronchioles was higher in smokers with mild emphysema than in smokers with no emphysema. This indicates an important role of Langerhans cells in cigarette smoking-induced early inflammatory responses occurring in the respiratory bronchioles. In smokers with greater degrees of emphysema than milder forms, the number of Langerhans cells tended to be lower. This substantiates a previous report of significantly decreased number of inflammatory cells in the sub-epithelium of severe COPD patients, compared to that in mildly/moderately diseased patients, although infiltration by Langerhans cells was not investigated [36]. An increased number of Langerhans cells was associated with COPD severity, whereas the number of Langerhans cell precursors in airway walls decreased with increasing disease severity [19,20]. The number of Langerhans cells were reported to be lower in COPD patients than in non-COPD patients, suggesting that cigarette smoking affected the maturation of DCs in the lungs [37]. The authors surmised that the altered differentiation of DCs in smokers and/or COPD led to the selective accumulation of Langerhans cells accompanied by a corresponding decrease in Langerhans cell precursors [19,37]. In the current study, the number of Langerhans cells in the respiratory bronchioles of smokers was not different between those with and without COPD. These results in smokers with/without emphysema and with/without COPD may be influenced by factors other than emphysema, such as airflow obstruction.

Although an association between the role of Langerhans cells and smoking is indicated, cellular mechanisms by which Langerhans cells impact emphysema development remain unknown.

Smoking is associated with subclinical lung parenchymal changes [14,38]. We identified various histological changes in smokers. Langerhans cells may be involved in initiating smoking-related lung inflammation; however, no association has been reported between the accumulation of Langerhans cells in the respiratory bronchioles and findings of pathological lung abnormalities. High frequencies of RB, parenchymal fibrosis, accumulation of macrophages, and SRIF were significantly associated with the higher LC group ($p < 0.05$). However, the impact exerted by the infiltration of Langerhans cells in the respiratory bronchioles on the parenchyma remains unclear. The precise association between Langerhans cells and cigarette smoking in terms of an immune response or lung pathology is not clear. Thus, further studies on the contribution of Langerhans cells to smoking-related lung abnormalities are warranted.

The limitations of the current study were: (i) The effect of primary lung diseases, such as cancer, on observed changes was not completely excluded, although normal lung tissue specimens located away from the lesions of primary diseases were sampled; (ii) All patients underwent lung resection for lung diseases, and patients with severely impaired lung function were excluded when surgery was indicated. (iii) The degree of emphysema was evaluated on chest CT, using the modified Goddard scoring system, by taking the average score in all lung fields. However, the number of Langerhans cells was evaluated in the local area of the resected lung and not in all lung fields. Therefore, the results should be interpreted carefully since they may not reflect all lung fields.

5. Conclusions

This study demonstrated for the first time that the number of Langerhans cells increases in the respiratory bronchioles and not in the membranous bronchioles of smokers. Such a selective accumulation of Langerhans cells may be implicated in the pathogenesis of lung parenchymal abnormalities as well as emphysematous changes. Further studies are needed to elucidate the mechanism underlying the accumulation of Langerhans cells in the airways and its effect on the development of smoking-related lung diseases.

Author contributions

AM, TT, and HI were involved in the conception and design of the study. EM, YH, SS, SH, TM, and MS collected the data. AM, TT, HI, HK, and TS interpreted the results and helped to write the manuscript. All the authors reviewed, revised, and approved the manuscript for submission.

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Ethical statement

The authors are accountable for all aspects of the study in ensuring that questions related to the accuracy or integrity of any part of the study are appropriately investigated and resolved. This study was approved by the Institutional Review Board of Kumamoto University Hospital (IRB No. 1831; July 7, 2014). Obtaining informed consent was not necessary because this was a retrospective analysis with data being collected in an anonymized fashion.

Conflict of Interest

The authors have no conflicts of interest to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.resinv.2021.03.001.

REFERENCES

[1] American Thoracic Society. Cigarette smoking and health. Am J Respir Crit Care Med 1996;153:861–5. https://doi.org/10.1164/ajrccm.153.2.8564146.
[2] Doll R, Peto R, Boreham J, Sutherland I. Mortality in relation to smoking: 50 years' observations on male British doctors. Br J Canc 2004;328:1519–27. https://doi.org/10.1136/bmj.38142.554479.
[3] Svanes C, Sunyer J, Plana E, Dharmage S, Heinrich J, Jarvis D, et al. Early life origins of chronic obstructive pulmonary disease. Thorax 2010;65:14–20. https://doi.org/10.1136/thx.2008.112136.
[4] Flaherty KR, Fell C, Aubry MC, Brown K, Colby T, Costabel U, et al. Smoking-related idiopathic interstitial pneumonia. Eur Respir J 2014;44:594–602. https://doi.org/10.1183/09031936.00166813.
[5] Franks TJ, Galvin JR. Smoking-related "interstitial" lung disease. Arch Pathol Lab Med 2015;139:974–7. https://doi.org/10.5858/arpa.2013-0384-RA.
[6] Myers JL, Veal CF, Shin MS, Katzenstein AL. Respiratory bronchiolitis causing interstitial lung disease. A clinicopathologic study of six cases. Am Rev Respir Dis 1987;135:880–4. https://doi.org/10.1164/arrd.1987.135.4.880.
[7] Travis WD, Costabel U, Hansell DM, King Jr TE, Lynch DA, Nicholson AG, et al. ATS/ERS committee on idiopathic interstitial pneumonias. An official American thoracic society/European respiratory society statement: update of the international multidisciplinary classification of the idiopathic interstitial pneumonias. Am J Respir Crit Care Med 2013;188:733–48. https://doi.org/10.1164/rccm.201308-1483ST.
[8] Konopka KE, Myers JL. A review of smoking-related interstitial fibrosis, respiratory bronchiolitis, and desquamative interstitial pneumonia: overlapping histology and confusing terminology. Arch Pathol Lab Med 2018;142:1177–81. https://doi.org/10.5858/arpa.2018-0240-RA.
[9] Tazi A. Adult pulmonary Langerhans' cell histiocytosis. Eur Respir J 2006;27:1272–85. https://doi.org/10.1183/09031936.06.00024004.
[10] Colby TV. Bronchiolitis. Pathologic considerations. Am J Clin Pathol 1998;109:101–9. https://doi.org/10.1093/ajcp/109.1.101.
[11] Fraig M, Shreesha U, Savici D, Katzenstein ALA. Respiratory bronchiolitis: a clinicopathologic study in current smokers, ex-smokers, and never-smokers. Am J Surg Pathol 2002;26:647–53. https://doi.org/10.1097/00000478-200205000-00011.
[12] Yousem SA. Respiratory bronchiolitis-associated interstitial lung disease with fibrosis is a lesion distinct from fibrotic nonspecific interstitial pneumonia: a proposal. Mod Pathol 2006;19:1474–9. https://doi.org/10.1038/modpathol.3800671.
[13] Kawabata Y, Hoshi E, Murai K, Ikeya T, Takahashi N, Saitou Y, et al. Smoking-related changes in the background lung of specimens resected for lung cancer: a semiquantitative study with correlation to postoperative course. Histopathology 2008;53:707–14. https://doi.org/10.1111/j.1365-2559.2008.03183.x.
[14] Katzenstein ALA, Mukhopadhyay S, Zanardi C, Dexter E. Clinically occult interstitial fibrosis in smokers: classification and significance of a surprisingly common finding in lobectomy specimens. Hum Pathol 2010;41:316–25. https://doi.org/10.1016/j.humpath.2009.09.003.
[15] Allen TC. Pathology of small airways disease. Arch Pathol Lab Med 2010;134:702–18. https://doi.org/10.1043/1543-2165-134.5.702.
[16] Niewoehner DE, Kleinerman J, Rice DB. Pathologic changes in the peripheral airways of young cigarette smokers. N Engl J Med 1974;291:755–8. https://doi.org/10.1056/NEJM197410102911503.
[17] Remy-Jardin M, Remy J, Gosselin B, Becette V, Edme JL. Lung parenchymal changes secondary to cigarette smoking: pathologic-CT correlations. Radiology 1993;186:643–51. https://doi.org/10.1148/radiology.186.3.8430168.
[18] Deckers J, Hammad H, Hoste E. Langerhans cells: sensing the environment in health and disease. Front Immunol 2018;9:1–14. https://doi.org/10.3389/fimmu.2018.00093.
[19] Demedts IK, Bracke KR, Van Pottelberge G, Testelmans D, Verleden GM, Vermassen FE, et al. Accumulation of dendritic cells and increased CCL20 levels in the airways of patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2007;175:998–1005. https://doi.org/10.1164/rccm.200608-1113OC.
[20] Van Pottelberge GR, Bracke KR, Demedts IK, De Rijck K, Reinartz SM, Van Drunen CM, et al. Selective accumulation of langerhans-type dendritic cells in small airways of patients with COPD. Respir Res 2010;11:1–21. https://doi.org/10.1186/1465-9921-11-35.
[21] Casolaro MA, Bernaudin JF, Saltini C, Ferrans VJ, Crystal RG. Accumulation of Langerhans' cells on the epithelial surface of the lower respiratory tract in normal subjects in association with cigarette smoking. Am Rev Respir Dis 1988;137:406–11. https://doi.org/10.1164/ajrccm/137.2.406.

[22] Bratke K, Klug M, Bier A, Julius P, Kuepper M, Virchow JC, et al. Function-associated surface molecules on airway dendritic cells in cigarette smokers. Am J Respir Cell Mol Biol 2008;38:655–60. https://doi.org/10.1165/rcmb.2007-0400OC.
[23] Stoll P, Heinz AS, Bratke K, Bier A, Garbe K, Kuepper M, et al. Impact of smoking on dendritic cell phenotypes in the airway lumen of patients with COPD. Respir Res 2014;15:1–10. https://doi.org/10.1186/1465-9921-15-48.
[24] Tazi A, Soler P, Hance AJ. Adult pulmonary Langerhans' cell histiocytosis. Thorax 2000;55:405–16. https://doi.org/10.1136/thorax.55.5.405.
[25] Weibel ER. It takes more than cells to make a good lung. Am J Respir Crit Care Med 2013;187:342–6. https://doi.org/10.1164/rccm.201212-2260OE.
[26] Mirsadraee M. Anthracosis of the lungs: etiology, clinical manifestations and diagnosis: a review. Tanaffos 2014;13:1–13. PMC4386010.
[27] Wanger J, Clausen JL, Coates A, Pedersen OF, Brusasco V, Burgos F, et al. Standardisation of the measurement of lung volumes. Eur Respir J 2005;26:511–22. https://doi.org/10.1183/09031936.05.00035005.
[28] Kubota M, Kobayashi H, Quanjer PH, Omori H, Tatsumi K, Kanazawa M, et al. Reference values for spirometry, including vital capacity, in Japanese adults calculated with the LMS method and compared with previous values. Respir Investig 2014;52:242–50. https://doi.org/10.1016/j.resinv.2014.03.003.
[29] Makita H, Nasuhara Y, Nagai K, Ito Y, Hasegawa M, Betsuyaku T, et al. Hokkaido COPD Cohort Study Group. Characterisation of phenotypes based on severity of emphysema in chronic obstructive pulmonary disease. Thorax 2007;62:932–7. https://doi.org/10.1136/thx.2006.072777.
[30] Sholl LM, Hornick JL, Pinkus JL, Pinkus GS, Padera RF. Immunohistochemical analysis of langerin in langerhans cell histiocytosis and pulmonary inflammatory and infectious diseases. Am J Surg Pathol 2007;31:947–52. 10.1097/01.pas.0000249443.82971.bb.
[31] Vassallo R, Walters PR, Lamont J, Kottom TJ, Yi ES, Limper AH. Cigarette smoke promotes dendritic cell accumulation in COPD; a Lung Tissue Research Consortium study. Respir Res 2010;11:1–13. https://doi.org/10.1186/1465-9921-11-45.
[32] Soler P, Moreau A, Basset F, Hance AJ. Cigarette smoking-induced changes in the number and differentiated state of pulmonary dendritic cells/Langerhans cells. Am Rev Respir Dis 1989;139:1112–7. https://doi.org/10.1164/ajrccm/139.5.1112.
[33] Tsoumakidou M, Koutsopoulos AV, Tzanakis N, Dambaki K, Tzortzaki E, Zakynthinos S, et al. Decreased small airway and alveolar CD83+ dendritic cells in COPD. Chest 2009;136:726–33. https://doi.org/10.1378/chest.08-2824.
[34] Liao SX, Ding T, Rao XM, Sun DS, Sun PP, Wang YJ, et al. Cigarette smoke affects dendritic cell maturation in the small airways of patients with chronic obstructive pulmonary disease. Mol Med Rep 2015;11:219–25. https://doi.org/10.3892/mmr.2014.2759.
[35] Hernandez JA, Anderson AE, Holmes WL, Foraker AG. Pulmonary parenchymal defects in dogs following prolonged cigarette smoke exposure. Am Rev Respir Dis 1966;93:78–83. https://doi.org/10.1164/arrd.1966.93.1.78.
[36] Di Stefano A, Capelli A, Lusuardi M, Caramori G, Balbo P, Ioli F, et al. Decreased T lymphocyte infiltration in bronchial biopsies of subjects with severe chronic obstructive pulmonary disease. Clin Exp Allergy 2001;31:893–902. https://doi.org/10.1046/j.1365-2222.2001.01098.x.
[37] Arellano-Orden E, Calero-Acuña C, Moreno-Mata N, Gómez-Izquierdo L, Sánchez-López V, López-Ramírez C, et al. Cigarette smoke decreases the maturation of lung myeloid dendritic cells. PLoS One 2016;11:e0152737. https://doi.org/10.1371/journal.pone.0152737.
[38] Lederer DJ, Enright PL, Kawut SM, Hoffman EA, Hunninghake G, van Beek EJ, et al. Cigarette smoking is associated with subclinical parenchymal lung disease: the Multi-Ethnic Study of Atherosclerosis (MESA)-lung study. Am J Respir Crit Care Med 2009;180:407–14. https://doi.org/10.1164/rccm.200812-1966OC.