Reduced Tolerogenic Program Death-Ligand 1 (PD-L1)-expressed Conventional Type 1 Dendritic cells (cDC1) Is Associated with A Rapid Decliner Phenotype of Chronic Obstructive Pulmonary Disease (COPD)

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**Keywords:** programmed death-ligand 1 (PD-L1), conventional type 1 dendritic cells (cDC1s), chronic obstructive pulmonary disease (COPD), rapid decliner phenotype
Abstract

Background

Chronic obstructive pulmonary disease (COPD) is characterized, at least in part, by autoimmunity through amplified T helper 1 and 17 (Th1 and Th17) immune response. Loss of immune tolerance controlled by programed death ligand 1 (PD-L1) may contribute to it.

Objectives

We studied the tolerogenic role of PD-L1+ dendritic cells (DCs) and its subtypes in relation to specific T cell immunity and clinical phenotypes of COPD.

Methods

We used flow cytometry to analyzed PD-L1 expression on DC and its subtypes in peripheral blood mononuclear cells (PBMCs) from normal and COPD participants. T cells proliferation and signature cytokines of T cell subtypes stimulated with elastin as autoantigens were measured by flow cytometry and enzyme-linked immunosorbent assays (ELISA), respectively.

Measurement and main results

A total of 83 participants were enrolled (normal, n = 29; COPD, n = 54). Reduced PD-L1+ conventional dendritic cell 1 (cDC1) in PBMCs of COPD patients was shown (13.7±13.7%, \( P = 0.03 \)). The decrease in PD-L1+ cDC1 was associated with a rapid decliner phenotype of COPD (\( P = 0.02 \)) and correlated with CD4+ T cells (\( r = -0.33, P = 0.02 \)). Functionally, PD-L1 blockade enhanced CD4+ T cells proliferation stimulated with CD3/elastin (31.2±22.3%, \( P = 0.04 \)) and interleukin (IL)-17A production with both CD3 (156.3±54.7, \( P = 0.03 \)) and CD3/elastin (148±64.9, \( P = 0.03 \)) from normal PBMCs. PD-L1 blockade failed to increase IL-17A production in cDC1-depleted PBMCs. By contrast, there was no significant change in interferon (IFN)-\( \gamma \), IL-4, and IL-10 after PD-L1 blockade.

Conclusion

Circulating PD-L1+ cDC1 was reduced in COPD, which the tolerogenic role was suppressed with susceptibility to self-antigens and linked to a rapid decliner phenotype through Th17-skewed chronic inflammation.

Introduction

Chronic obstructive pulmonary disease (COPD) is the third leading cause of mortality globally (1). Smoking is the most common contributor to COPD, which can lead to persistent airway inflammation even after smoking cessation (2-4). The underlying mechanism of the self-perpetuating airway to lung parenchyma inflammation is still unclear, but macrophages, neutrophils, B and T lymphocytes...
inflammatory response increase as COPD progresses (3, 5). Among T-cell immunity, there appear to be CD8<sup>+</sup> cytotoxic T (Tc) cells, CD4<sup>+</sup> T helper 1 (Th1) cells and CD4<sup>+</sup> T helper 17 (Th17) cells predominant in COPD and might sustain macrophagic and neutrophilic inflammation (6, 7). Activated CD8<sup>+</sup> Tc cells produce cytotoxic granule enzymes and Th1 cells activate macrophages to release matrix metalloelastases (MMPs), which will lead to apoptosis of alveolar cells and degradation of extracellular matrix of lung tissues, aggravating the process of COPD (8, 9). Th17 cells contribute to the pathogenesis of COPD mainly by producing IL-17A which facilitates unresolving neutrophilic inflammation in the small airways and induces lymphoid neogenesis formation by B-cell attracting chemokine C-X-C motif ligand (CXCL) 12 in the progression of COPD (10, 11). More complicated, lung tissue component destructed by CD8<sup>+</sup> Tc and Th1 cells might release auto-antigens, such as elastin peptides, and drive the development of lymphoid neogenesis similar to autoimmune responses (12). In support of this theory, cigarette smoke could induce Th1 and Th17 immune response to autoantigen elastin peptides and was associated with pathologies of bronchitis and emphysema from both human and mice COPD studies (13, 14).

Dysregulation of the inhibitory immune checkpoints have shown to cause autoimmune disease (15). Programmed death-1 (PD-1)/Programmed death ligand 1 (PD-L1) axis is critical inhibitory immune checkpoint but less studies in autoimmunity phenomenon in COPD as mentioned above. Furthermore, COPD is a heterogeneous disease, with specialized clinical phenotype related to treatment and prognosis (16). There is currently no research on the role of the PD-1/PD-L1 axis in different COPD phenotypes, particular in its relation to autoimmune feature triggered by Th17 immunity.

Dendritic cells (DCs), a major link between innate and adaptive immunity, are well equipped to drive the pathogenesis of COPD through the earliest to end-stage stages (17). There are three main subtypes of human DCs, including type 1 and type 2 classical/conventional DCs (cDC1 and cDC2, respectively) and plasmacytoid DCs (pDCs) with both shared and distinct functions (18). Whilst cDC1 is able to trigger a Th1 immune response and cross-present antigens related to apoptotic cells, cDC2 primarily produces pro-inflammatory chemokines and recruits inflammatory cells (17). In mouse lung, CD103<sup>+</sup> DCs, the equivalent of human cDC1, predominantly elicit Th1 and Th17 responses, whereas CD11b<sup>high</sup> lung dendritic cells (LDCs) primarily provoke a Th2 response (19). DCs also have a tolerogenic function. In addition to human cDC2, mouse intestinal mucosal CD103<sup>+</sup> DCs play a crucial role in tolerance (20). Relative inefficiency of those DCs to mediate Th17 cell differentiation could be reversed by the retinoic acid receptor antagonist LE135 (21). It is not clear whether PD-L1 plays a role in tolerogenic DCs, particularly in different subtypes, neither is their clinical relevance.

We hypothesized that impairment in PD-L1-mediated immune tolerance has impact in pathogenesis of COPD. During an initial screen test using PBMCs from normal subjects and patients with COPD, we found PD-L1 expression in COPD was decreased in DCs, particularly in cDC1. We then sophisticatedly studied the clinical relevance of this reduction and linked to the CD4<sup>+</sup> T cell differentiation. In vitro experiments were also conducted to test the functional role of PD-L1.
Methods

A total of 83 participants, aged 40-80 years, were enrolled (COPD, n = 54; Healthy, n = 29) in this study. Some COPD patients had undergone high resolution computed tomography (HRCT) at Shuang Ho Hospital, Taipei Medical University (New Taipei, Taiwan) between March 2015 and February 2021. COPD severity was based on the Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines. Patients were in a stable condition without acute exacerbations for the previous 3 months, defined as no requirement for admission, antibiotics or systemic oral corticosteroid therapy and no change in respiratory symptoms. Normal subjects were defined as those without any known medical diseases, and they had not experienced any acute infections, including upper airway infection, in the previous 3 months. Full informed consent was obtained from all participants before sample collection. The study protocol was approved by the Taipei Medical University-Joint Institutional Review Board (N201902021). All experiments were performed in accordance with the relevant guidelines and regulations.

Classification of COPD phenotype

Rapid decliner

We define a loss of forced expiratory volume in one second (FEV1) ≥ 60 ml/year as rapid decline of lung function in COPD (22), therefore, lung function required to be tracked for at least one year. Based on the definition, a total of 9 rapid decliner and 35 non-rapid decliner in the study. The lung function was followed for an average of 3.5±1.4 years in total, 3.9±1.3 years for those with rapid decliner, and 3.4±1.4 years for those with non-rapid decline, and there was no significant difference of the follow-up years between the two.

Frequent exacerbator

According to GOLD report (23), COPD patients were divided into the frequent exacerbator (≥2 acute exacerbations/year or ≥1 severe acute exacerbation/year) and the infrequent exacerbator. In the study, we took patient’s medical history in the year before we obtained PBMCs, there were 12 frequent exacerbator and 40 non-frequent exacerbators.

Eosinophilic COPD

Eosinophilic COPD is defined as a blood eosinophil count >300 cells/μl based on treatment considerations from GOLD report recommendations (23). There were 7 eosinophilic and 47 non-eosinophilic COPD patients in this study.

COPD with emphysema

Emphysema was represented by the percentage of low attenuation areas (LAA) calculated based on quantitative computed tomography (CT) via a density mask method with a threshold of ≤−950
Hounsfield units (HU). We define mild (LAA<=15%) and severe (>15%) emphysema groups based on previous study (24). There were 17 mild and 15 severe emphysematous COPD patients in our study.

**Peripheral blood mononuclear cells (PBMCs) isolation**

After obtaining informed consent, 30 ml whole blood from 29 normal subjects and 54 COPD patients with different phenotypes was collected. Plasma then be collected from whole blood with an anticoagulant by centrifuging 2000´g, 10 min, 4°C before PBMC separation and stored at −80°C until use. The rest blood cells will be used for PBMC isolation and the following in vitro experiments. PBMCs were isolated from whole peripheral blood by gradient centrifugation (Lymphoprep™, Catalog #07801, StemCell Technologies Inc, Vancouver, Canada).

**Flow cytometry**

PBMCs were stained according to the manufacturer's recommendations. To measure the expression of PD-L1, 5x10^5 cells were incubated for 30 minutes at 4°C with specific mouse anti-human monoclonal antibodies conjugated with fluorochromes. Anti-human HLA-DR-APC/Fire™ 750 and anti-lineage-FITC were used to define DCs. Anti-human 11c-Alexa Fluor® 700 in combination with anti-human 141-PE-Cy7, anti-human 1c-Alexa Fluor® 647 and anti-human 123- PerCP/Cyamine5.5 used to define cDC1, cDC2 and pDC, respectively. Anti-CD4-BV786 and anti-CD8- BV711 were used to identify CD4 and CD8 T cell subsets. The data were analyzed with FlowJo™ Software v10.0. To determine the effect of PD-L1+ cDC1 on lymphocyte proliferative status, PBMC were stimulated with anti-CD3 (10 µg/mL) or anti-CD3+ elastin peptide (30 ng/mL) with or without anti-PD-L1 (2 µg/mL) for 96 hours and then use anti-Ki-67 antibody as a proliferation marker under the indicated culture conditions.

**ELISA**

Culture supernatants were harvested from PBMC of healthy controls and COPD patients stimulated with anti-CD3 antibody (10 mg/mL) or anti-CD3 combined with elastin peptide (30 ng/mL), with or without anti-PD-L1 (2 mg/mL) for 48 h, and the levels of IFN-γ, IL-4, IL-17A, and IL-10 were measured by commercial ELISA kits (R&D Systems; Minneapolis, MN, USA). Briefly, 100 µL of sample or standards added per well containing diluent were incubated at 24°C for 2 h. This was followed by carefully washing with cold 1´PBS 3 times, addition of 100 µL substrate solution to each well, and incubation at 24°C for 20 min in dark room. Thereafter, 50 µL of stop solution was added per well, and optical density determined immediately in a microplate reader set to 450 nm.

**Statistical analysis**

All statistical analyses were performed using GraphPad Prism V5.0. Results are expressed as means ± standard deviation of assays performed at least three independent experiments. Comparison between 2 groups of normally distributed data was by two-tailed unpaired t-test, otherwise, Mann-Whitney U test was
used. For groups of 3 or more, one-way ANOVA test was used. Spearman's rank correlation coefficient aided analysis of correlations between variables. P-value < 0.05 was considered statistically significant.

Results

**PD-L1^+ cDC1 was decreased in PBMCs of COPD patients**

During an initial screen of PD-L1 expression on variable PBMCs, we found a trend of decreased PD-L1 expressing dendritic cells in patients with COPD compared with normal subjects. After expanding n numbers, we confirmed that the ratio of PD-L1^+ cDC1 was lower in COPD patients (n = 54) than that in normal subjects (n = 29) (13.7± 13.7% vs. 32.7± 33.1%, respectively; P = 0.03, Fig. 1A). In contrast, PD-L1 expression on both cDC2 and pDC was similar between COPD patients and normal subjects. The reduction in PD-L1^+ cDC1 was not attributable to a change in total numbers of cDC1-distributions of total DCs (Fig. 1B). In addition to the quantitative finding that the ratio of PD-L1 expressed on cDC1 are significantly reduced in COPD patients, visual display of representative images, which the quantitative morphology of PD-L1 fluorescent staining (shown with green color) in cDC1 is lower in COPD patients than normal subjects by digital microcopy (Fig. 1C). We therefore confirmed the decreased PD-L1 expression in cDC1 in COPD patients.

**The Decrease in PD-L1^+ cDC1 was associated with a phenotype of rapid lung function decline in COPD**

We next asked whether the decrease in PD-L1^+ cDC1 link to any clinical characteristics, particularly distinct phenotypes of COPD. We found that the proportion of PD-L1^+ cDC1 was significantly lower in COPD patients with rapid lung function decline compared with those without rapid decline in lung function (4.6±4.7% vs 15.5±18.6%, respectively; P=0.02, Fig. 2A). This association was specific to the subtype cDC1, which was consistent with the selective decrease in PD-L1 in this subtype of DCs (Fig. 1A). PD-L1 expression on DCs were compared among COPD with different GOLD stages and normal subjects. The results showed that a statistically significant reduction of PD-L1^+ cDC1 cells was only seen in patients with GOLD stage II COPD compared with normal subjects (10.6±10.8% vs 32.5±33.1%, respectively, P=0.01, Fig.2B). These observations were in line with the previous studies on trajectory of lung function showing the rapidest decline of lung function in the same stage of the disease (25). There was no significant difference in the expression of PD-L1 on total and three subtypes of DCs in the phenotypes examined, including frequent exacerbators, eosinophilic, and emphysema (Fig. 2C-E). PD-L1 expression on cDC1 is thus distinctively associated with rapid decline of lung function.

**PD-L1^+ cDC1 was negative correlated with CD4^+ T cells**

Based on our previous results, we further investigated the mechanism through which the reduction in the ratio of PD-L1^+ cDC1 lead to loss of lung function. As both CD4^+ and CD8^+ T cells are implicated in the pathogenesis of COPD, we asked whether PD-L1 expression on cDC1 was associated with the ratio of both T cells in PBMC. We observed a significantly negative correlation of the ratio of PD-L1^+cDC1 with
that of CD4$^+$ T cells in PBMC from COPD patients ($r=-0.37$, $P=0.049$, Fig. 3A). Consistent with our previous findings, there was no association of cDC2 and pDC with CD4$^+$ T cells. By contrast, and unexpectedly, we did not see linkages between either subtype of DCs and CD8$^+$ T cells (Fig. 3B). To support this finding, we used NCBI GEO database accession number GSE56766 and analyzed the relationship between PD-L1 and CD4 or CD8 gene expression. This data set included microarray data from whole blood transcriptomics in 49 patients with COPD. In agreement with our FACS findings, the gene expression of PD-L1 and CD4, but not CD8, was negatively correlated ($r = -0.43$, $P=0.002$ in Fig. 3C).

**PD-L1 was involved in suppression of CD4$^+$ T cells proliferation and Th17 cells differentiation**

To understand the functional role of PD-L1 in the activation and differentiation of CD4$^+$ T cells, we conducted a series of in vitro studies using PBMC from normal subjects and patients with COPD. Previous reports indicated an involvement of elastin-specific T cell response in COPD (14). We thus used elastin peptide as a stimulation. In the absence of elastin, the specific anti-PD-L1 blocking antibody had no effect on the proliferation of CD3-activated CD4$^+$ T cells, measured by the proliferation marker Ki-67. In the presence of elastin, CD3 stimulation induced the proliferation of CD4 T$^+$ cells to a level similar to CD3 stimulation only. However, PD-L1 blockade robustly enhanced the proliferation (14.9±15.4% vs. 31.2±22.3%, respectively; $P=0.04$, Fig 4A). In PBMC from patients with COPD, CD3 plus elastin co-stimulation induced stronger proliferation of CD4$^+$ T cells compared with those from normal subjects (35.2±21.5 vs 14.9±15.4%, respectively, $P=0.03$ in Fig.4). Interestingly, in the absence of the PD-L1 blocking antibody, this proliferation in COPD was similar to that in normal subjects with PD-L1 blockade. These data suggest that PD-L1 is functionally involved in the tolerance of CD4 T cell response to elastin. Reduced PD-L1 expression on cDC1 might impair this tolerance, leading to autoimmunity in COPD. Because CD4$^+$ T cells dysregulation in COPD involves polarization of distinct subtypes (26), we further tested their association with the expression of PD-L1. Again, we use NCBI GEO database accession number GSE56766 to analyze the relationship between gene expression level of PD-L1 and subtypes of CD4 T cells, including Th1, Th2, Th17 and Treg cells. The results showed that only the expression of RORC, a master transcription factor driving Th17 cells, was significantly negatively correlated to PD-L1 ($r = -0.33$, *$p=0.02$, Fig 5A).

In functional studies with in vitro experiments, it was shown that PD-L1 blockade augmented the production of IL-17A in both CD3-(156.3±54.7 vs. 108.2±45.0 pg/mL, $P=0.03$, Fig.5B) and CD3/elastin-stimulated(148±64.9 vs 106.5±43.2 pg/mL, respectively; $P=0.03$, Fig.5B) PBMCs from normal subjects. By contrast, there was no significant change in IFN-γ, IL-4, and IL-10 after PD-L1 blockade. To further confirm the contribution of cDC1 in the IL-17A production and the tolerogenic role of PD-L1$^+$ cDC1, cDC1 cells were removed from PBMCs by cell sorting. In the deletion of cDC1, neither CD3 or CD3/elastin was able to stimulate IL-17A production (Fig.5C). Although CD3 stimulation seemed to induce weak IL-17A production, it was not statistically significant. Importantly, PD-L1 blockade did not affect the production of IL-17A. Taken together, PD-L1 in PBMC is functionally related to suppression of CD4$^+$ T cells proliferation and Th17 differentiation.
Discussion

Our study demonstrated that PD-L1⁺ cDC1 was reduced in COPD patients and was associated with the phenotype of rapid decline in lung function. The underlying mechanisms might be through loss of PD-L1-mediated tolerance leading to activation of CD4⁺ lymphocytes with Th17 polarization. Our study suggests a tolerogenic role of PD-L1⁺ cDC1 and its clinical relevance in COPD.

PD-L1 is constitutionally expressed on antigen presenting cells—PD-L proteins on tolerogenic DCs limit self-reactive T cell activation to maintain peripheral tolerance and prevent autoimmunity (27). Our data revealed that PD-L1 expression was specifically reduced in circulating cDC1 of patients with COPD. This reduction was negatively correlated with CD4⁺ T cells, suggesting a tolerogenic role of PD-L1⁺ cDC1. Importantly, we found a link between PD-L1⁺ cDC1 reduction and lung function rapid decliners. The unique role of the PD-L1⁺ cDC1 among three subtypes of DCs in COPD was consistent across variable observations, including its ratio and the relationship with clinical phenotypes and CD4⁺ T cells, which makes those findings more convincing. Our results suggest that loss of PD-L1⁺ cDC1-mediated tolerance may lead to perpetuating inflammation in response to self-antigens, such as elastin.

One previous report demonstrated that PD-L1 were decreased in circulating pDC from COPD patients (28), which was not seen in our study. It could be due to different cohorts of patients, particularly with different ethnic groups. It is not clear whether PD-L1 in cDC1 was reduced in this report. In addition to DCs, decreased virus-induced expression of PD-L1 on COPD macrophages was reported with corresponding increase in IFN-γ release (29).

In contrast to the clear association with rapid decliner phenotype of COPD, the lower PD-L1⁺ cDC1 was only statistically reduced in patients with GOLD stage II disease. Nevertheless, this finding is consistent with the fastest decline in lung function is in this stage of COPD (23, 25). It is likely that loss of PD-L1⁺ cDC1-mediated tolerance becomes severe in this stage and induces aberrant adaptive immune response to self-antigens (30), leading to airway remodeling and lung damage. In the later stages, PD-L1 should be induced by persistent inflammation; however, it did not seem to reach the extent as the normal in our observation. It is not clear why PD-L1 is reduced in cDC1. One possibility is through an epigenetic mechanism. For example, oxidative stress in COPD could increase microRNA-34a (miR-34a) and downregulate the expression of PD-L1(31, 32). Alternatively, we could not exclude the possibility that PD-L1⁺ cDC1 constitutes a specific lineage of DCs.

Two lines of evidence support a functional role of PD-L1 in suppression of Th17 in PBMC—negative correlation between RORC and PD-L1 gene expression in COPD, and anti-PD-L1 enhancement of IL-17A production in normal PBMCs. Thus PD-L1 in PBMC play the role of Th17 tolerance in the steady state. In COPD, reduced PD-L1⁺ cDC1 might lose this tolerance and leads to Th17 inflammation. In a mouse model of cigarette smoke exposure, MMP-12-generated elastin fragments could serve as a autoantigen to induce autoimmune processes by IL-17A (14). In line with this, decreased PD-L1⁺ cDC1 in COPD might
lose the tolerance and mount Th17-skewed CD4\(^+\) T cells reaction in response to such self-antigens. Indeed, when cDC1 was removed from PBMC, PD-L1 blockade failed to enhance Th17 immunity, supporting this scenario. However, conventionally, it is cDC2 that induces Th17 polarization (33). Nevertheless, the function of cDC1 and cDC2 seems to be plastic and can drive specific T cell immunity according to different environmental stimuli. It was shown in a mouse model that intranasal immunization with an adenosine diphosphate (ADP)-ribosylating adjuvant modified cDC1 cells to effectively prime Th17 cells (34). More direct evidence shown that vitamin D receptor (VDR) KO mice increased expression of Th17 cells and was associated with a reduction in tolerogenic CD103\(^+\) dendritic cells (35). In addition, mouse CD103\(^+\) subset of tolerogenic gut DCs acted directly on T cells to reduce their capacity of IL-17 production, by producing thymic stromal lymphopoietin (TSLP) (36). As CD103\(^+\) DCs are the mouse equivalent of human cDC1, it is tempting to suggest that PD-L1\(^+\) cDC1 is tolerogenic to Th17.

Therefore, this research provides a new immunopathogenic mechanism for COPD, in which reduced PD-L1\(^+\) cDC1 may lose immune tolerance to self-antigens, induce Th17 immunity and lead to decline of lung function. However, there are some limitations in our study. Firstly, in the selection of control groups, we did not specifically match age and smoking status with COPD patients. Age and smoking will affect the expression of PD-L1 in conditions such as obstructive sleep apnea (37) and rheumatoid arthritis (38), but it is not clear whether it affects normal subjects. Second, the results of decreased PD-L1\(^+\) cDC1 linked to the COPD phenotype of rapid lung function decline and CD4 T cells may not solid enough due to small sample size. Nevertheless, this novel concept is worth further exploring.

In conclusion, we found reduced circulating PD-L1\(^+\) cDC1 in COPD, which was linked to the phenotype of rapid lung function decline. We proposed a theory in which PD-L1\(^+\) cDC1 is a tolerogenic DC controlling Th17 response to self-antigens in steady conditions. In COPD, reduction in PD-L1\(^+\) cDC1 loses this self-tolerance and enhances susceptibility to self-antigens, particularly from the apoptotic cells, leading to a Th17-skewed inflammation. PD-L1\(^+\) cDC1 might provide a novel treatment strategy for COPD.

**Abbreviation**

ADP: adenosine diphosphate  
DC: dendritic cell  
BMI: body mass index  
ELISA: enzyme-linked immunosorbent assay  
cDC1: conventional type 1 DC  
FOXP3: forkhead box protein P3
cDC2: conventional type 2 DC
GEO: gene expression omnibus
CD: cluster of differentiation
GSE: GEO series
COPD: chronic obstructive pulmonary disease
CT: computer tomography
GATA-3: GATA binding protein 3
CXCL: (C-X-C motif) ligand
GOLD: global initiative for obstructive lung disease
HLA: human leukocyte antigen
T-bet: T-box expressed in T cells
IL: interleukin
Th1: type 1 T helper
IFN-γ: interferon-gamma
Th2: type 2 T helper
KO: knockout
Th17: type 17 T helper
LAA: low attenuation area
Treg: regulatory T cells
LDCs: lung dendritic cells
Tc: cytotoxic T cell
LE135: retinoic acid antagonist
TSLP: thymic stromal lymphopoietin
MMPs: matrix metalloproteinases
RORC: retinoic-acid-orphan-receptor-C
PD-1: programmed cell death protein 1
PD-L1: programmed cell death ligand 1
pDC: plasmacytoid dendritic cell
VDR: vitamin D receptor

Declarations

Ethics approval and consent to participate: The study was approved by the Joint Institutional Review Board of Taipei Medical University (TMU-JIRB Approval No.20190202) and was conducted compliant with guidelines from the Declaration of Helsinki regarding studies involving human subjects.

Informed Consent Statement: Written informed consent was obtained from all participants before sample collection.

Consent for publication: Not applicable.

Availability of data and materials: The data used in the current study are all contained in the manuscript and may be obtained upon reasonable request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

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Authors’ contributions: K.Y.L. and C.F.L. initiated study conception and instructed study design, W.L.S., P.H.F. and T.T.C. collected and corrected data, S.C.H., Y.H.L. and Y.H. C. performed analysis of date. K.Y.C. and S.M. W. wrote the main manuscript text. All authors read and approved the final manuscript.

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Tables

Table 1. Characteristics of normal and COPD participants
Data are presented with mean ± standard deviation (SD); BMI: body mass index; COPD: chronic obstructive pulmonary disease; FEV1: forced expiratory volume in 1 s; % pred: % predicted; FVC: forced vital capacity; NA: not available. Differences between groups were determined using the Fisher’s exact test and differences between individual variables from two groups were analyzed by the Mann–Whitney U-test. *: p<0.05

**Table 2 Antibodies for flow cytometry analysis**
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**Figures**
Figure 1

PD-L1 expression and distribution on DCs and subsets in normal and COPD participants

Flow cytometric analysis showing PD-L1 expression on DCs and subsets in normal (n=29) and COPD (n=54) participants. DCs were defined as HLA-DR\(^+\)lin\(^-\), whereas plasmacytoid dendritic cells (pDCs) were defined as CD123\(^+\)HLA-DR\(^+\)lin\(^-\). Conventional type 1 DC (cDC1) and conventional type 2 DC (cDC2) were defined as CD141\(^+\)HLA-DR\(^+\)lin\(^-\) and CD1C\(^+\)HLA-DR\(^+\)lin\(^-\), respectively. PBMCs stained directly with indicated antibodies for analysis. (A) The percentage of PD-L1\(^+\) DCs and subsets in normal and COPD subjects
were shown. (B) The ratio of DCs and subsets to PBMC were indicated in normal and COPD participants. (C) Representative images of PD-L1+ cDC1 (green) from normal and COPD participants, shown with spatial resolution and quantitative morphology by digital microscopy. Data are shown as means ± SEM.

Figure 2

The association of PD-L1+ DCs and its subsets with COPD phenotypes
A loss of forced expiratory volume in one second (FEV1) $\geq$ 60 ml/year was defined as rapid decline of lung function in COPD. Ratios of PD-L1$^+$ expression on DCs and subpopulations in COPD patients with rapid decliner (n=9) or non-rapid decliner (n=35) are shown. (B) The percentage of PD-L1$^+$ expression in DCs and subsets of normal (n=29) and GOLD stage I-IV COPD participants (n=54), including 6 stage I, 28 stage II, 17 stage III, and 3 stage IV patients are shown. (C) Percentage of COPD patients with frequent or infrequent exacerbations showing PD-L1$^+$ expression on DCs and subsets. According to the number of acute exacerbations per year, COPD patients were divided into the frequent exacerbation group (n=12) ($\geq$ 2 acute exacerbations/year or $\geq$ 1 severe acute exacerbation/year) and the infrequent acute exacerbation group (n=40). (D) Percentage of PD-L1$^+$ expression on DCs and subsets in 7 eosinophilic or 47 non-eosinophilic COPD patients. Eosinophilic COPD is defined as a blood eosinophil count $>$300 cells/μl. (E) Emphysema was represented by the percentage of low attenuation areas (LAA) calculated based on quantitative computed tomography (CT) via a density mask method with a threshold of $\leq$ -950 Hounsfield units (HU). PD-L1$^+$ expressing cells were shown on DCs and subsets in mild (LAA$\leq$15%, n=17) and severe (>15%, n=15) emphysema groups. Data are shown as means $\pm$ SEM.

Figure 3

The association of PD-L1$^+$ DCs and subsets with CD4$^+$ or CD8$^+$ T lymphocytes of PBMC
(A) Flow cytometry analysis of the correlation between PD-L1$^+$ DCs and subsets and CD4$^+$ T in PBMCs of 29 COPD patients. (B) Furthermore, flow cytometric analysis demonstrated a correlation between PD-L1$^+$ DCs and subsets and CD8$^+$ T cells in COPD PBMCs. (C) The expression of CD4 and CD8 genes in whole blood of 49 COPD patients (GSE56766 dataset) was correlated with PD-L1 expression.

*p< 0.05

Figure 4
**PD-L1 is involved in inhibiting CD4 T cell proliferation**

Representative proliferation marker (Ki67) for CD4\(^+\) cells from normal subjects (n = 6-8) and COPD patients (n=4) with and without anti–PD-L1 blocking antibodies after in vitro stimulation with anti-CD3 and anti-CD3/elastin peptide (30ng/ml).

\*p< 0.05

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**Figure 5**

**PD-L1 is involved in inhibiting the differentiation of Th17 cells**
(A) The expression levels of T-bet (represented as Th1), GATA-3 (represented as Th2), RORC (represented as Th17) and FOXP3 (represented as Treg) genes in the whole blood of 49 COPD patients (GSE56766 dataset) were correlated with the expression of PD-L1. (B) Th1 cytokine (interferon gamma, IFN-gamma), Th2 cytokines (interleukin 4, IL-4), Th17 cytokine (interleukin 17A, IL-17A) and Treg cytokine (interleukin 10, IL-10) levels in CD4\(^+\) subset cells from 6 normal participants stimulated with CD3 or CD3/elastin peptide alone or with anti-PD-L1 blocking antibody were compared with vehicle controls. These cytokine levels produced by the CD4\(^+\) subset cells in normal participants were measured by ELISA. (C) Comparison of the Th17 cytokines (interleukin 17A, IL-17A) between normal subjects stimulated with CD3 or CD3/elastin peptide alone or with anti-PD-L1 blocking antibody (n=4) from cDC1-depleted PBMC. * p<0.05

**Supplementary Files**

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- SupplFigures.docx