Peripheral Tc17 and Tc17/Interferon-γ Cells are Increased and Associated with Lung Function in Patients with Chronic Obstructive Pulmonary Disease

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Abstract

Background: Chronic obstructive pulmonary disease (COPD) is characterized by progressive loss of lung function and local and systemic inflammation, in which $CD8^+$ T cells are believed to play a key role. Activated $CD8^+$ T cells differentiate into distinct subpopulations, including interferon-γ (IFN-γ)-producing Tc1 and interleukin (IL)-17-producing Tc17 cells. Recent evidence indicates that Tc17 cells exhibit considerable plasticity and may convert into IL-17/IFN-γ-double producing (Tc17/IFN-γ) cells when driven by inflammatory conditions. The aim of this study was to investigate the Tc17/IFN-γ subpopulation in peripheral blood of patients with COPD and to evaluate their potential roles in this disease. Methods: Peripheral blood samples were collected from 15 never-smokers, 23 smokers with normal lung function, and 25 patients with COPD (Global Initiative for Chronic Obstructive Lung Disease 2-4). Proportions of the IL-17/IFN-γ-double expressing subpopulation were assessed using flow cytometry. Plasma concentrations of cytokines favoring Tc17/IFN-γ differentiation were measured by enzyme-linked immunosorbent assay.

Results: Patients with COPD had higher proportions of Tc17 cells and Tc17/IFN-γ cells in the peripheral blood than smokers and never-smokers. The plasticity of Tc17 cells was higher than that of Th17 cells. The percentages of Tc17 cells and Tc17/IFN-γ cells showed negative correlations with forced expiratory volume in 1 s % predicted value ($r = -0.418$, $P = 0.03$; $r = -0.596$, $P = 0.002$, respectively). The plasma concentrations of IL-6, transforming growth factor-β1, and IL-12 were significantly higher in patients with COPD compared with smokers and never-smokers.

Conclusions: Peripheral Tc17 cells are increased and more likely to convert to Tc17/IFN-γ cells in COPD, suggesting that Tc17 cell plasticity may be involved in persistent inflammation of the disease.

Key words: $CD8^+$ T-cells; Interferon-γ; Interleukin-17; Plasticity

IntroductIon

Chronic obstructive pulmonary disease (COPD) is associated with enhanced and chronic inflammatory responses of the lungs to tobacco smoking and other noxious particles or gasses.[1] The inflammation in COPD, both in the lungs and in the systemic circulation, plays critical roles in disease development and progression.[2,3]

$CD8^+$ T-cells are major players in the inflammation and lung destruction of COPD.[4] It is believed that $CD8^+$ T-cells induce lung inflammation and emphysema in COPD by the production of granzyme B, perforin, and many other injurious and pro-inflammatory mediators. Studies in COPD patients have demonstrated that $CD8^+$ T-cells are accumulated in lung parenchyma and airways and correlate with the degree of airway obstruction.[5-8]

Activated $CD8^+$ T-cells differentiate into distinct subpopulations, including interferon-γ (IFN-γ)-producing

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Tc1, interleukin (IL)駿4 producing Tc2, and IL駿17駿producing Tc17 cells, defined by selected sets of cytokines and transcription factors production.[9-11] Studies have shown that most $CD8^+$ T駿cells isolated from the lung parenchyma of COPD patients are IFN駿γ駿producing Tc1 cells, exhibiting greater cytotoxicity compared with Tc2 cells.[12,13] Compared to Tc1 cells, Tc17 cells exhibit strikingly suppressed cytotoxic activity by secreting low levels of the cytotoxic T lymphocytes markers: T駿bet, IFN駿γ, perforin, and granzyme B.[14] Tc17 cells are shown to share some phenotypical properties with Th17, including retinoic acid receptor駿related orphan receptor γt, CCR6, and IL駿23R, and express tumor necrosis factor駿α (TNF駿α), IL駿21, and IL駿22.[15,16] Recent studies, however, have revealed that Tc17 cells are increased in several autoimmune diseases, such as rheumatoid arthritis (RA), multiple sclerosis, psoriasis, and are implicated in the pathogenesis of these diseases.[17-19]

Tc17 cells possess a high plasticity and can convert to IL駿17/ IFN駿γ駿double producing cells (Tc17/IFN駿γ cells) permitted by IL駿12 signaling, with distinct properties from Tc1 lineage.[20] Tc17/IFN駿γ cells are highly cytotoxic and exhibit strong antitumor activity *in vitro* and *in vivo*. Interestingly, this unique subpopulation of Tc17 was found implicated in various inflammatory conditions in both humans and mice.[20-26]

Previous studies reported that IL駿17A and IL駿17F expressions by $CD8^+$ T駿cells were increased in the airways of COPD patients.[27,28] However, little is known about the frequency of circulating Tc17 cells, particularly Tc17/IFN駿γ cells and their associations with disease progression in COPD. Given that COPD is a lung disease with significant extrapulmonary effects, exploring the differentiation of peripheral $CD8^+$ T駿cells, particularly the newly recognized Tc17 cells, may provide revealing evidence for the understanding of the mechanisms underlying systemic inflammation of the disease. Therefore, we assessed the signature cytokine IL駿17 and IFN駿γ expressions by $CD8^+$ T lymphocyte in peripheral blood from patients with COPD and analyzed the difference in the plasticity between Tc17 cells and Th17 cells. The cytokines believed to favor Tc17/IFN駿γ differentiation were measured in plasma from the study subjects. Our results revealed higher proportions of Tc17 cells and Tc17/IFN駿γ cells in peripheral blood from COPD patients, which could be explained by increased concentrations of IL駿6, transforming growth factor β1 (TGF駿β1) and IL駿12. Importantly, the percentages of Tc17 cells and Tc17/IFN駿γ cells were correlated negatively with forced expiratory volume in 1 s (FEV_1) % predicted. These results indicate that more studies are warranted to reveal the potential involvement of Tc17/IFN駿γ cells in the pathogenesis of COPD.

Methods

Study subjects

Twenty駿five male patients with COPD, all current or former smokers, were recruited for the study in the Beijing Tongren Hospital, Capital Medical University, China. Twenty駿three smokers and 15 never駿smokers with normal lung function were also included. The diagnosis of COPD was made according to clinical symptoms, a history of tobacco smoking, and impaired pulmonary function (postbronchodilator FEV_1/forced vital capacity <70%), according to the diagnostic criteria of the Global Initiative for Chronic Obstructive Lung Disease (2013).[1] All subjects with COPD were clinically stable and had not suffered any exacerbations for ≥3 months prior to enrollment. Smokers with normal lung function (FEV_1 >80% predicted) had a smoking history of ≥20 pack駿years. Individuals with asthma, restrictive lung diseases, lung surgery, other chronic systemic inflammatory diseases, such as RA, type 1 diabetes mellitus, and inflammatory bowel disease, were excluded. The demographic and baseline clinical characteristics of the study participants are summarized in Table 1.

The study was approved by the local research ethics committee (TRECKT 2008駿14). Written informed consent was obtained from all subjects.

Cell collection and flow cytometry

Peripheral blood samples were collected into ethylenediaminetetraacetic acid駿treated tubes by venipuncture from the subjects after an 8駿h fasting and were layered on the Ficoll駿Paque Plus solution (Amersham Biosciences, Amersham, Bucks, UK) in a centrifuge tube, centrifuged at 400 × *g* for 20 min at 21°C, and peripheral blood mononuclear cells (PBMCs) were harvested. Then, divalent cation駿free Hanks balanced salt solution was used for washing of cells at 300 × *g* for 5 min at 4°C. PBMCs were resuspended at 10^6 cells/ml in RPMI 駿1640 medium and prepared for the following procedures.

Freshly processed human PBMCs were stimulated with 50 ng/ml of phorbol 12駿myristate 13駿acetate and 500 ng/ml of ionomycin in the presence of 5 μg/ml brefeldin A for 5 h

Table 1: The demographic and clinical characteristics of all participants

Items	Healthy nonsmokers	Smokers	Patients with COPD
Number of subjects	15	23	25
Age (years)	67.3 ± 6.5	66.4 ± 8.2	67.9 ± 7.7
Male/female (*n*/*n*)	15/0	23/0	25/0
Current/ex-smokers (*n*/*n*)	0	16/7	10/15
Pack-years, median (IQR)	0	39 (28–50)	46 (30–72)
FEV_1% predicted, mean ± SD	95.8 ± 6.2	91.3 ± 8.7	51.7 ± 15.5
FEV_1/FVC%, mean ± SD	83.2 ± 3.4	80.8 ± 4.9	55.6 ± 11.0
ICS use (*n*)	0	0	19
Bronchodilator use (*n*)	0	0	20
Exacerbations/year, mean ± SD	0	0	1.1 ± 0.3

Values are presented median (IQR) for smoking history, mean, and standard deviation for all others. COPD: Chronic obstructive pulmonary disease; FEV_1: Forced expiratory volume in 1 s; FVC: Forced vital capacity; ICS: Inhaled corticosteroids; IQR: Interquartile range; SD: Standard deviation.

at 37°C as described by others.[29] The cells were harvested and stained with anti-hCD4-PE (BD Biosciences, San Jose, California, USA) and anti-hCD8-Percp (BD Biosciences) for 30 min at room temperature, followed by staining with anti-hIL-17A-FITC (eBioscience, San Diego, California, USA) and anti-hIFN-γ-APC (eBioscience) after fixation and permeabilization. $CD8^+$ subpopulations were determined using FACS-Calibur (BD Biosciences). A total of 1×10^5 events were collected for each subject and data were analyzed by FlowJo software (Tree Star, Ashland, OR, USA).

Cytokine enzyme-linked immunosorbent assay

The concentrations of IL-6, IL-12, and TGF-β1 in the plasma from the study subjects were measured by enzyme-linked immunosorbent assay (ELISA, eBioscience, San Diego, CA, USA) according to the manufacturer's recommendations with the sensitivity of 2 pg/ml, 2.1 pg/ml, and 8.6 pg/ml, respectively.

Statistical analysis

Group data were depicted as a mean and standard error of the mean or median and interquartile range when appropriate. Comparisons of three groups were performed using one-way analysis of variance (ANOVA) for group data distributed normally, and when the test detected statistical significance, *post hoc* analysis between two groups was performed by the use of the Tukey test. The correlation was analyzed using Pearson's rank correlation coefficients. A P value < 0.05 was considered statistically significant. All analyses were performed by Prism 5.02 (GraphPad, La Jolla, CA, USA) and SPSS for Windows standard version released 17.0 (SPSS Inc, Chicago, Illinois, USA).

results

The frequency of Tc1 cells and Tc17 cells is increased in chronic obstructive pulmonary disease patients

We first examined the frequencies of IFN-γ-producing $CD8^+$ T-cells in peripheral blood from the study subjects using flow cytometry. There was a higher proportion of Tc1 cells in circulating $CD8^+$ T-cells in COPD patients (median, 68.50%) compared with smokers (median, 56.60%, $P < 0.05$) and never-smokers (median, 47.20%, $P < 0.001$), and there was a trend for increase in smokers compared with never-smokers [Figure 1a and 1c]. The percentage of Tc17 cells in total circulating $CD8^+$ T lymphocytes was increased in patients with COPD (median, 0.562%) compared with smokers (median, 0.434%, $P < 0.01$) and never-smokers (median, 0.33%, $P < 0.001$) [Figure 1b and 1d].

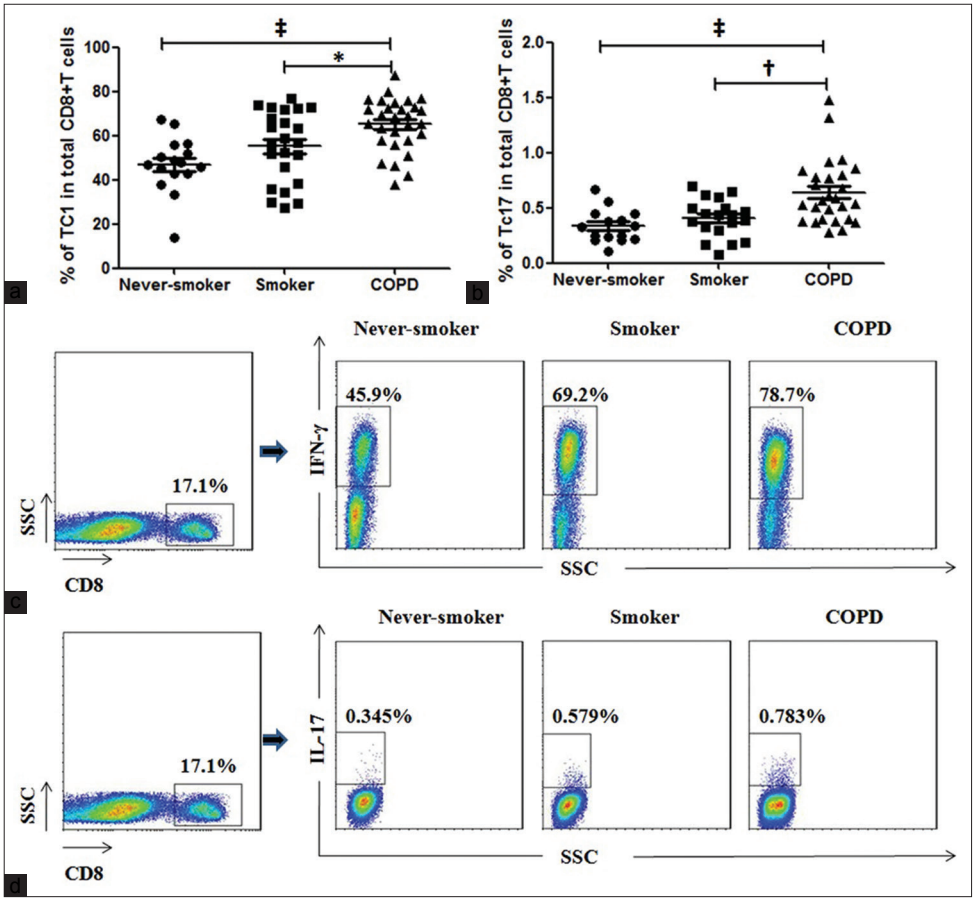

Figure 1: $CD8^+$ T-cell subpopulations in peripheral blood from patients with the chronic obstructive pulmonary disease, smokers, and never-smokers. $CD8^+$ cells were analyzed for production of interferon-γ or interleukin-17. (a and b) The percentages of Tc1 and Tc17 cells among $CD8^+$ T-cells in peripheral blood from patients with chronic obstructive pulmonary disease, smokers, and never-smokers. (c and d) Representative flow cytometry of Tc1 and Tc17 cells. Horizontal lines indicate median values. SSC: Side scatter. COPD: Chronic obstructive pulmonary disease. *$P < 0.05$, †$P < 0.01$, ‡$P < 0.001$.

The frequency of dual-positive Tc17/interferon-γ cells is increased in chronic obstructive pulmonary disease patients

In patients with COPD, a significantly higher percentage of Tc17/IFN-γ cells among $CD8^+$ T-cells (median, 0.268%) in the peripheral blood was found as compared to smokers (median, 0.128%, $P < 0.001$) and never-smokers (median, 0.074%, $P < 0.001$) [Figure 2a and 2c]. Furthermore, a significantly higher percentage of Tc17/IFN-γ cells among Tc17 cells was seen in patients with COPD [median, 48.09%; Figure 2b] compared with smokers [median, 31.25%, $P < 0.001$; Figure 2b] and never-smokers [median, 26.67%, $P < 0.001$; Figure 2b], which indicated increased differentiation of Tc17 cells to Tc17/IFN-γ cells in COPD.

Plasticity of Tc17 cells is higher than that of Th17 cells in chronic obstructive pulmonary disease patients

As demonstrated previously, similar to Th17 cells, the Tc17 phenotype was unstable. Tc17 cell plasticity (converting to Tc17/IFN-γ cells), driven by the inflammatory milieu, especially by IL-12,[22,28] is higher than Th17 plasticity.[10] Therefore, we investigated whether the plasticity of Tc17 cells was also higher in patients with COPD [Figure 3a]. As shown in Figure 3b, the frequency of Tc17/IFN-γ cells (median, 48.09%) in Tc17 cells was significantly higher than the frequency of Th17/Th1 cells (median, 15.44%, $P < 0.001$) in Th17 cells in COPD patients, indicating that Tc17 plasticity was greater than Th17 plasticity in COPD. Consistent with previous studies[10] similar results were also seen in both smokers and never-smokers (data not shown).

Increased expression of dual-positive Tc17/interferon-γ cells is inversely correlated with forced expiratory volume in 1 s

The increased percentage of Tc17 cells among $CD8^+$ T-cells in peripheral blood from COPD patients was inversely correlated with FEV_1% predicted values [$r = -0.418$, $P = 0.03$; Figure 4a]. More importantly, the higher frequency of Tc17/IFN-γ cells among $CD8^+$ T-cells in peripheral blood from COPD patients was also inversely correlated with FEV_1% predicted values [$r = -0.596$, $P = 0.002$; Figure 4b].

The concentrations of interleukin-6, transforming growth factor-β1, and interleukin-12 are increased in chronic obstructive pulmonary disease

We next examined the concentrations of plasma cytokines believed to drive Tc17/IFN-γ cell differentiation. The concentrations of IL-6, TGF-β1, and IL-12 were significantly higher in plasma from patients with COPD compared with smokers and never-smokers [$P < 0.01$, Figure 5a-5c]. These suggest that the Tc17 plasticity in COPD may be driven by the inflammatory environment of the disease.

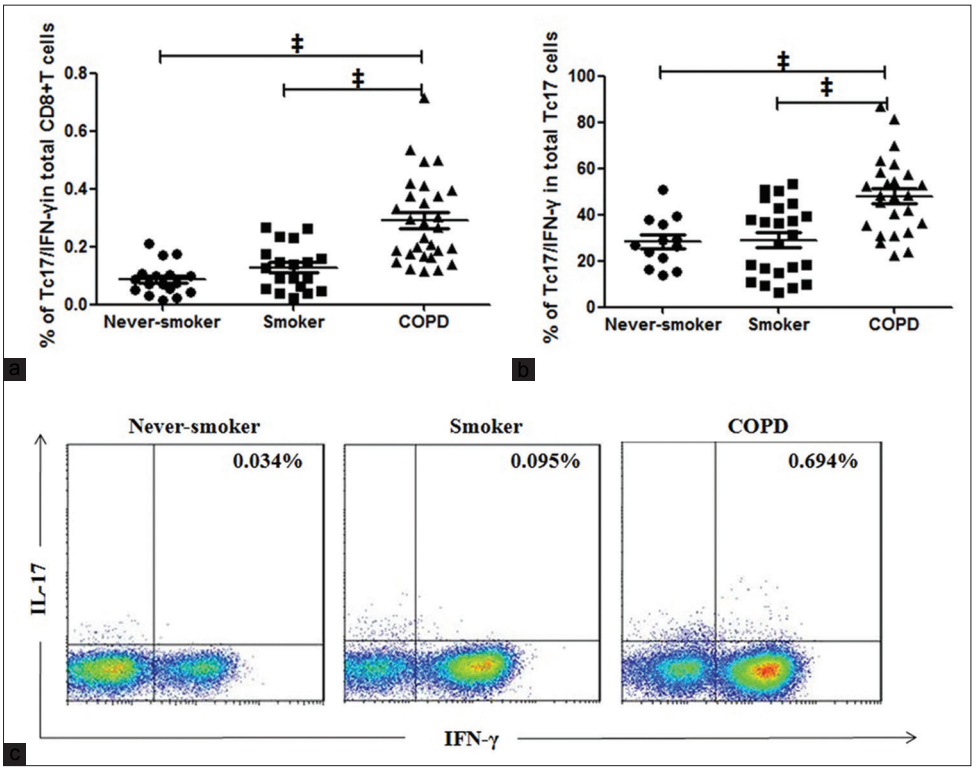

Figure 2: Proportions of the Tc17/interferon-γ cell subpopulation in peripheral blood from patients with the chronic obstructive pulmonary disease, smokers, and never-smokers. $CD8^+$ cells were analyzed for production of interleukin-17 and interferon-γ cells after 5 h of stimulation with phorbol 12-myristate 13-acetate/ionomycin and GolgiStop. (a and b) The percentages of Tc17/interferon-γ cells among $CD8^+$ T-cells and Tc17 cells in peripheral blood from patients with the chronic obstructive pulmonary disease, smokers, and never-smokers. (c) Representative flow cytometry of Tc17/interferon-γ cells. COPD: Chronic obstructive pulmonary disease. Horizontal lines indicate median values, ‡$P < 0.001$.

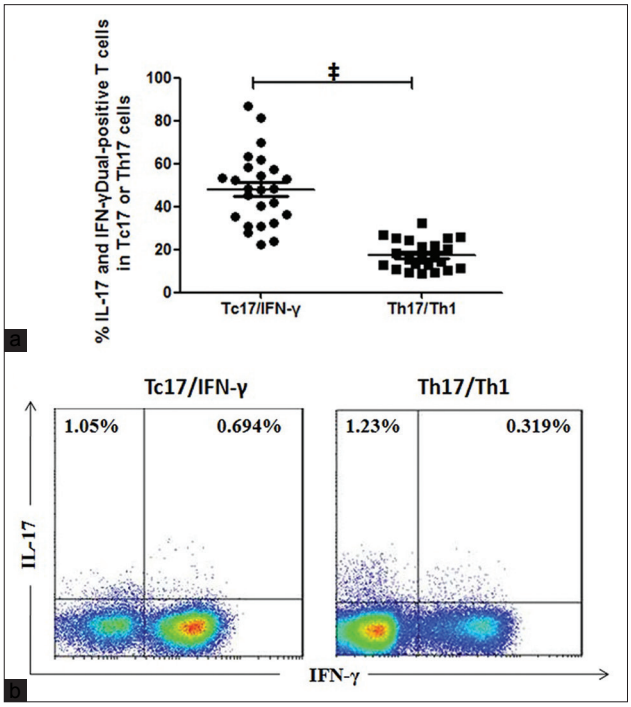

Figure 3: Frequencies of interleukin駸17/interferon駸γ駸double positive subpopulations among Tc17 cells and Th17 cells in peripheral blood from patients with chronic obstructive pulmonary disease. $CD4^+$ cells and $CD8^+$ cells were analyzed for production of interleukin駸17 and interferon駸γ after 5 h of stimulation with phorbol 12駸myristate 13駸acetate/ionomycin and GolgiStop. (a) The percentages of Th17/ Th1 cells and Tc17/interferon駸γ cells among $CD4^+$ T駸cells and $CD8^+$ T駸cells in peripheral blood from patients with chronic obstructive pulmonary disease, respectively. (b) Representative flow cytometry of Th17/Th1 and Tc17/interferon駸γ cells. Horizontal lines indicate median values, $^{‡}P < 0.001$.

dIscussIon

$CD8^+$ T駸cells have long been recognized as the major pathogenic T駸cells in airway inflammation and lung destruction in COPD. In contrast to the plenty of evidence supporting the accumulation and activation of $CD8^+$ T駸cells in the lungs of COPD, little is known about the characteristics and functions of circulating $CD8^+$ T駸cells in COPD, and the limited findings are often controversial. DomagaⅡa駸Kulawik *et al*. reported that frequencies of circulating $CD8^+$ T駸cells were increased in COPD while others found no differences in COPD patients compared with healthy subjects.[30-32] While these discrepancies might be due to the heterogeneous nature of the disease and different patient populations enrolled, however, functional plasticity and phenotype heterogeneity of $CD8^+$ T駸cells are likely to be implicated.

Following the identification of an IL駸17駸producing subset within $CD8^+$ T駸cells, studies have demonstrated that the so駸called Tc17 cells are involved in a wide spectrum of immune diseases.[17-19,21,23-28] More recent evidence confirmed that the Tc17 lineage possessed a late developmental plasticity, that is, converting to Tc17/IFN駸γ cells, in response to inflammatory signals.[20,22,26,33] As COPD is a lung disease with significant systemic inflammation, it is likely that circulating $CD8^+$ T駸cells and/or their subpopulations are implicated in these pathogenic processes. We therefore hypothesized that Tc17 cells in COPD might show high plasticity and differentiate more to Tc17/IFN駸γ cells, which, unlike Tc17 cells in general, are highly toxic, a property similar to Tc1 cells. We demonstrated here that the proportions of Tc17 cells, and more importantly, the multifunctional subpopulation Tc17/IFN駸γ cells, were significantly increased in the peripheral blood of patients with COPD, and both were correlated negatively with FEV_1, a hallmark of severity of the disease.

Our findings of a higher proportion of Tc1 cells in peripheral blood from COPD patients compared with smokers and never駸smokers are consistent with previous reports.[31,34] But our result of increased percentage of Tc17 cells in peripheral blood from patients with COPD was different from the study by Paats *et al*., who had found that the proportion of IL駸17A positive $CD8^+$ T駸cells was negligible in the peripheral blood, and no difference existed between COPD patients and healthy controls.[31] This discrepancy may be due to differences in disease severity (our patients had a higher mean FEV_1) and gender of the patients (our patients were all males). Moreover, we showed that the elevated frequency of circulating Tc17 cells was correlated significantly with COPD severity, suggesting that these cells may be involved in the pathogenesis of COPD. This is supported by animal studies demonstrating that the number of Tc17 cells was significantly increased in lungs of cigarette smoke駸exposed mice, even after smoking cessation, and was correlated with lung emphysematous lesions.[31,35,36]

As mentioned earlier, Tc17 cells are far less cytotoxic as compared to Tc1 cells, and then by what mechanisms they may be pathogenic in COPD? As a novel subset of Tc17 cells, Tc17/IFN駸γ cells are capable of acquiring strong cytotoxic function similar to Tc1 cells and expressing pro駸inflammatory cytokines similar to their Th17/Th1 counterparts and therefore are believed to augment the pathogenic capability of Tc17 cells and promote exacerbation of a variety of autoimmune diseases.[20,22,24-26,37-40] Saxena *et al*. revealed that Tc17/ IFN駸γ cells might be indispensable for the aggravation of diabetes by direct cytotoxicity on the β駸islet cells and expressing pro駸inflammatory cytokines apart from IFN駸γ in an experimental model of autoimmune diabetes.[24] Tajima *et al*. reported that Tc17/IFN駸γ cells were rapidly generated in mesenteric lymph nodes, and IL駸17 acted synergistically with IFN駸γ to recruit effector $CD8^+$ T駸cells and other inflammatory cells to colon tissues in a colitis model.[25] Here, we demonstrated for the first time to our knowledge that the percentages of Tc17/IFN駸γ cells among $CD8^+$ T cells and Tc17 cells were significantly higher in peripheral blood from patients with COPD and correlated with FEV_1, suggesting that circulating Tc17/IFN駸γ cells might be involved in persistent inflammation and loss of lung function in COPD. In addition, we found that Tc17 cells exhibited higher developmental plasticity than

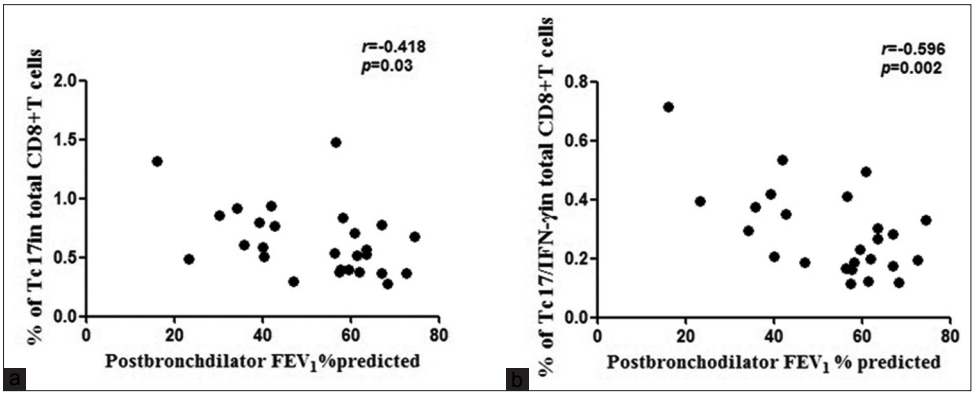

Figure 4: Correlations of Tc17/interferon駸γ cells and lung function (n=25). (a) Frequencies of Tc17 cells in $CD8^+$ T駸cells and (b) frequencies of Tc17/interferon駸γ cells in $CD8^+$ T駸cells correlated with forced expiratory volume in 1 s (FEV1)% predicted values in patients with chronic obstructive pulmonary disease. A P value < 0.05 was considered statistically significant.

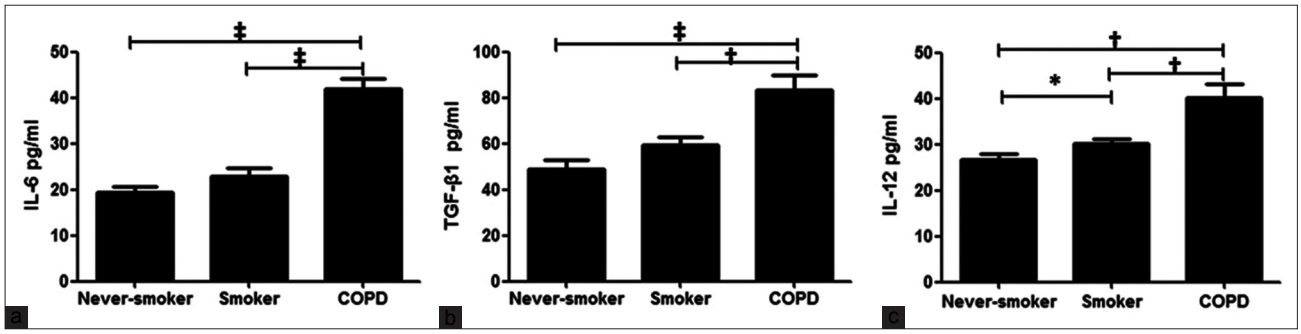

Figure 5: The concentrations of plasma cytokines. Interleukin駸6 (a), transforming growth factor駸β1 (b), and interleukin駸12 (c) from patients with chronic obstructive pulmonary disease, smokers, and never駸smokers. COPD: Chronic obstructive pulmonary disease. Horizontal lines indicate median values, *P < 0.05, †P < 0.01, ‡P < 0.001.

Th17 cells, although the implications of Tc17 plasticity in the pathogenesis of COPD remain speculative.

Since Tc17 plasticity is driven by inflammatory conditions, we supposed that the higher Tc17 plasticity was related to the systemic inflammation in COPD. Previous studies showed that in the presence of IL駸6 and TGF駸β1, human naïve $CD8^+$ T駸cells acquired the Tc17 phenotype, and IL駸12 was able to permit Tc17 cells to acquire the potential to produce IFN駸γ, thus differentiating to Tc17/IFN駸γ cells.[20,29,33] In the current study, we found increased concentrations of IL駸6 and TGF駸β1 in the plasma from patients of COPD. More importantly, consistent with other studies, the concentration of IL駸12 was higher in patients with COPD as compared to the controls.[41,42] These results indicate that further studies are needed to explore the mechanisms by which these cytokines induce Tc17 plasticity in COPD.

Our study has several limitations. First, we did not examine the frequency of Tc17 and Tc17/IFN駸γ cells in the lungs, for example, in bronchoalveolar lavage, which may be more relevant to airway diseases of COPD; hence, further investigations to this issue are needed. Second, we have only shown increased plasma levels of IL駸6, TGF駸β1, and IL駸12 in COPD; whether and by what mechanism these cytokines promote Tc17 plasticity is still speculative. Third, some of our patients had used inhaled corticosteroids, and therefore the possibility of an effect of this medication on our results cannot be excluded, although Paats *et al*. found no effect of inhaled corticosteroids on peripheral $CD8^+$ T駸cells in COPD.[31]

In summary, this study provides a comprehensive analysis of circulating $CD8^+$ T駸cells and their subpopulations in COPD, with a novel finding that the circulating Tc17/IFN駸γ cells, in addition to Tc17 cells, are significantly increased and correlated to the severity of disease, suggesting that these cells may be involved in the pathogenesis of COPD. Further studies are needed to elucidate the underlying mechanisms of $CD8^+$ T駸cell heterogeneity and Tc17 cell plasticity in COPD, which may shed new light on the understanding of local and systemic inflammation characteristic of the disease.

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Conflicts of interest

There are no conflicts of interest.

references

1. Vestbo J, Hurd SS, Agustí AG, Jones PW, Vogelmeier C, Anzueto A, *et al.* Global strategy for the diagnosis, management, and prevention

of chronic obstructive pulmonary disease: GOLD executive summary. Am J Respir Crit Care Med 2013;187:347駁65. doi: 10.1164/ rccm.201204駁0596PP. 2. Willemse BW, ten Hacken NH, Rutgers B, Lesman駁Leegte IG, Postma DS, Timens W. Effect of 1駁year smoking cessation on airway inflammation in COPD and asymptomatic smokers. Eur Respir J 2005;26:835駁45. doi: 10.1183/09031936.05.00108904. 3. Miller M, Cho JY, Pham A, Friedman PJ, Ramsdell J, Broide DH. Persistent airway inflammation and emphysema progression on CT scan in ex駁smokers observed for 4 years. Chest 2011;139:1380駁7. doi: 10.1378/chest.10駁0705. 4. Cosio MG, Majo J, Cosio MG. Inflammation of the airways and lung parenchyma in COPD: Role of T cells. Chest 2002;121 5 Suppl: 160S駁5S. doi: 10.1378/chest.121.5_suppl.160S. 5. Saetta M, Baraldo S, Corbino L, Turato G, Braccioni F, Rea F, *et al.* CD8+ve cells in the lungs of smokers with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 1999;160:711駁7. doi: 10.1164/ajrccm.160.2.9812020. 6. O駟Shaughnessy TC, Ansari TW, Barnes NC, Jeffery PK. Inflammation in bronchial biopsies of subjects with chronic bronchitis: Inverse relationship of CD8+T lymphocytes with FEV1. Am J Respir Crit Care Med 1997;155:852駁7. doi: 10.1164/ajrccm.155.3.9117016. 7. Saetta M, Di Stefano A, Turato G, Facchini FM, Corbino L, Mapp CE, *et al.* CD8+T駁lymphocytes in peripheral airways of smokers with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 1998;157 (3 Pt 1):822駁6. doi: 10.1164/ajrccm.157.3.9709027. 8. Forsslund H, Mikko M, Karimi R, Grunewald J, Wheelock ÅM, Wahlström J, *et al.* Distribution of T駁cell subsets in BAL fluid of patients with mild to moderate COPD depends on current smoking status and not airway obstruction. Chest 2014;145:711駁22. doi: 10.1378/chest.13駁0873. 9. Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. J Immunol 2005;136:2348駁57. doi: 0022駁1767/86/1367駁2348802.00/0. 10. Liang Y, Pan HF, Ye DQ. IL駁17A駁producing CD8(+) T cells as therapeutic targets in autoimmunity. Expert Opin Ther Targets 2015;19:651駁61. doi: 10.1517/1472 8222.20 14.997710. 11. Mosmann TR, Li L, Sad S. Functions of CD8 T駁cell subsets secreting different cytokine patterns. Semin Immunol 1997;9:87駁92. doi: 10.1006/smim.1997.0065. 12. Gill D, Tan PH. Induction of pathogenic cytotoxic T lymphocyte tolerance by dendritic cells: A novel therapeutic target. Expert Opin Ther Targets 2010;14:797駁824. doi: 10.1517/14728222.2010.499360. 13. Chrysofakis G, Tzanakis N, Kyriakoy D, Tsoumakidou M, Tsiligianni I, Klimathianaki M, *et al.* Perforin expression and cytotoxic activity of sputum CD8+lymphocytes in patients with COPD. Chest 2004;125:71駁6. doi: 10.1378/chest.125.1.71. 14. Huber M, Heink S, Grothe H, Guralnik A, Reinhard K, Elflein K, *et al.* A Th17駁like developmental process leads to CD8(+) Tc17 cells with reduced cytotoxic activity. Eur J Immunol 2009;39:1716駁25. doi: 10.1002/eji.200939412. 15. Langrish CL, Chen Y, Blumenschein WM, Mattson J, Basham B, Sedgwick JD, *et al*. IL駁23 drives a pathogenic T cell population that induces autoimmune inflammation. J Exp Med 2005;201:233駁40. doi: 10.1084/jem.20041257. 16. Acosta駁Rodriguez EV, Rivino L, Geginat J, Jarrossay D, Gattorno M, Lanzavecchia A, *et al.* Surface phenotype and antigenic specificity of human interleukin 17駁producing T helper memory cells. Nat Immunol 2007;8:639駁46. doi: 10.1038/ni1467.17. Henriques A, Gomes V, Duarte C, Pedreiro S, Carvalheiro T, Areias M, *et al.* Distribution and functional plasticity of peripheral blood Th(c) 17 and Th(c) 1 in rheumatoid arthritis. Rheumatol Int 2013;33:2093駁9. doi: 10.1007/s00296駁013駁2703駁6. 18. Peelen E, Thewissen M, Knippenberg S, Smolders J, Muris AH, Menheere P, *et al.* Fraction of IL駁10+and IL駁17+CD8 T cells is increased in MS patients in remission and during a relapse, but is not influenced by immune modulators. J Neuroimmunol 2013;258:77駁84. doi: 10.1016/j.jneuroim.2013.02.014. 19. Cheuk S, Wikén M, Blomqvist L, Nylén S, Talme T, Ståhle M, *et al.* Epidermal Th22 and Tc17 cells form a localized disease memory in clinically healed psoriasis. J Immunol 2014;192:3111駁20. doi: 10.4049/jimmunol.1302313. 20. Tajima M, Wakita D, Satoh T, Kitamura H, Nishimura T. IL駁17/IFN駁γ double producing CD8+T (Tc17/IFN駁γ) cells: A novel cytotoxic T駁cell subset converted from Tc17 cells by IL駁12. Int Immunol 2011;23:751駁9. doi: 10.1093/intimm/dxr086. 21. Ortega C, Fernández AS, Carrillo JM, Romero P, Molina IJ, Moreno JC, *et al.* IL駁17駁producing CD8+T lymphocytes from psoriasis skin plaques are cytotoxic effector cells that secrete Th17駁related cytokines. J Leukoc Biol 2009;86:435駁43. doi: 10.1189/JLB.0109046. 22. Hamada H, Garcia駁Hernandez Mde L, Reome JB, Misra SK, Strutt TM, McKinstry KK, *et al.* Tc17, a unique subset of CD8 T cells that can protect against lethal influenza challenge. J Immunol 2009;182:3469駁81. doi: 10.4049/jimmunol.0801814. 23. Henriques A, Inês L, Couto M, Pedreiro S, Santos C, Magalhães M, *et al.* Frequency and functional activity of Th17, Tc17 and other T駁cell subsets in systemic lupus erythematosus. Cell Immunol 2010;264:97駁103. doi: 10.1016/j.cellimm.2010.05.004. 24. Saxena A, Desbois S, Carrié N, Lawand M, Mars LT, Liblau RS. Tc17 CD8+T cells potentiate Th1駁mediated autoimmune diabetes in a mouse model. J Immunol 2012;189:3140駁9. doi: 10.4049/ jimmunol.1103111. 25. Tajima M, Wakita D, Noguchi D, Chamoto K, Yue Z, Fugo K, *et al.* IL駁6駁dependent spontaneous proliferation is required for the induction of colitogenic IL駁17駁producing CD8+T cells. J Exp Med 2008;205:1019駁27. doi: 10.1084/jem.20071133. 26. Satoh T, Tajima M, Wakita D, Kitamura H, Nishimura T. The development of IL駁17/IFN駁γ駁double producing CTLs from Tc17 cells is driven by epigenetic suppression of Socs3 gene promoter. Eur J Immunol 2012;42:2329駁42. doi: 10.1002/eji.201142240. 27. Chang Y, Nadigel J, Boulais N, Bourbeau J, Maltais F, Eidelman DH, *et al.* CD8 positive T cells express IL駁17 in patients with chronic obstructive pulmonary disease. Respir Res 2011;12:43. doi: 10.1186/1465駁9921駁12駁43. 28. Eustace A, Smyth LJ, Mitchell L, Williamson K, Plumb J, Singh D. Identification of cells expressing IL駁17A and IL駁17F in the lungs of patients with COPD. Chest 2011;139:1089駁100. doi: 10.1378/ chest.10駁0779. 29. Nistala K, Adams S, Cambrook H, Ursu S, Olivito B, de Jager W, *et al.* Th17 plasticity in human autoimmune arthritis is driven by the inflammatory environment. Proc Natl Acad Sci U S A 2010;107:14751駁6. doi: 10.1073/pnas.1003852107. 30. Domagala駁Kulawik J, Hoser G, Dabrowska M, Chazan R. Increased proportion of Fas positive CD8+cells in peripheral blood of patients with COPD. Respir Med 2007;101:1338駁43. doi: 10.1016/j. rmed.2006.10.004. 31. Paats MS, Bergen IM, Hoogsteden HC, van der Eerden MM, Hendriks RW. Systemic CD4+and CD8+T駁cell cytokine profiles correlate with GOLD stage in stable COPD. Eur Respir J 2012;40:330駁7. doi: 10.1183/09031936.00079611. 32. Barceló B, Pons J, Fuster A, Sauleda J, Noguera A, Ferrer JM, *et al.* Intracellular cytokine profile of T lymphocytes in patients with chronic obstructive pulmonary disease. Clin Exp Immunol 2006;145:474駁9. doi: 10.1111/j.1365駁2249.2006.03167.x. 33. Yen HR, Harris TJ, Wada S, Grosso JF, Getnet D, Goldberg MV, *et al.* Tc17 CD8 T cells: Functional plasticity and subset diversity. J Immunol 2009;183:7161駁8. doi: 10.4049/jimmunol.0900368. 34. Shirai T, Suda T, Inui N, Chida K. Correlation between peripheral blood T駁cell profiles and clinical and inflammatory parameters in stable COPD. Allergol Int 2010;59:75駁82. doi: 10.2332/allergolint.09駁OA駁0126. 35. Duan MC, Tang HJ, Zhong XN, Huang Y. Persistence of Th17/ Tc17 cell expression upon smoking cessation in mice with cigarette smoke駁induced emphysema. Clin Dev Immunol 2013;2013:162駁71. doi: 10.1155/2013/350727. 36. Zhou H, Hua W, Jin Y, Zhang C, Che L, Xia L, *et al.* Tc17 cells are associated with cigarette smoke駁induced lung inflammation and emphysema. Respirology 2015;20:426駁33. doi: 10.1111/resp.12486. 37. Yeh N, Glosson NL, Wang N, Guindon L, McKinley C, Hamada H, *et al.* Tc17 cells are capable of mediating immunity to vaccinia virus by acquisition of a cytotoxic phenotype. J Immunol 2010;185:2089駁98. doi: 10.4049/jimmunol.1000818.

38. Lee Y, Awasthi A, Yosef N, Quintana FJ, Xiao S, Peters A, *et al.* Induction and molecular signature of pathogenic TH17 cells. Nat Immunol 2012;13:991駁9. doi: 10.1038/ni.2416. 39. Boniface K, Blumenschein WM, Brovont駁Porth K, McGeachy MJ, Basham B, Desai B, *et al.* Human Th17 cells comprise heterogeneous subsets including IFN駁gamma駁producing cells with distinct properties from the Th1 lineage. J Immunol 2010;185:679駁87. doi: 10.4049/ jimmunol.1000366. 40. Kondo T, Takata H, Matsuki F, Takiguchi M. Cutting edge: Phenotypic characterization and differentiation of human CD8+T cells producing IL駁17. J Immunol 2009;182:1794駁8. doi: 10.4049/jimmunol.0801347. 41. Bade G, Khan MA, Srivastava AK, Khare P, Solaiappan KK, Guleria R, *et al.* Serum cytokine profiling and enrichment analysis reveal the involvement of immunological and inflammatory pathways in stable patients with chronic obstructive pulmonary disease. Int J Chron Obstruct Pulmon Dis 2014;9:759駁73. doi: 10.2147/COPD.S61347. 42. Pinto駁Plata V, Toso J, Lee K, Park D, Bilello J, Mullerova H, *et al.* Profiling serum biomarkers in patients with COPD: Associations with clinical parameters. Thorax 2007;62:595駁601. doi: 10.1136/ thx.2006.064428.