Original Article

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

Wei-Han Xu[1], Xiao-Ling Hu[1], Xiao-Fang Liu[1], Peng Bai[1], Yong-Chang Sun[1,2]

[1]Department of Respiratory Medicine, Beijing Tongren Hospital, Capital Medical University, Beijing 100730, China
[2]Department of Respiratory and Critical Care Medicine, Peking University Third Hospital, Beijing 100191, China

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

Access this article online

Quick Response Code:

Website: www.cmj.org

DOI: 10.4103/0366-6999.179798

Address for correspondence: Prof. Yong-Chang Sun, Department of Respiratory Medicine, Beijing Tongren Hospital, Capital Medical University, Beijing 100730, China
Department of Respiratory and Critical Care Medicine, Peking University Third Hospital, Beijing 100191, China
E-Mail: suny@ccmu.edu.cn

Received: 08-12-2015 **Edited by:** Li-Min Chen
How to cite this article: Xu WH, Hu XL, Liu XF, Bai P, Sun YC. Peripheral Tc17 and Tc17/Interferon-γ Cells are Increased and Associated with Lung Function in Patients with Chronic Obstructive Pulmonary Disease. Chin Med J 2016;129:909-16.

周围TC17和TC17/Interferon-γ细胞增加并与肺部功能有关慢性阻塞性肺部疾病患者

wei-han xu [1]，xiao-ling hu [1]，小豆liu [1]，peng bai [1]，yong-chang sun [1,2] [1,2] [1]中国北京
北京第三北京大学医学

抽象的

背景：慢性阻塞性肺疾病（COPD）的特征是肺功能的进行性丧失以及局部和全身性炎症，其中CD8 + T细胞被认为起着关键作用。激活的CD8 + T −细胞分化为不同的亚群，包括干扰素− γ（IFN- γ） - 产生Tc1和interleukin（IL）−17 − 17-生产的TC17细胞。最近的证据表明，TC17细胞表现出相当大的可塑性，并且在受炎症条件驱动时可能会转化为IL - 17/IFN- γ - 双重产生（TC17/IFN- γ）细胞。这项研究的目的是研究COPD患者外周血中TC17/IFN- γ亚群，并评估其在该疾病中的潜在作用。方法：从15位从未吸烟者，23名具有正常肺功能的吸烟者和25例COPD患者（全球慢性阻塞性肺疾病2-4）中收集外周血样本。使用流式细胞术评估了IL - 17/IFN- γ - 双重表达亚群的比例。通过酶连接的免疫吸附测定法测量了有利于TC17/IFN- γ分化的细胞因子的血浆浓度。

结果：COPD患者的TC17细胞比例更高，而外周血中的TC17/IFN- γ细胞比吸烟者和从未吸烟者的细胞更高。 TC17细胞的可塑性高于Th17细胞的可塑性。 TC17细胞和TC17/IFN- γ细胞的百分比显示出与1 s%预测值（$r = -0.418$， $P = 0.03$; $r = r = - - - 0.596$ 0.596，v352222252220222， $P = 0.03$; 与吸烟者和从未吸烟者相比，COPD患者的IL − 6，转化生长因子β 1和IL - 12的血浆浓度明显更高。

结论：周围TC17细胞增加，并且更有可能将COPD中的TC17/IFN- γ细胞转换为TC17 C中的TC17/IFN- γ细胞 Ell可塑性可能与疾病的持续炎症有关。

关键词：cd8 + t -cells; Interferon − γ;白介素17;可塑性

介绍

慢性阻塞性肺疾病（COPD）与肺对吸烟和其他有害颗粒或其他有害的颗粒和其他有害的颗粒和其他有害的颗粒或慢性炎症反应有关。

CD8 + T孔是COPD炎症和肺部破坏的主要参与者。[4]人们认为，CD8 + T − cells诱导COPD肺部炎症和肺气肿，这是通过生产Granzyme B，Perforin，perforin，Perforin，以及许多其他不利的和其他造物主义者和许多人的杀虫剂。 在COPD患者中的研究表明，CD8 + T细胞是积聚在肺实质和气道中，与气道阻塞程度相关。[5-8]

激活的CD8 + T −细胞分化为不同的亚群，包括Interferon − γ（IFN- γ） - 生产

通讯地址：北京汤顿医院呼吸医学系的Yong -chang Sun教授，北京医科大学，北京100730，中国呼吸道和重症监护医学系，北京第三医院，北京第三医院，北京100191，中国电子书：suny@ccmu.edu.edu.ccmu.ccmu.ccmu.ccmu.ccn

Received: 08-12-2015 **Edited by:** Li-Min Chen
How to cite this article: Xu WH, Hu XL, Liu XF, Bai P, Sun YC. Peripheral Tc17 and Tc17/Interferon-γ Cells are Increased and Associated with Lung Function in Patients with Chronic Obstructive Pulmonary Disease. Chin Med J 2016;129:909-16.

Access this article online

Quick Response Code:

Website: www.cmj.org

DOI: 10.4103/0366-6999.179798

Tc1, interleukin (IL)-4 producing Tc2, and IL-17-producing Tc17 cells, defined by selected sets of cytokines and transcription factors production.[9-11] Studies have shown that most $CD8^+$ T-cells isolated from the lung parenchyma of COPD patients are IFN-γ-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-bet, IFN-γ, perforin, and granzyme B.[14] Tc17 cells are shown to share some phenotypical properties with Th17, including retinoic acid receptor-related orphan receptor γt, CCR6, and IL-23R, and express tumor necrosis factor-α (TNF-α), IL-21, and IL-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-17/IFN-γ-double producing cells (Tc17/IFN-γ cells) permitted by IL-12 signaling, with distinct properties from Tc1 lineage.[20] Tc17/IFN-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-17A and IL-17F expressions by $CD8^+$ T-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-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-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-17 and IFN-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-γ differentiation were measured in plasma from the study subjects. Our results revealed higher proportions of Tc17 cells and Tc17/IFN-γ cells in peripheral blood from COPD patients, which could be explained by increased concentrations of IL-6, transforming growth factor β1 (TGF-β1) and IL-12. Importantly, the percentages of Tc17 cells and Tc17/IFN-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-γ cells in the pathogenesis of COPD.

Methods

Study subjects

Twenty-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-three smokers and 15 never-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-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 2008-14). Written informed consent was obtained from all subjects.

Cell collection and flow cytometry

Peripheral blood samples were collected into ethylenediaminetetraacetic acid-treated tubes by venipuncture from the subjects after an 8-h fasting and were layered on the Ficoll-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-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-1640 medium and prepared for the following procedures.

Freshly processed human PBMCs were stimulated with 50 ng/ml of phorbol 12-myristate 13-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.

TC1，介体（IL）−4产生Tc2和IL − 17 · 产生的TC17细胞，由选定的细胞因子和转录因子产生的选定集定义。[9-11]研究表明，大多数CD8 + T -cells与COPD患者的肺部较高型细胞分离出来。与TC2细胞相比，细胞毒性。[12,13]与TC1细胞相比，TC17细胞通过分泌低水平的细胞毒性T淋巴细胞标记来表现出明显抑制的细胞毒性活性：T -bet，T -bet，Ifn- ifn- γ，portorin和granzyme B. Th17，包括视黄酸受体与孤儿受体γ T，CCR6和IL − 23r，以及表达肿瘤坏死因子− α（tnf − α），IL - 21和IL - 21和IL − 22和IL - 22。类风湿关节炎（RA），多发性硬化症，牛皮癣，与这些疾病的发病机理有关。[17-19]

TC17细胞具有较高的可塑性，可以转换为IL -12信号传导允许的IL -17 γ - 双重产生细胞（TC17/IFN- γ细胞），具有与TC1谱系不同的特性。活动 *in vitro* 和*in vivo*。有趣的是，发现TC17的这种独特的亚群涉及人类和小鼠的各种炎症条件。[20-26]

先前的研究报道，CD8 + T − t -cells的IL -17A和IL - 17F表达在COPD患者的气道中增加了。[27,28] [27,28]，对于循环TC17细胞的频率，尤其是TC17/IFN- γ细胞及其与COPD中的疾病相关的频率。鉴于COPD是一种肺部疾病，具有明显的肺外影响，探索了周围CD8 + T-细胞的分化，尤其是新认识的TC17细胞，可能会为理解这种疾病全身性炎症的机制提供启示的证据。因此，我们评估了COPD患者的外周血中CD8 + T淋巴细胞的签名细胞因子IL - 17和IFN- γ表达，并分析了TC17细胞和Th17细胞之间可塑性的差异。从研究对象中测量了在血浆中测量的据信TC17/IFN- γ分化的细胞因子。我们的结果表明，COPD患者的外周血中TC17细胞和TC17/IFN- γ细胞的比例较高，可以通过IL − 6的浓度增加来解释，从而转化生长因子β 1（TGF- β 1）和IL −12。 重要的是，TC17细胞和TC17/IFN- γ细胞的百分比与1 s（FEV $_1$）%的强制呼气量负相关。 这些结果表明，有必要进行更多的研究，以揭示TC17/IFN- γ细胞在COPD发病机理中的潜在参与。

方法

研究科目

在首都医科大学的北京汤期医院招募了25名患有COPD的男性COPD患者，所有现任或以前的吸烟者都被招募中国。 还包括二十三名吸烟者和15名具有正常肺功能的从未吸烟者。 COPD的诊断是根据临床症状，烟草吸烟的病史和肺功能受损的诊断（支支支架后FEV $_1$/强制生命能力< 70%），根据全球诊断标准，根据临床的临床copterabilitive and the Global Sighative with of Copt and Copt and Copt and Copt。入学前3个月遭受了≥的任何加重。具有正常肺功能的吸烟者（FEV $_1$ > 80%预测）的吸烟病史为≥ 20包年。 患有哮喘，限制性肺部疾病，肺手术，其他慢性全身性炎症性疾病（例如RA，1型糖尿病和炎症性肠病）的患者被排除在外。 表1总结了研究参与者的人口统计学和基线临床特征。

该研究得到当地研究伦理委员会的批准（Treckt 2008-14）。从所有科目获得书面知情同意书。

细胞收集和流式细胞术

Peripheral blood samples were collected into ethylenediaminetetraacetic acid-treated tubes by venipuncture from the subjects after an 8-h fasting and were layered on the Ficoll-Paque Plus solution (Amersham Biosciences, Amersham, Bucks, UK) in a centrifuge tube,在21°C下以400 × *g*离心20分钟，并收集周围血液单核细胞（PBMC）。然后，将二价阳离子汉克斯平衡盐溶液用于300 × *g*的细胞在4°C下持续5分钟。将PBMC重悬于RPMI −1640培养基中的10 ^{6}细胞/mL中，并准备以下步骤。

在5 μ g/ml brefeldin a的存在下，用50 ng/ml的12-乙酸盐和500 ng/ml的雌雄同体刺激新鲜加工的人PBMC刺激13-乙酸盐和500 ng/ml的离子霉素

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

为吸烟史，平均值和所有其他人的标准偏差提出了中位数（IQR）。 COPD：慢性阻塞性肺疾病； FEV $_1$：1 s的强制呼气量； FVC：强迫生命力； ICS：吸入的皮质类固醇； IQR：四分位数范围； SD：标准偏差。

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].

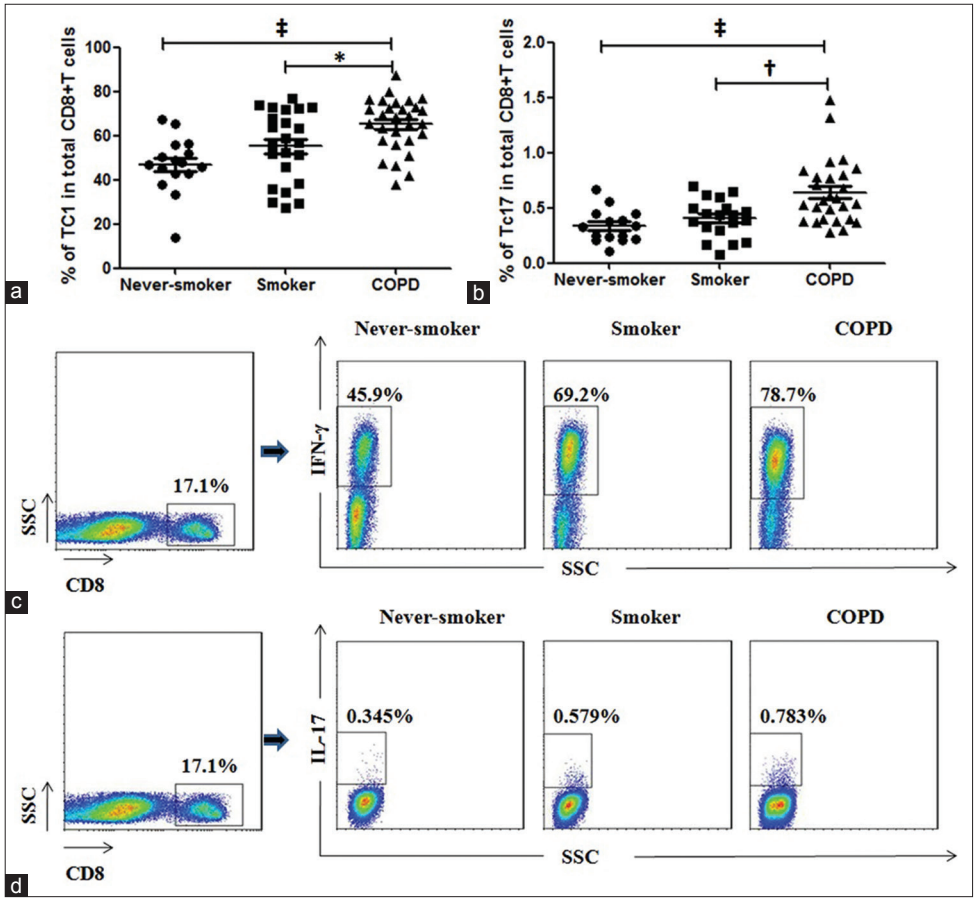

Figure 1: $CD8^+$ T-cell subpopulations in peripheral blood from patients with the chronic obstructive pulmonary disease, smokers, and never-smokers. $CD8^+$ cells were analyzed for production of interferon-γ or interleukin-17. (a and b) The percentages of Tc1 and Tc17 cells among $CD8^+$ T-cells in 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. *$P < 0.05$, †$P < 0.01$, ‡$P < 0.001$.

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固定和透化后，抗hifn-γ−APC（EBISOSCIENCE）。 CD8⁺亚群是使用FACS -Calibur（BD Biosciences）确定的。总共为每个主题收集了总共1个× 10⁵事件，并通过FlowJo软件（Tree Star，Ashland，OR，USA）分析数据。

细胞因子酶连接的免疫吸附测定法

根据制造商的建议，通过酶联免疫吸附测定法（ELISA，Ebioscience，CA，CA，CA，美国，CA，CA，CA，CA，USA）的建议，根据制造商的建议，通过具有2 pg/ml，2.1 pg/Ml，2.1 pg/ML，ML，ML，ML，ML，ML，ML，ML，2.1 pg/6，通过酶联免疫吸附测定法（ELISA，CA，CA，美国，CA，CA，CA，CA，CA，CA，CA，CA，CA）测量IL -6，IL -12和TGF -β1的浓度。

统计分析

在适当的情况下，将组数据描述为平均值或中位数和四分位间范围的平均值和标准误差。使用单向方差分析（ANOVA）对正态分布进行了三组的比较，当检测到统计显着性时，两组之间的*post hoc*分析是通过使用Tukey测试执行。使用Pearson的等级相关系数分析了相关性。 *P*值< 0.05被认为具有统计学意义。所有分析均由Prism 5.02（GraphPad，La Jolla，CA，USA，美国）和SPSS进行的Windows Standard版本（SPSS Inc，美国芝加哥，伊利诺伊州）发行。

结果

慢性阻塞性肺部疾病患者中TC1细胞和TC17细胞的频率增加

我们首先使用流式细胞仪研究了研究受试者的外周血中的IFN-γ的频率。与吸烟者（中位数为56.60%， $P < 0.05$）相比，COPD患者（中位数为68.50%）的循环CD8⁺T细胞的TC1细胞比例更高。 Never -smokers [图1A和1C]。 与吸烟者（中位数为0.434%， $P < 0.01$）相比，COPD患者（中位数为0.562%）的患者（中位数为0.562%）患者的总循环CD8⁺T淋巴细胞的TC17细胞百分比增加了。

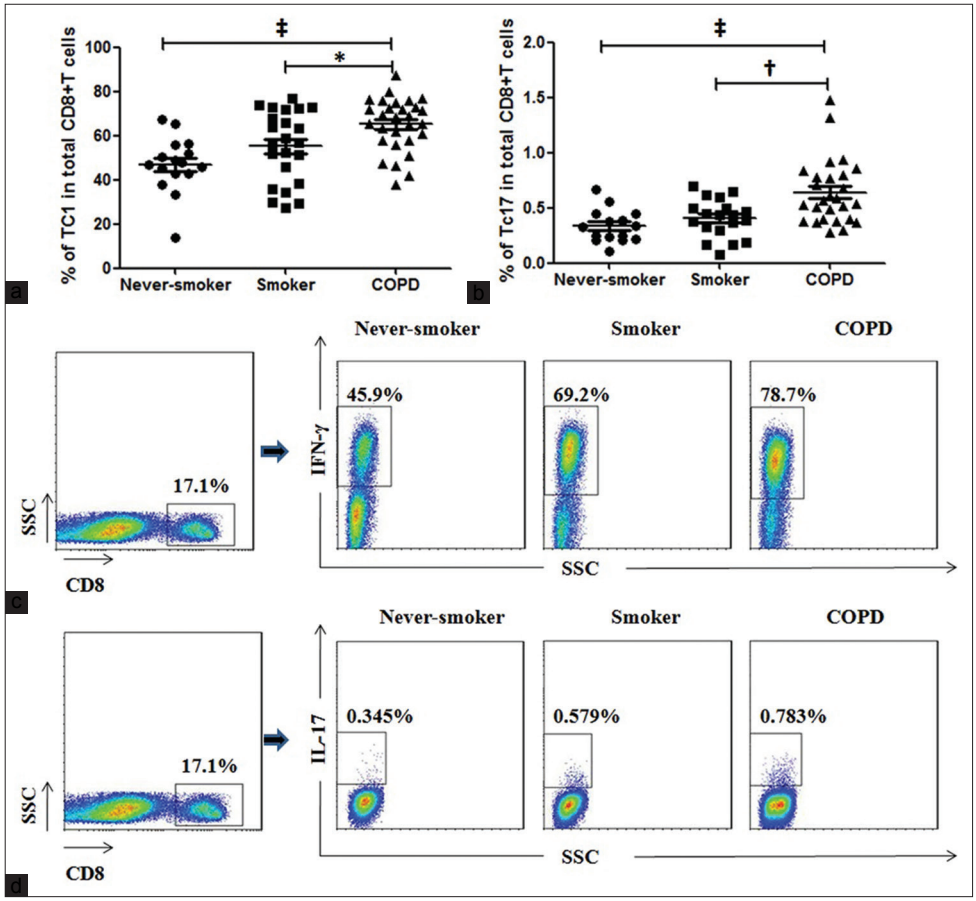

图1：来自慢性阻塞性肺部疾病，吸烟者和从未吸血的患者的外周血中的CD8⁺T细胞亚群。 分析了CD8⁺细胞的产生干扰素γ或白介素−17。 （a和b）来自慢性阻塞性肺部疾病，吸烟者和永无止境的患者的外周血中CD8⁺T细胞中TC1和TC17细胞的百分比。 （C和D）TC1和TC17细胞的代表性流式细胞仪。水平线表示中值。 SSC：侧面散射。 COPD：慢性阻塞性肺部疾病。 *$P < 0.05$，†$P < 0.01$，‡$P < 0.001$。

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.

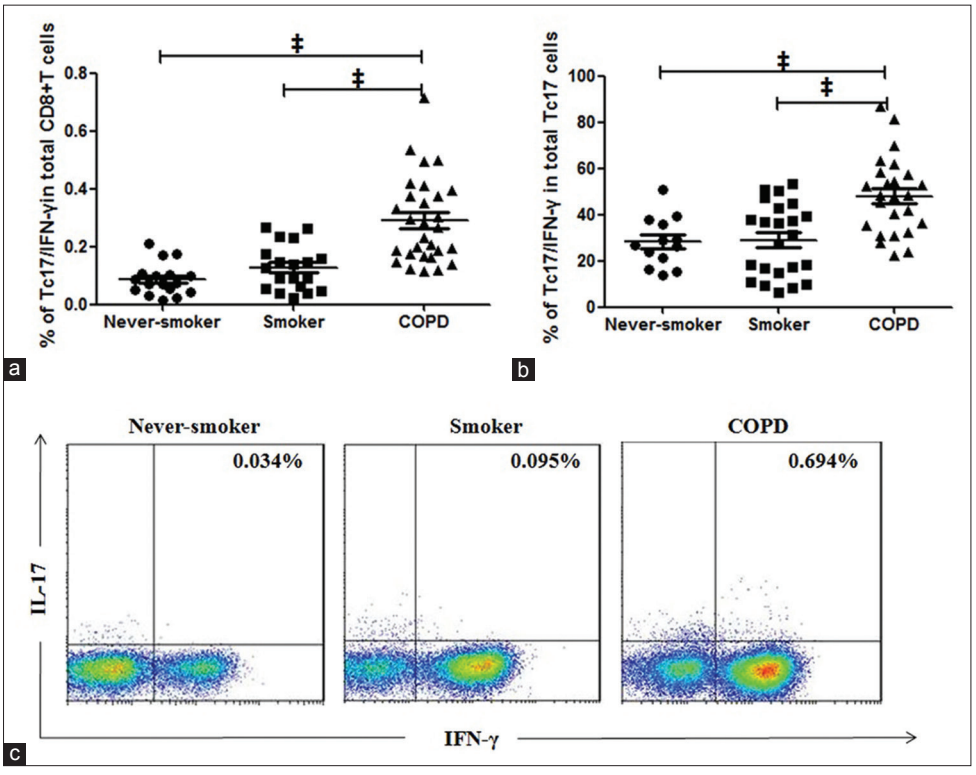

Figure 2: Proportions of the Tc17/interferon-γ cell subpopulation in peripheral blood from patients with the chronic obstructive pulmonary disease, smokers, and never-smokers. $CD8^+$ cells were analyzed for production of interleukin-17 and interferon-γ cells after 5 h of stimulation with phorbol 12-myristate 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, ‡$P < 0.001$.

慢性阻塞性肺部疾病患者的双阳性TC17/Interferon - γ细胞的频率增加

在COPD的患者中，与吸烟者相比，周围血液中CD8 + T -cells（中位数为0.268%）中TC17/IFN- γ细胞的百分比明显更高。 0.001）[图2A和2C]。此外，在COPD患者中，观察到TC17细胞中TC17/IFN- γ细胞的百分比明显更高[中位数为48.09%；图2b]与吸烟者相比[中位数，31.25%， $P < 0.001$；图2b]和Never -Mmoker [中位数，26.67%， $P < 0.001$；图2b]，这表明在COPD中，TC17细胞与TC17/IFN- γ细胞的分化增加。

TC17细胞的可塑性高于慢性阻塞性肺疾病患者中Th17细胞的可塑性

如前所述，类似于Th17细胞，TC17表型不稳定。 TC17细胞可塑性（转换为TC17/IFN- γ细胞），由炎症环境驱动，尤其是由IL - 12，[22,28]高于Th17可塑性。[10]高于Th17的可塑性。 如图3B所示，TC17细胞中TC17/IFN- γ细胞（中位数为48.09%）的频率显着高于Th17细胞中Th17细胞中Th17/Th1细胞的频率（中位数，15.44%，$P < 0.001$）

在COPD患者中，表明TC17可塑性大于COPD中的Th17可塑性。 与以前的研究一致[10]在吸烟者和从未吸烟者中都可以看到相似的结果（数据未显示）。

双阳性TC17/Interferon- γ细胞的表达增加与1 s的强迫呼气量成反比

来自COPD患者的外周血中CD8 + T细胞中TC17细胞的百分比增加与FEV_1%预测值[$r = - - 0.418$， $P = P = 0.03$；图4a]。 更重要的是，COPD患者的CD8 + T −细胞中TC17/IFN- γ细胞的较高频率也与FEV_1%预测值[$r = - - - 0.596$，V29} 02;图4b]。

慢性阻塞性肺疾病中的白介素6，变化生长因子的浓度，转化生长因子β 1和白介素12增加

接下来，我们检查了据信驱动TC17/IFN- γ细胞分化的血浆细胞因子的浓度。 与吸烟者和从未吸烟者[$P < 0.01$，图5A - 5C]相比，COPD患者的血浆中IL - 6，TGF − β 1和IL - 12的浓度明显更高。这些表明，COPD中的TC17可塑性可能是由疾病的炎症环境驱动的。

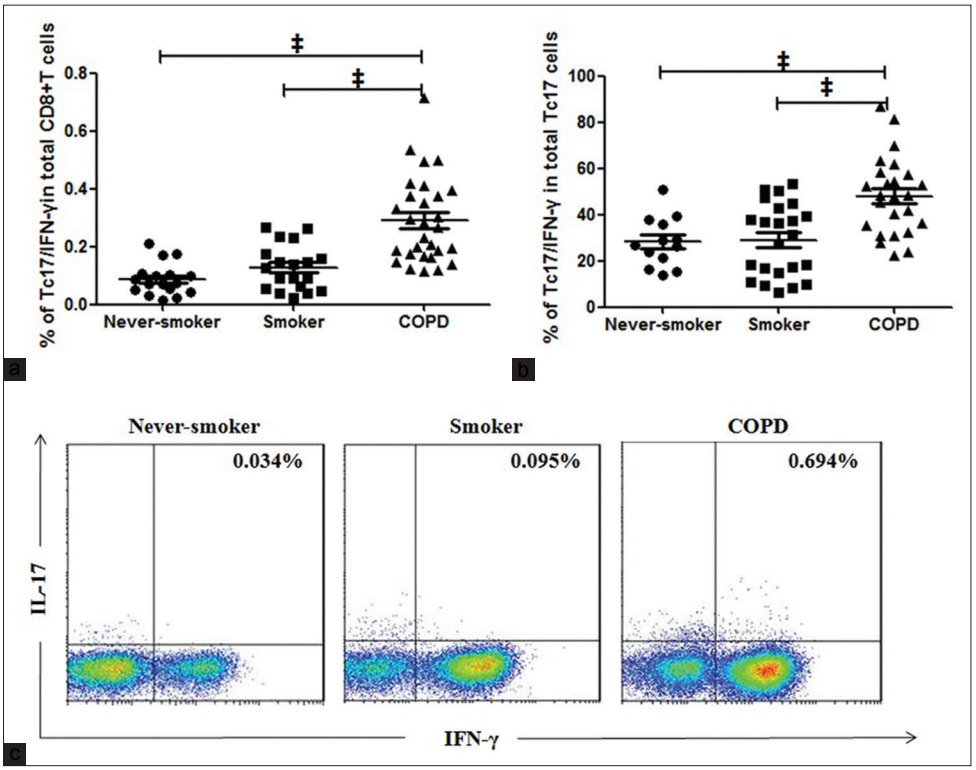

图2：患有慢性阻塞性肺部疾病，吸烟者和从未吸烟的患者的外周血中TC17/干扰素γ细胞亚群的比例。分析CD8 +细胞的生产白介素−17和干扰素− γ细胞在用Phorbol刺激5小时12-羟基苯二醇酯13-乙酸盐/离子霉素和Golgistop后5小时。 （a和b）来自慢性阻塞性肺部疾病，吸烟者和从不熟练的患者的CD8 + T -Cells和TC17细胞中TC17/干扰素γ细胞的百分比。 （c）TC17/干扰素γ细胞的代表性流式细胞仪。 COPD：慢性阻塞性肺部疾病。水平线表示中值值，‡$P < 0.001$。

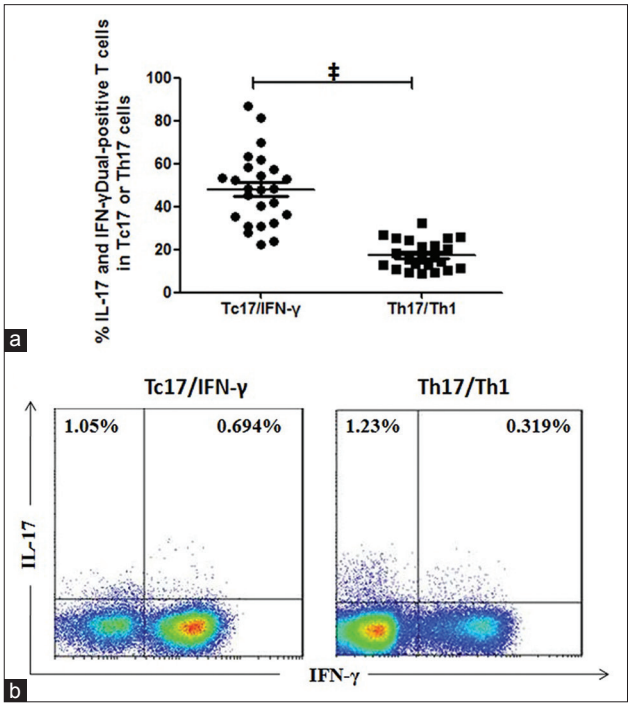

Figure 3: Frequencies of interleukin-17/interferon-γ-double positive subpopulations among Tc17 cells and Th17 cells in peripheral blood from patients with chronic obstructive pulmonary disease. $CD4^+$ cells and $CD8^+$ cells were analyzed for production of interleukin-17 and interferon-γ after 5 h of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

Discussion

$CD8^+$ T-cells have long been recognized as the major pathogenic T-cells in airway inflammation and lung destruction in COPD. In contrast to the plenty of evidence supporting the accumulation and activation of $CD8^+$ T-cells in the lungs of COPD, little is known about the characteristics and functions of circulating $CD8^+$ T-cells in COPD, and the limited findings are often controversial. Domagała-Kulawik *et al*. reported that frequencies of circulating $CD8^+$ T-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-cells are likely to be implicated.

Following the identification of an IL-17-producing subset within $CD8^+$ T-cells, studies have demonstrated that the so-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-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-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-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-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-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-17A positive $CD8^+$ T-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-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-γ cells are capable of acquiring strong cytotoxic function similar to Tc1 cells and expressing pro-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-γ cells might be indispensable for the aggravation of diabetes by direct cytotoxicity on the β-islet cells and expressing pro-inflammatory cytokines apart from IFN-γ in an experimental model of autoimmune diabetes.[24] Tajima *et al*. reported that Tc17/IFN-γ cells were rapidly generated in mesenteric lymph nodes, and IL-17 acted synergistically with IFN-γ to recruit effector $CD8^+$ T-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-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-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

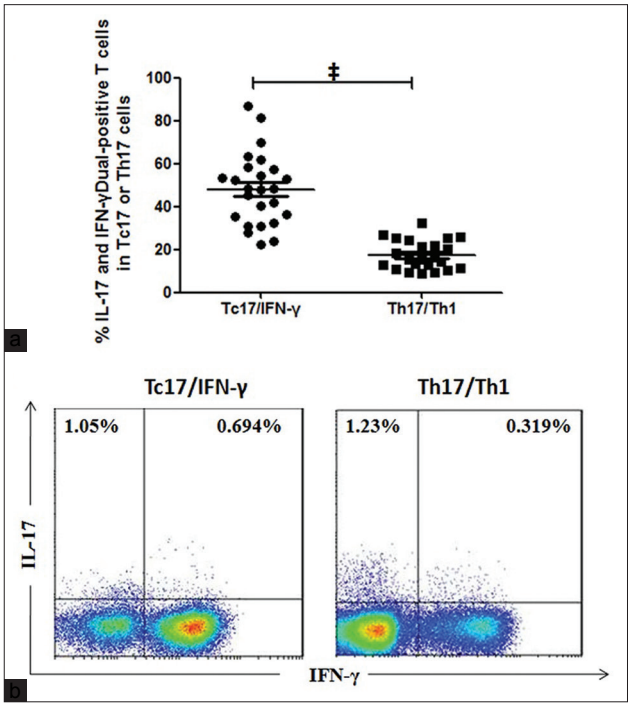

图3：来自慢性阻塞性肺疾病的患者的TC17细胞和Th17细胞中TC17细胞和Th17细胞中TC17细胞和Th17细胞中的白介素−17/Interferon − γ - 双重阳性亚群的频率。用凤凰刺激5小时12-丙酸13-乙酸酯/离子霉素和戈尔吉斯托普刺激5小时后，分析了CD4 +细胞和CD8 +细胞的生产白介素−17和干扰素− γ。（a）分别来自慢性阻塞性肺部疾病的患者的CD4 + T -cells和CD8 + T细胞中Th17/ Th1细胞和TC17/干扰素的百分比。（b）Th17/Th1和Tc17/Interferon − γ细胞的代表性流式细胞仪。水平线表示中值值，‡$P < 0.001$。

讨论

长期以来，CD8 + T细胞被认为是COPD中气道炎症和肺部破坏的主要致病性T细胞。与支持COPD肺中CD8 + T细胞的积累和激活的大量证据相反，关于COPD中循环CD8 + T核的特征和功能知之甚少，有限发现通常是有争议的。 domaga a -kulawik *et al*。报道说，COPD的循环CD8 + T细胞的频率增加，而其他人则发现COPD患者与健康受试者相比没有差异。[30-32]，尽管这些差异可能是由于疾病的异质性质引起的，但患者人群的异质性和不同的可塑性和现象型cd8均可能是cd8}的可能性。

Following the identification of an IL-17-producing subset within CD8+ T-cells, studies have demonstrated that the so-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-γ cells, in response to炎症信号。[20,22,26,33]作为COPD是一种肺部疾病，具有严重的全身性炎症，很可能很可能

循环CD8 + T细胞和/或它们的亚群与这些致病过程有关。 因此，我们假设COPD中的TC17细胞可能显示出很高的可塑性，并且更多地与TC17/IFN- γ细胞区分开，该细胞通常与TC17细胞一般不同，它具有剧毒，类似于TC1细胞。 我们在这里证明了TC17细胞的比例以及更重要的是，多功能亚群TC17/IFN- γ细胞在COPD患者的外围血液中显着增加，并且两者都与FEV_1负面相关，这是疾病严重疾病的单位。

与吸烟者和从未吸烟者相比，我们对COPD患者外周血中TC1细胞比例较高的发现与以前的报告一致。[31,34]，但是我们发现，COPD患者中周围血液中TC17细胞的百分比增加与PAATS *et al*的研究不同于研究的研究。在周围血液中可以忽略不计，COPD患者和健康对照组之间没有差异。[31]这种差异可能是由于疾病严重程度的差异（我们的患者的平均FEV_1）和患者的性别较高（我们的患者都是男性）。此外，我们表明循环TC17细胞的频率升高与COPD的严重程度显着相关，这表明这些细胞可能参与COPD的发病机理。这是由动物研究支持的，表明即使在戒烟后，香烟烟雾暴露的小鼠肺的TC17细胞数量也显着增加，并且与肺部肿瘤病变相关。[31,35,36]}

如前所述，与TC1细胞相比，TC17细胞的细胞毒性要小得多，然后通过哪种机制在COPD中可能具有致病性？作为TC17细胞的新型子集，TC17/IFN- γ细胞能够获得与TC1细胞相似的强细胞毒性功能，并表达与TH17/TH1相似的促炎细胞因子，因此可以认为可以增强TC17细胞的病原体和exac17 Cellice的病态能力。疾病。[20,22,24-26,37-40] saxena *et al*。 揭示TC17/ IFN- γ细胞在β - ISLET细胞上通过直接细胞毒性加剧糖尿病可能是必不可少的，并且在自动透射模型的IFN- γ中表达了促炎性细胞因子。 *et al*。报道说，在肠系膜淋巴结中迅速产生TC17/IFN- γ细胞，IL - 17与IFN- γ协同作用，以招募效应的CD8 + t -cells t -cells和其他炎症细胞的结合范围的Interiation Insive for Insive for colam Inswort formities formities。 Tc17/IFN-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-γ cells might be involved in persistent inflammation and loss of lung function in COPD. 此外，我们发现TC17细胞表现出比

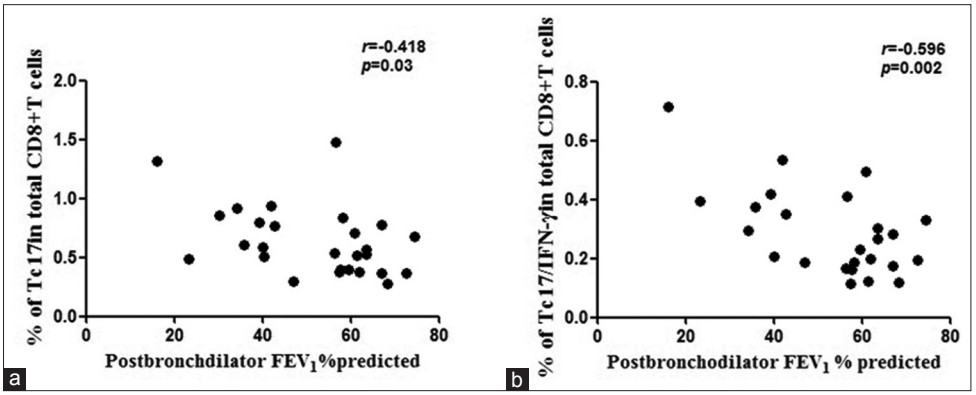

Figure 4: Correlations of Tc17/interferon-γ cells and lung function (n=25). (a) Frequencies of Tc17 cells in CD8$^+$ T-cells and (b) frequencies of Tc17/interferon-γ cells in CD8$^+$ T-cells correlated with forced expiratory volume in 1 s (FEV1)% predicted values in patients with chronic obstructive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

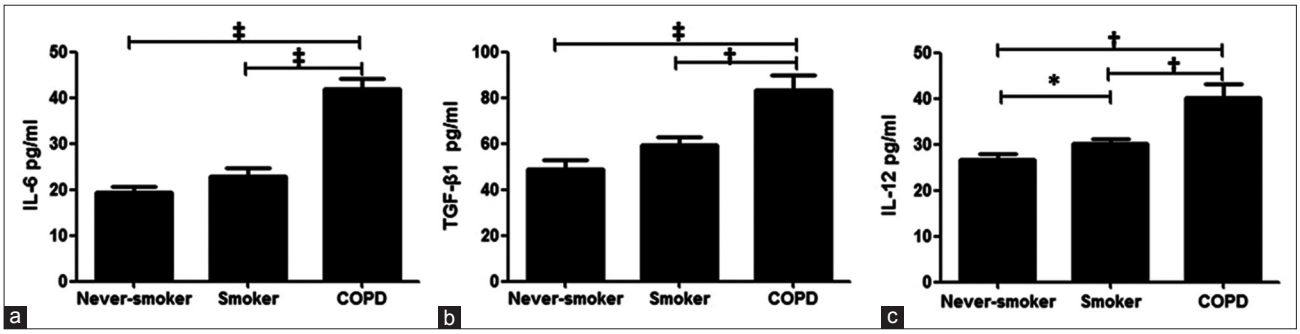

Figure 5: The concentrations of plasma cytokines. Interleukin-6 (a), transforming growth factor-β1 (b), and interleukin-12 (c) from patients with chronic obstructive pulmonary disease, smokers, and never-smokers. COPD: Chronic obstructive pulmonary disease. Horizontal lines indicate median values, *P < 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-6 and TGF-β1, human naïve CD8$^+$ T-cells acquired the Tc17 phenotype, and IL-12 was able to permit Tc17 cells to acquire the potential to produce IFN-γ, thus differentiating to Tc17/IFN-γ cells.[20,29,33] In the current study, we found increased concentrations of IL-6 and TGF-β1 in the plasma from patients of COPD. More importantly, consistent with other studies, the concentration of IL-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-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-6, TGF-β1, and IL-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-cells in COPD.[31]

In summary, this study provides a comprehensive analysis of circulating CD8$^+$ T-cells and their subpopulations in COPD, with a novel finding that the circulating Tc17/IFN-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-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.

Financial support and sponsorship

This study was supported by grants from the National Natural Science Foundation of China (No. 81170039 and No. 81470239).

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

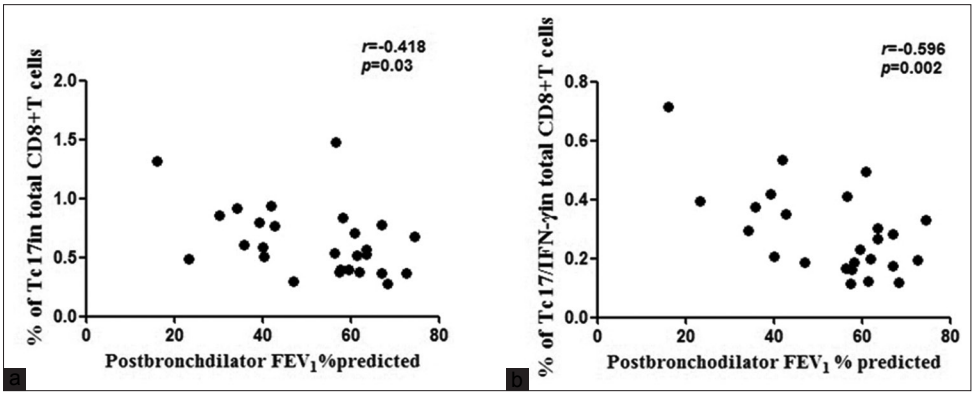

图4：TC17/Interferon－γ细胞和肺功能（*n*= 25）的相关性。（a）CD8⁺T -cells和（b）CD8⁺T -Cells中TC17细胞的频率和（b）TC17/Interferon γ细胞的频率与1 s（FEV1）%预测的Christic bessitive Chormicary Chormonary疾病患者的强制性量相关。*P*值< 0.05被认为具有统计学意义。

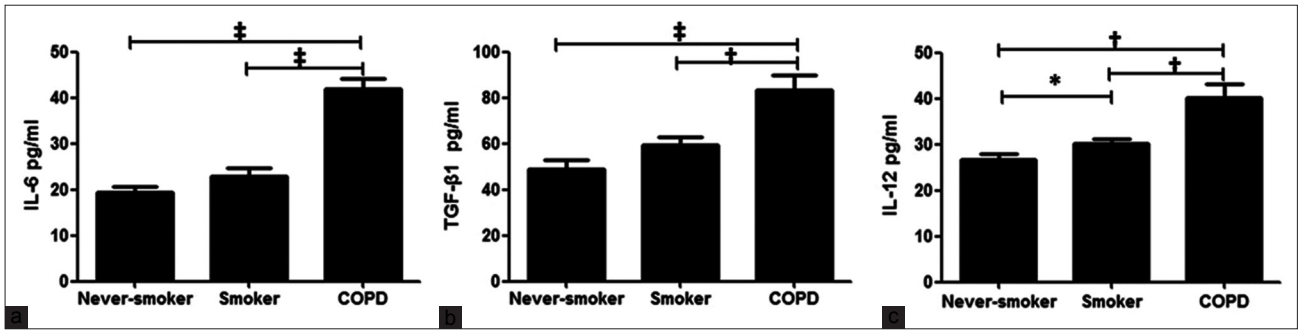

图5：血浆细胞因子的浓度。白介素−6（a），转化生长因子−β1（b）和白介素−12（c），来自慢性阻塞性肺部疾病，吸烟者和永不脱毛的患者。 COPD：慢性阻塞性肺部疾病。水平线表示中位数，*$P < 0.05$，†$P < 0.01$，‡$P < 0.001$。

Th17细胞，尽管TC17可塑性在COPD发病机理中的影响仍然是推测性的。

由于TC17可塑性是由炎症条件驱动的，因此我们认为较高的TC17可塑性与COPD的全身性炎症有关。先前的研究表明，在存在IL－6和TGF-β1的情况下，人类幼稚的CD8⁺T −细胞获得了TC17表型，并且IL - 12能够允许TC17细胞获取潜在的潜力，从而产生IFN-γ，从而对TC17/iifn -ifn -ifn -ifn -ifn -ifn}进行区分。发现COPD患者的血浆中IL－6和TGFβ1的浓度增加。 更重要的是，与其他研究一致的是，与对照组相比，COPD患者的IL - 12的浓度更高。[41,42]这些结果表明，需要进一步的研究来探索这些细胞因子在COPD中诱导TC17可塑性的机制。

我们的研究有几个局限性。首先，我们没有检查肺中TC17和TC17/IFN-γ细胞的频率，例如，在支气管肺泡灌洗中，这可能与COPD的气道疾病有关。因此，需要对此问题进行进一步的调查。其次，我们仅显示COPD中的IL - 6，TGFβ1和IL - 12的血浆水平升高。这些细胞因子是否以及通过什么机制促进TC17可塑性仍然是推测性的。第三，我们的一些患者使用了吸入的皮质类固醇，因此，尽管Paats *et al*}，但不能排除这种药物对我们结果的影响。发现吸入的皮质类固醇对COPD中的外围CD8⁺T -cells没有影响。[31]

总而言之，这项研究对COPD中的循环CD8⁺T型细胞及其亚群进行了全面分析，并有一个新颖的发现，即除TC17/IFN-γ细胞外，除了TC17细胞外，还显着增加了这些细胞，表明这些细胞可能涉及COPT的病例，并且表明这些细胞可能与疾病的严重程度相关。需要进一步的研究来阐明CD8⁺T-细胞异质性和COPD中TC17细胞可塑性的潜在机制，这可能使人们对疾病的局部和全身炎症特征有了新的了解。

财政支持和赞助

这项研究得到了中国国家自然科学基金会（第81170039号和第81470239号）的资助。

利益冲突

没有利益冲突。

参考

1。VestboJ，Hurd SS，AgustíAG，Jones PW，Vogelmeier C，Anzueto A，*et al.*全球诊断，管理和预防策略

of chronic obstructive pulmonary disease: GOLD executive summary. Am J Respir Crit Care Med 2013;187:347-65. doi: 10.1164/rccm.201204-0596PP.
2. Willemse BW, ten Hacken NH, Rutgers B, Lesman-Leegte IG, Postma DS, Timens W. Effect of 1-year smoking cessation on airway inflammation in COPD and asymptomatic smokers. Eur Respir J 2005;26:835-45. 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-smokers observed for 4 years. Chest 2011;139:1380-7. doi: 10.1378/chest.10-0705.
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-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;160:711-7. doi: 10.1164/ajrccm.160.2.9812020.
6. O'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;155:852-7. 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-lymphocytes in peripheral airways of smokers with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 1998;157 (3 Pt 1):822-6. 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-cell subsets in BAL fluid of patients with mild to moderate COPD depends on current smoking status and not airway obstruction. Chest 2014;145:711-22. doi: 10.1378/chest.13-0873.
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;136:2348-57. doi: 0022-1767/86/1367-2348802.00/0.
10. Liang Y, Pan HF, Ye DQ. IL-17A-producing CD8(+) T cells as therapeutic targets in autoimmunity. Expert Opin Ther Targets 2015;19:651-61. doi: 10.1517/1472 8222.20 14.997710.
11. Mosmann TR, Li L, Sad S. Functions of CD8 T-cell subsets secreting different cytokine patterns. Semin Immunol 1997;9:87-92. 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;14:797-824. 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;125:71-6. doi: 10.1378/chest.125.1.71.
14. Huber M, Heink S, Grothe H, Guralnik A, Reinhard K, Elflein K, *et al.* A Th17-like developmental process leads to CD8(+) Tc17 cells with reduced cytotoxic activity. Eur J Immunol 2009;39:1716-25. doi: 10.1002/eji.200939412.
15. Langrish CL, Chen Y, Blumenschein WM, Mattson J, Basham B, Sedgwick JD, *et al*. IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. J Exp Med 2005;201:233-40. doi: 10.1084/jem.20041257.
16. Acosta-Rodriguez EV, Rivino L, Geginat J, Jarrossay D, Gattorno M, Lanzavecchia A, *et al.* Surface phenotype and antigenic specificity of human interleukin 17-producing T helper memory cells. Nat Immunol 2007;8:639-46. 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;33:2093-9. doi: 10.1007/s00296-013-2703-6.
18. Peelen E, Thewissen M, Knippenberg S, Smolders J, Muris AH, Menheere P, *et al.* Fraction of IL-10+and IL-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;258:77-84. 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;192:3111-20. doi: 10.4049/jimmunol.1302313.
20. Tajima M, Wakita D, Satoh T, Kitamura H, Nishimura T. IL-17/IFN-γ double producing CD8+T (Tc17/IFN-γ) cells: A novel cytotoxic T-cell subset converted from Tc17 cells by IL-12. Int Immunol 2011;23:751-9. doi: 10.1093/intimm/dxr086.
21. Ortega C, Fernández AS, Carrillo JM, Romero P, Molina IJ, Moreno JC, *et al.* IL-17-producing CD8+T lymphocytes from psoriasis skin plaques are cytotoxic effector cells that secrete Th17-related cytokines. J Leukoc Biol 2009;86:435-43. doi: 10.1189/JLB.0109046.
22. Hamada H, Garcia-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;182:3469-81. 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-cell subsets in systemic lupus erythematosus. Cell Immunol 2010;264:97-103. 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-mediated autoimmune diabetes in a mouse model. J Immunol 2012;189:3140-9. doi: 10.4049/jimmunol.1103111.
25. Tajima M, Wakita D, Noguchi D, Chamoto K, Yue Z, Fugo K, *et al.* IL-6-dependent spontaneous proliferation is required for the induction of colitogenic IL-17-producing CD8+T cells. J Exp Med 2008;205:1019-27. doi: 10.1084/jem.20071133.
26. Satoh T, Tajima M, Wakita D, Kitamura H, Nishimura T. The development of IL-17/IFN-γ-double producing CTLs from Tc17 cells is driven by epigenetic suppression of Socs3 gene promoter. Eur J Immunol 2012;42:2329-42. 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-17 in patients with chronic obstructive pulmonary disease. Respir Res 2011;12:43. doi: 10.1186/1465-9921-12-43.
28. Eustace A, Smyth LJ, Mitchell L, Williamson K, Plumb J, Singh D. Identification of cells expressing IL-17A and IL-17F in the lungs of patients with COPD. Chest 2011;139:1089-100. doi: 10.1378/chest.10-0779.
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;107:14751-6. doi: 10.1073/pnas.1003852107.
30. Domagala-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;101:1338-43. 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-cell cytokine profiles correlate with GOLD stage in stable COPD. Eur Respir J 2012;40:330-7. 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;145:474-9. doi: 10.1111/j.1365-2249.2006.03167.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;183:7161-8. doi: 10.4049/jimmunol.0900368.
34. Shirai T, Suda T, Inui N, Chida K. Correlation between peripheral blood T-cell profiles and clinical and inflammatory parameters in stable COPD. Allergol Int 2010;59:75-82. doi: 10.2332/allergolint.09-OA-0126.
35. Duan MC, Tang HJ, Zhong XN, Huang Y. Persistence of Th17/Tc17 cell expression upon smoking cessation in mice with cigarette smoke-induced emphysema. Clin Dev Immunol 2013;2013:162-71. 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-induced lung inflammation and emphysema. Respirology 2015;20:426-33. 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;185:2089-98. doi: 10.4049/jimmunol.1000818.

慢性阻塞性肺部疾病：黄金执行摘要。 Am J Respir Crit Care Med 2013; 187：347-65。 doi：10.1164/ rccm.201204-0596pp。 2。WillemseBW，Ten Hacken NH，Rutgers B，Lesman -Leegte IG，Postma DS，Timens W. 1年吸烟戒烟对COPD和无症状吸烟者气道炎症的影响。 Eur Respir J 2005; 26：835-45。 doi：10.1183/09031936.05.00108904。 3。MillerM，Cho JY，Pham A，Friedman PJ，Ramsdell J，Broide DH。 在观察到4年的前蒸发器中，持续的气道炎症和肺气肿进展。胸部2011; 139：1380-7。 doi：10.1378/cast.10-0705。 4。CosioMG，Majo J，Cosio Mg。气道和肺实质在COPD中的炎症：T细胞的作用。 Chest 2002; 121 5补充：160S - 5。 doi：10.1378/cast.121.5_suppl.160s。 5。SaettaM，Baraldo S，Corbino L，Turato G，Braccioni F，Rea F，*et al.* CD8 + VE细胞中慢性阻塞性肺部疾病的吸烟者肺中的VE细胞。 Am J Respir Crit Care Med 1999; 160：711-7。 doi：10.1164/ajrccm.160.2.9812020。 6。O’Shaughnessy TC，Ansari TW，Barnes NC，Jeffery PK。患有慢性支气管炎受试者支气管活检的炎症：CD8 + T淋巴细胞与FEV1的反比关系。 Am J Respir Crit Care Med 1997; 155：852-7。 doi：10.1164/ajrccm.155.3.9117016。 7。SaettaM，Di Stefano A，Turato G，Facchini FM，Corbino L，Mapp CE，*et al.* CD8 + T -lymphocytes在吸烟者的慢性阻塞性肺部疾病的吸烟者的外围气道中。 Am J Respir Crit Care Med 1998; 157（3 pt 1）：822-6。 doi：10.1164/ajrccm.157.3.9709027。 8。ForsslundH，Mikko M，Karimi R，Grunewald J，WheelockÅm，WahlströmJ，*et al.*在轻度至中度COPD患者的BAL流体中T -Cell亚群的分布取决于当前的吸烟状况，而不是气道阻塞。 胸部2014; 145：711-22。 doi：10.1378/cast.13-0873。 9。MosmannTR，Cherwinski H，Bond MW，Giedlin MA，Coffman RL。 两种类型的鼠辅助T细胞克隆。 I.根据淋巴因子活性和分泌蛋白质的特征定义。 J Immunol 2005; 136：2348-57。 doi：0022-1767/86/1367-2348802.00/0。 10。LiangY，Pan HF，Ye DQ。 IL -17A生产CD8（+）T细胞作为自身免疫性的治疗靶标。 Expert Opin Ther Targets 2015; 19：651-61。 doi：10.1517/1472 8222.20 14.997710。 11。MosmannTR，Li L，Sad S. CD8 T细胞子集的功能分泌不同的细胞因子模式。 Semin Immunol 1997; 9：87-92。 doi：10.1006/smim.1997.0065。 12。Gilld，Tan ph。 树突状细胞诱导致病性细胞毒性T淋巴细胞耐受性：一种新型的治疗靶标。 Expert Opin Ther Targets 2010; 14：797-824。 doi：10.1517/14728222.2010.499360。 13。ChrysofakisG，Tzanakis N，Kyriakoy D，Tsoumakidou M，Tsiligiannis I，Klimathianaki M，*et al.* Pultorin CD8 +淋巴细胞的细胞毒素表达和细胞毒性活性。 Chest 2004; 125：71-6。 doi：10.1378/cast.125.1.71。 14。HuberM，Heink S，Grothe H，Guralnik A，Reinhard K，Elflein K，*et al.* A Th17 -th17 -th17 -th17样发育过程会导致CD8（+）TC17细胞，其细胞毒性活性降低。 Eur J Immunol 2009; 39：1716-25。 doi：10.1002/eji.200939412。 15。LangrishCL，Chen Y，Blumenschein WM，Mattson J，Basham B，Sedgwick JD，*et al*。 IL - 23驱动致病性T细胞群，诱导自身免疫性炎症。 J Exp Med 2005; 201：233-40。 doi：10.1084/jem.20041257。 16. Acosta − Rodriguez EV，Rivino L，Geginat J，Jarrossay D，Gattorno M，Lanzavecchia A，*et al.* 表面表型和人白介素17-生产的T Helper Memory细胞的表面表型和抗原特异性。 Nat Immunol 2007; 8：639-46。 doi：10.1038/ni1467。 17。 Rheumatol Int 2013; 33：2093-9。 doi：10.1007/s00296-013-2703前。 18。PeelenE，Thewissen M，Knippenberg S，Smolders J，Muris AH，Menheere P，*et al.* IL - 10 +和IL - 17 + CD8 T细胞的MS患者的缓解和不受免疫模量的影响，MS患者在MS患者中增加。 J Neuroimmunol 2013; 258：77-84。 doi：10.1016/j.jneuroim.2013.02.014。 19。CheukS，WikénM，Blomqvist L，NylénS，Talme T，StåhleM，*et al.*表皮TH22和TC17细胞在临床治愈的牛皮癣中形成局部疾病记忆。 J Immunol 2014; 192：3111-20。 doi：10.4049/jimmunol.1302313。 20。TajimaM，Wakita D，Satoh T，Kitamura H，Nishimura T. IL - 17/IFN- γ双产生CD8 + T（TC17/IFN- γ）细胞：一种新型的Cytototoxic T -Cell TCELL CONVERTED CONTED tTCC17细胞。 INT Immunol 2011; 23：751-9。 doi：10.1093/intimm/dxr086。 21。OrtegaC，FernándezAS，Carrillo JM，Romero P，Molina IJ，Moreno JC，*et al.* IL - 17-产生的CD8 + T lymphocytes来自银屑病皮肤的plaquess plaques plaques plaques plaques是细胞毒性效应细胞，是细胞毒性效应的细胞，这些细胞是分泌th17 -th17 -cyokine cy的细胞。 J Leukoc Biol 2009; 86：435-43。 doi：10.1189/ jlb.0109046。 22。HamadaH，Garcia -Hernandez Mde L，Reome JB，Misra SK，Strutt TM，McKinstry KK，*et al.* TC17，CD8 T细胞的独特子集，可以防止致命的流感挑战。 J Immunol 2009; 182：3469-81。 doi：10.4049/jimmunol.0801814。 23. Henriques A，InêsL，Couto M，Pedreiro S，Santos C，MagalhãesM，*et al.* TH17，TC17和其他T -Cell子集的频率和功能活动。 细胞疫苗2010; 264：97-103。 doi：10.1016/j.cellimm.2010.05.004。 24。SaxenaA，Desbois S，CarriéN，Lawand M，Mars LT，Liblau RS。 TC17 CD8 + T细胞在小鼠模型中增强了Th1介导的自身免疫性糖尿病。 J Immunol 2012; 189：3140-9。 doi：10.4049/ jimmunol.1103111。 25。TajimaM，Wakita D，Noguchi D，Chamoto K，Yue Z，Fugo K，*et al.* IL - 6-依赖性自发增殖需要诱导共构成IL -17-产生的CD8 + T细胞。 J Exp Med 2008; 205：1019-27。 doi：10.1084/jem.20071133。 26。SatohT，Tajima M，Wakita D，Kitamura H，NishimuraT。由TC17细胞产生的IL - 17/IFN- γ - 双倍的CTL的发展是由SOCS3基因启动子的表观抑制作用驱动的。 Eur J Immunol 2012; 42：2329-42。 doi：10.1002/eji.201142240。 27。ChangY，Nadigel J，Boulais N，Bourbeau J，Maltais F，Eidelman DH，*et al.* CD8阳性T细胞在慢性阻塞性肺部疾病的患者中表达IL -17。 Respir Res 2011; 12：43。 doi：10.1186/1465-9921-12-43。 28。EustaceA，Smyth LJ，Mitchell L，Williamson K，Plumb J，SinghD。鉴定COPD患者肺中表达IL − 17A和IL - 17F的细胞。 胸部2011; 139：1089-100。 doi：10.1378/ cast.10-0779。 29。NistalaK，Adams S，Cambrook H，Ursu S，Olivito B，De Jager W，*et al.* TH17人类自身免疫性关节炎中的可塑性是由炎症环境驱动的。 Proc Natl Acad Sci u s a 2010; 107：14751-6。 doi：10.1073/pnas.1003852107。 30。Domagala -Kulawik J，Hoser G，Dabrowska M，ChazanR。COPD患者外周血中Fas阳性CD8 +细胞的比例增加。 Respir Med 2007; 101：1338-43。 doi：10.1016/j。 RMED.2006.10.004。 31。PaatsMS，Bergen IM，Hoogsteden HC，Van der Eerden MM，Hendriks RW。 系统性CD4 +和CD8 + T −细胞因子剖面与稳定COPD中的金阶段相关。 Eur Respir J 2012; 40：330-7。 doi：10.1183/09031936.00079611。 32。BarcelóB，Pons J，Fuster A，Sauleda J，Noguera A，Ferrer JM，*et al.*慢性阻塞性肺病患者的T淋巴细胞的细胞内细胞因子谱。 Clin Exp Immunol 2006; 145：474-9。 doi：10.1111/j.1365-2249.2006.03167.x。 33。Yen HR，Harris TJ，Wada S，Grosso JF，Getnet D，Goldberg MV，*et al.* TC17 CD8 T细胞：功能可塑性和子集多样性。 J Immunol 2009; 183：7161-8。 doi：10.4049/jimmunol.0900368。 34。ShiraiT，Suda T，Inui N，Chida K.稳定COPD中外周血T细胞谱与临床和炎症参数之间的相关性。 Allergol INT 2010; 59：75-82。 doi：10.2332/allergo lint.09 - OA - 0126。 35。DuanMC，Tang HJ，Zhong XN，Huang Y.带有香烟烟雾引起的肺气肿的小鼠吸烟时Th17/ TC17细胞表达的持久性。 Clin Dev Immunol 2013; 2013：162-71。 doi：10.1155/2013/350727。 36. Zhou H，Hua W，Jin Y，Zhang C，Che L，Xia L，*et al.* TC17细胞与香烟烟雾引起的肺部炎症和肺气肿有关。 呼吸学2015; 20：426-33。 doi：10.1111/resp。 12486。37。YehN，Glosson NL，Wang N，Guindon L，McKinley C，Hamada H，*et al.* TC17细胞能够通过审查细胞毒性表型来介导对疫苗病毒的免疫力。 J Immunol 2010; 185：2089-98。 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;13:991-9. doi: 10.1038/ni.2416.
39. Boniface K, Blumenschein WM, Brovont-Porth K, McGeachy MJ, Basham B, Desai B, *et al.* Human Th17 cells comprise heterogeneous subsets including IFN-gamma-producing cells with distinct properties from the Th1 lineage. J Immunol 2010;185:679-87. 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-17. J Immunol 2009;182:1794-8. 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;9:759-73. doi: 10.2147/COPD.S61347.
42. Pinto-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;62:595-601. doi: 10.1136/thx.2006.064428.

38。 Nat Immunol 2012; 13：991-9。 doi：10.1038/ni.2416。 39。BonifaceK，Blumenschein WM，Brovont -Porth K，McGeachy MJ，Basham B，Desai B，*et al.*人Th17细胞包含异构子集，包括具有IFN-伽马生成细胞，具有与Th1谱系不同特性的细胞。 J Immunol 2010; 185：679-87。 doi：10.4049/ jimmunol.1000366。 40。KondoT，Takata H，Matsuki F，Takiguchi M.尖端：人类CD8 + T细胞产生的表型表征和分化

IL -17。 J Immunol 2009; 182：1794-8。 doi：10.4049/jimmunol.0801347。 41。BadeG，Khan MA，Srivastava AK，Khare P，Solaiapean KK，Guleria R，*et al.*血清细胞因子分析和富集分析揭示了稳定患者与慢性阻塞性肺疾病的免疫学和炎症途径的参与。 Int J Chron阻塞肺部2014年； 9：759-73。 doi：10.2147/copd.S61347。 42。Pinto -Plata V，Toso J，Lee K，Park D，Bilello J，Mullerova H，*et al.* COPD患者的血清生物标志物分析：与临床参数的关联。 Thorax 2007; 62：595-601。 doi：10.1136/ thx.2006.064428。