# Imbalance between subsets of CD8$^+$ peripheral blood T cells in patients with chronic obstructive pulmonary disease

**Long Chen** [1], **Gang Chen** [1], **Ming-Qiang Zhang** [1], **Xian-Zhi Xiong** [Corresp., 1], **Hong-Ju Liu** [1], **Jian-Bao Xin** [1], **Jian-Chu Zhang** [1], **Jiang-Hua Wu** [1], **Zhao-Ji Meng** [1], **Sheng-Wen Sun** [1]

[1] Department of Respiratory and Critical Care Medicine, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, China

Corresponding Author: Xian-Zhi Xiong
Email address: xxz0508@hust.edu.cn

**Background.** CD8$^+$ T lymphocytes are known to play a critical role in the pathogenesis of chronic obstructive pulmonary disease (COPD). However, systematic analyses of CD8$^+$ T cell ( Cytotoxic T cells, Tc) subsets in COPD patients have yet to be well conducted.

**Methods.** The whole Tc subsets, including Tc1/2/10/17, CD8$^+$ regulatory T cells (Tregs) and CD8$^+$α7$^+$ T cells, were quantified by flow cytometry in peripheral blood from 24 stable COPD subjects (SCOPD), 14 patients during acute exacerbations (AECOPD) , and 14 healthy nonsmokers (HN).

**Results.** Acute exacerbations of COPD were accompanied by elevated levels of circulating CD8$^+$ T cells. Tc1 cells were increased in both SCOPD and AECOPD patients, whereas the percentage of Tc2 cells was decreased in SCOPD patients but remained normal in AECOPD patients. Tc17 cells were increased only in AECOPD patients, and the percentage of Tc10 cells was reduced in both SCOPD and AECOPD patients. The imbalances of pro/anti-inflammatory Tc subsets observed in COPD may be caused by the lack of Tc10 cells and the impaired anti-inflammatory capacity of CD8$^+$ Tregs.

**Discussion.** The imbalances between subsets of CD8$^+$ peripheral blood T cells contribute to the immune response dysfunction in COPD pathogenesis.

# Imbalance between subsets of CD8$^+$ peripheral blood T cells in patients with chronic obstructive pulmonary disease

Long Chen[1,2], Gang Chen[1,2], Ming-Qiang Zhang[1], Xian-Zhi Xiong[1], Hong-Ju Liu[1], Jian-Bao Xin[1], Jian-Chu Zhang[1], Jiang-Hua Wu[1], Zhao-Ji Meng[1] and Sheng-Wen Sun[1]

[1] Department of Respiratory Medicine, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, 1277 JieFang Avenue, Wuhan 430022, China.

[2] Long Chen and Gang Chen contributed equally to this work.

Corresponding author:

Xian-Zhi Xiong[1]

1277 Jiefang Avenue, Wuhan, Hubei, 430022, China.

E-mail address: xxz0508@hust.edu.cn

## Abstract

**Background.** $CD8^+$ T lymphocytes are known to play a critical role in the pathogenesis of chronic obstructive pulmonary disease (COPD). However, systematic analyses of $CD8^+$ T cell (Cytotoxic T cells, Tc) subsets in COPD patients have yet to be well conducted.

**Methods.** The whole Tc subsets, including Tc1/2/10/17, $CD8^+$ regulatory T cells (Tregs) and $CD8^+\alpha7^+$ T cells, were quantified by flow cytometry in peripheral blood from 24 stable COPD subjects (SCOPD), 14 patients during acute exacerbations (AECOPD), and 14 healthy nonsmokers (HN).

**Results.** Acute exacerbations of COPD were accompanied by elevated levels of circulating $CD8^+$ T cells. Tc1 cells were increased in both SCOPD and AECOPD patients, whereas the percentage of Tc2 cells was decreased in SCOPD patients but remained normal in AECOPD patients. Tc17 cells were increased only in AECOPD patients, and the percentage of Tc10 cells was reduced in both SCOPD and AECOPD patients. The imbalances of pro/anti-inflammatory Tc subsets observed in COPD may be caused by the lack of Tc10 cells and the impaired anti-inflammatory capacity of $CD8^+$ Tregs.

**Discussion.** The imbalances between subsets of $CD8^+$ peripheral blood T cells contribute to the immune response dysfunction in COPD pathogenesis.

## Introduction

Chronic obstructive pulmonary disease (COPD) is characterized by poorly reversible airflow limitation and progressive airway inflammation. Currently, no cure or effective treatment is available to stop the progression of COPD. Airway inflammation is triggered by the inhalation of a variety of hazardous gases and particles, with tobacco smoke serving as the leading contributing factor (Hogg et al. 2004; Lakshmi et al. 2014; Mortaz et al. 2008).

Increasing evidence indicates that T cell-mediated inflammation, as a key component, is involved in the pathogenesis of COPD (Saetta et al. 1993). Previous studies have shown that the numbers of $CD8^+$ T cells (Cytotoxic T cells, Tc) in the lung parenchyma and small airways are inversely correlated with pulmonary function (O'Shaughnessy et al. 1997; Saetta et al. 1999; Saetta et al. 1998), and depleting these cells may protect against alveolar destruction due to chronic cigarette smoke exposure (Podolin et al. 2013); together, these findings suggest that $CD8^+$ T lymphocytes may play a critical role in COPD pathogenesis. Several studies have focused on $CD8^+$ T cells in the blood of COPD patients (Freeman et al. 2015; Makris et al. 2008; Mathai & Bhat 2013); however, a comprehensive study on circulating $CD8^+$ T cell subsets in patients with this disease has yet to be conducted. Although some in vitro research has indicated that soluble components extracted from cigarette smoke can significantly reduce T cell activation and proliferation (Glader et al. 2006; Lambert et al. 2005), substantial evidence has shown that large numbers of $CD8^+$ T cells are present in the airways and parenchyma of smokers with COPD (Saetta et al. 1999). Thus, the precise influence of cigarette smoke on $CD8^+$ T cells remains unclear.

Although pro-inflammatory $CD8^+$ T cell pool (mainly including $CD8^+$ IFN-$\gamma^+$ Tc1, $CD8^+$ IL-$4^+$ Tc2, and $CD8^+$ IL-$17^+$ Tc17) and anti-inflammatory $CD8^+$ T cell pool (mainly including $CD8^+$ $Foxp3^+$ Treg, and $CD8^+$ IL-$10^+$ Tc10) have been considered to be involved in COPD pathogenesis, existing studies regarding this association vary widely in their designs, population sizes and experimental methods. Meanwhile, the α7 nicotinic acetylcholine receptors (α7 nAChRs) are recently discovered to be required for inhibition of TNF-α (Wang et al. 2003). However, the expression and function of α7 nAChRs in $CD8^+$ T cell, especially in the development of COPD is yet to be illuminated.

In the present study, we attempted to investigate systemic $CD8^+$ T cell subsets, including Tc1/2/10/17, $CD8^+$ regulatory T cells (Tregs) and $CD8^+\alpha7^+$ T cells, from healthy nonsmokers

and patients with either stable COPD (SCOPD) or during acute exacerbations (AECOPD). Especially, the data shown here are derived from the same sample set published earlier dealing with an in-depth analysis of the $CD4^+$ T cell compartment in COPD patients (Zhang et al. 2014). To the best of our knowledge, this is the first report showing the complete subsets of circulating $CD8^+$ T cells in the pathogenesis of COPD.

## Materials and methods

### Subjects

This study was approved by the Ethics Committee of Tongji Medical School, Huazhong University of Science and Technology (# 2013/S048), and was conducted in accordance with the Declaration of Helsinki. Informed written consent was obtained from all subjects. The demographic characteristics of subjects clinical characteristics have been presented in detail previously (Zhang et al. 2014). In brief, according to the diagnostic criteria from the Global Initiative for Chronic Obstructive Lung Disease (GOLD) (Rabe et al. 2007), we collected 24 SCOPD (mean age, 66.5 ± 7.2 years; smoking history, 41.9 ± 17.6 pack-years) and 14 AECOPD patients (mean age, 69.1 ± 9.6 years; smoking history, 47.2 ± 19.5 pack-years). Meanwhile, 14 healthy nonsmokers (HN; mean age, 65.6 ± 7.8 years) without any lung or systemic disease were also recruited. Patients with SCOPD were free of exacerbation for at least 4 weeks at the time of blood draw. AECOPD patients were identified by the initiation of symptoms diagnostic for COPD exacerbation in the past 3 days without any new therapeutic intervention. Exclusion criteria for the study included the following characteristics: other respiratory diseases apart from COPD, systemic autoimmune diseases and malignant tumors.

### Sample collection

Peripheral blood samples were collected in heparin-treated tubes from each subject within 24 h after arrival at the hospital. Blood samples were immediately placed on ice and then centrifuged at 1,200 g for 5 min. Then peripheral blood mononuclear cells (PBMCs) were isolated from the heparinized blood by Ficoll-Hypaque gradient centrifugation (Pharmacia, Uppsala, Sweden) as previously described (Zhang et al. 2014). Isolated peripheral blood cells were used for subsequent experiments.

### Flow cytometric analysis

The expression of surface and intracellular markers by T cells was assessed using flow cytometry as previously described (Zhang et al. 2014). Cell staining was performed using the following anti-human-specific antibodies (Abs): anti-CD3 PerCP-Cy5.5 (clone OKT3; eBioscience, San Diego, CA, USA), anti-CD8 FITC (Clone RPA-T8; BD Biosciences, San Jose, CA, USA), anti-Foxp3 PE (Clone 236A/E7; eBioscience), anti–IFN-γ PE (Clone 4S.B3; eBioscience), anti-IL-4 PE-Cy7 (Clone 8D4-8; eBioscience), anti-IL-17A eFluor660 (Clone eBio64DEC17; eBioscience), and anti-IL-10 Alexa Fluor647 (Clone JES3-9D7; eBioscience). Meanwhile, the expression of α7 AChRs on T cells was detected by its binding to α-bungarotoxin labeled with Alexa Fluor647 (Invitrogen, Carlsbad, CA, USA). Intracellular cytokines staining was performed after the T cells were stimulated with PMA (50 ng/ml; Sigma-Aldrich, St. Louis, MO, USA) and ionomycin (1 μM; Sigma-Aldrich) in the presence of GolgiStop (BD Biosciences) for 5 h. Appropriate species matched Abs were used as isotype controls. Lymphocyte population was discriminated in the light-scattering measurement (FSC/SSC dot plot) by surrounding them with a polygon gate to exclude debris and dead/adhesive cells. Flow cytometry was performed on a FACS Canto II (BD Biosciences) and analyzed using BD FCSDiva Software and FCS Express 4 software (De Novo Software, Los Angeles, CA, USA).

**Statistical analysis**

Data are expressed as the mean ± SD (unless indicated in the figure legends). Comparisons of data between different groups were performed using a Kruskal-Wallis one-way analysis of variance (ANOVA) based on rank, with Tukey and Dunn's post-hoc tests for between-group comparisons. Data analysis was performed using GraphPad Prism v.5.01 software (GraphPad Software, La Jolla, CA, USA), and two-tailed P values of less than 0.05 were considered statistically significant.

## Results

**Acute exacerbations of COPD are accompanied by elevated levels of circulating $CD8^+$ T cells**

We first determined the percentages of $CD8^+$ T cells in blood samples obtained from healthy nonsmokers and SCOPD and AECOPD patients using flow cytometry (Fig. 1A, 1B). We found that AECOPD patients (39.51±8.55%) showed the highest percentage of $CD8^+$ T cells in the

peripheral blood compared with the other two groups, with both comparisons reaching statistical significance. However, the percentage of $CD8^+$ T cells in SCOPD patients (30.36±10.25%) was similar to that in healthy nonsmokers (29.99±8.49%).

**Imbalance of peripheral Tc1, Tc2 and Tc17 cells in COPD**

To investigate changes in $CD8^+$ T cell subsets in COPD patients, we analyzed the following subsets of T cells in the blood: $CD3^+CD8^+IFN\text{-}\gamma^+$ (Tc1), $CD3^+CD8^+IL\text{-}4^+$ (Tc2), $CD3^+CD8^+IL\text{-}17A^+$ (Tc17), $CD3^+CD8^+Foxp3^+$ ($CD8^+$ Tregs), $CD3^+CD8^+IL\text{-}10^+$ (Tc10) and $CD3^+CD8^+\alpha7^+$ ($CD8^+\alpha7^+$) (Figs. 2 and 3).

As shown in Fig. 2A-B, Tc1 cells were markedly elevated in both the SCOPD (37.10±21.26%) and AECOPD (53.11±18.03%) patients compared with the healthy nonsmokers (17.56±13.90%). Furthermore, the frequency of Tc1 cells was higher in AECOPD patients than in SCOPD patients. Similar to Tc1 cells, the percentage of Tc17 cells was highest in AECOPD patients (1.13±0.83%); however, the levels of Tc17 cells were similar between healthy nonsmokers and SCOPD patients (0.33±0.12% and 0.38±0.38%, respectively). Additionally, the percentage of Tc2 cells was significantly reduced in SCOPD patients (0.76±0.69%) compared with healthy nonsmokers and AECOPD patients (2.12±1.22% and 2.68±1.96%, respectively).

**Decreased Tc10 percentage in both SCOPD and AECOPD patients**

The levels of $CD8^+$ Tregs appeared to be increased in SCOPD and AECOPD patients (2.05±1.93% and 2.56±2.17%, respectively) compared with healthy nonsmokers (0.76±0.48%), although the difference was not statistically significant. Meanwhile, the percentages of $CD8^+\alpha7^+$ T cells in SCOPD and AECOPD patients (0.85±0.83% and 0.59±0.38%, respectively) showed a trend toward up-regulation when compared with healthy controls (1.03±0.91%), but this was not statistically significant.

Unsurprisingly, as can be seen in fig. 3, the frequencies of Tc10 cells in SCOPD and AECOPD patients (0.45±0.54% and 0.42±0.21%, respectively) were significantly reduced to half the level observed in healthy nonsmokers (1.06±0.34%).

**Comprehensive analysis of the relative percentages of $CD8^+$ T cell subsets**

To precisely clarify which changes occur in different $CD8^+$ T cell subsets in COPD, we performed a comprehensive analysis of the relative proportion from mean value of each subset.

However, since the immunoregulatory function and key transcription factors of $CD8^+\alpha7^+$ T cells have not been elucidated, we could not firmly establishing $CD8^+\alpha7^+$ population as an independent cytotoxic T cell lineage in human. Moreover, $CD8^+\alpha7^+$ T cells could even overlap with other subsets, so this population is disregarded in the summary fig. 4.

As shown in Fig. 4, Tc1 cells accounted for the majority of pro-inflammatory $CD8^+$ T cells in both SCOPD and AECOPD patients (91% and 89%, respectively), and both of these values were higher than that observed in healthy nonsmokers (80%). Tc2 cells were radically reduced in SCOPD (2%) patients while only modestly reduced in AECOPD (4%) patients compared with healthy nonsmokers (10%). Notably, Tc17 cells were not increased in SCOPD or AECOPD patients (1% and 2%, respectively) compared with the healthy nonsmokers (2%). We also observed that the percentage of anti-inflammatory $CD8^+$ T cells ($CD8^+$ Tregs and Tc10 cells) was highest in healthy nonsmokers (8%) but was reduced in SCOPD (6%) and progressively decreased in AECOPD patients (5%). Although $CD8^+$ Tregs were increased modestly in SCOPD (5%) and AECOPD (4%) patients compared with healthy nonsmokers (3%), Tc10 cells were remarkably decreased in both SCOPD (1%) and AECOPD (1%) patients compared with healthy nonsmokers (5%).

Together, this comprehensive analysis showed that, compared with healthy nonsmokers, relatively more Tc1 cells and $CD8^+$ Tregs, but less Tc2 and Tc10 cells, were found in both the SCOPD and AECOPD patients and that the overall population of anti-inflammatory $CD8^+$ T cells was reduced in both COPD groups.

## Discussion

Previous studies have mainly focused on the sizes of the $CD8^+$ T cell pools and partial Tc subsets in the development of COPD (Chang et al. 2011; Freeman et al. 2015; Makris et al. 2008; Saetta et al. 1999). However, the collaborative relationship between overall Tc subsets is not well understood. To the best of our knowledge, this is the first study showing the complete subsets of circulating pro/anti-inflammatory $CD8^+$ T cells in the pathogenesis of COPD. Combined with our previous findings of an imbalance of pro/anti-inflammatory $CD4^+$ T pools in COPD patients, which revealed increased Th17 cells (only in AECOPD) and Th1 cells and reduced Th2 cells (only in SCOPD) and Th10 cells, as well as an impaired capacity of Tregs, our current research of $CD8^+$ compartment would contribute to the overall interpretation of the true role of T cell

pools in COPD.

COPD is considered to be a multi-component disease with systemic manifestations in addition to local pulmonary inflammation. This inflammation contributes to repeated injury and repair cycles, finally resulting in the remodeling of tissue with altered structure and function (Barnes et al. 2003). In conflict with published findings (Freeman et al. 2015), the percentage of peripheral $CD8^+$ T cells was elevated in AECOPD but not in SCOPD patients. Combined with previous findings that the percentage of sputum $CD8^+$ T lymphocytes was significantly increased at the onset of exacerbations (Makris et al. 2008), we speculate that the increased frequency of $CD8^+$ T cells is limited to the lungs, whereas the expanded $CD8^+$ T lymphocyte population spreads throughout the body during an acute exacerbation.

Tc1 cells were also significantly elevated in the peripheral blood of both COPD groups, which was consistent with a previous report (Zhu et al. 2009). Interestingly, the percentage of circulating Tc2 cells was significantly decreased in SCOPD patients but returned to normal during an acute exacerbation. One prior study revealed that decreased Tc1/Tc2 cell ratios were found in the sputum at the onset of an exacerbation compared with the stable state (Makris et al. 2008). Another study however reported a greater Tc2 response in the bronchoalveolar lavage fluid of COPD patients (Barcelo et al. 2006). Different sample sources and experimental conditions could possibly account for this discrepancy. Tc17 cells have recently been identified as pro-inflammatory lymphocytes and are implicated in the pathogenesis of some human autoimmune diseases such as psoriasis (Res et al. 2010) and systemic lupus erythematosus (Henriques et al. 2010). Tc17 cells are associated with cigarette smoke-induced lung inflammation and COPD (Chang et al. 2011; Zhou et al. 2015). Additionally, our results showed that the Tc17 cell population in the peripheral blood remarkably increased only in AECOPD patients; our findings indicate that Tc17 cells may participate in the acute exacerbation of COPD. From the perspective of the immune system, COPD mainly involves a Tc1 reaction; Tc2 and Tc17 reactions are attenuated in stable COPD. The above observations indicate that in the inhibitory state of the acute inflammatory reaction in COPD patients during the stable period, Tc2 and Tc17 cell levels remain unchanged unless the patient suffers from an acute irritation such as an infection.

We also assessed the anti-inflammatory $CD8^+$ T cells, including $CD8^+Foxp3^+$ Tregs and Tc10 in both COPD groups. $\alpha$7 nAChR activation was recently found to be capable of

suppressing inflammation by inhibiting specific cytokines such as TNF-α (Wang et al. 2003), thereby increasing the suppressive capacity of Tregs (Wang et al. 2010). Unfortunately, no significant changes were found in the levels of $CD8^+\alpha7^+$ T cells and $CD8^+$ Tregs in SCOPD or AECOPD patients. However, Tc10 cells were reduced in both SCOPD and AECOPD patients, revealing a lack of this anti-inflammatory cell throughout the disease course. Consistently, the concentration of IL-10 was reported to be reduced in the sputum of COPD patients (Takanashi et al. 1999), and less IL-10 was released from lung tissue of COPD patients after LPS stimulation compared with that of healthy smokers (Hackett et al. 2008).

Further comprehensive analyses were performed by calculating the relative proportions of each of the $CD8^+$ T cell subsets in the three groups. In both SCOPD and AECOPD patients, the proportions of pro-inflammatory Tc1 cells were robustly increased, whereas those of Tc2 cells were relatively reduced. Local recruitment of an overwhelming number of Tc1 cells to the lungs and airway mucosa may lead to persistent chronic inflammation; meanwhile, polarization from Tc2 toward Tc1 could promote inflammation through the secretion of IFN-γ (Mosmann et al. 1997). Considering the modestly increased quantity of $CD8^+$ Tregs in SCOPD and AECOPD patients, together with the IL-10-producing capacity of $CD8^+$ Tregs (Dinesh et al. 2010; Suzuki et al. 2008), we speculate that an increased percentage of $CD8^+$ Tregs may lose their suppressive ability and may be, to some extent, compensating for the significantly low proportion of Tc10 cells in this environment (Profita et al. 2009; Suzuki et al. 2008). Additionally, given that human $CD4^+$ Tregs are heterogeneous and that an imbalance exists between $CD4^+$ Treg subsets in COPD (Hou et al. 2013), we also presume that $CD8^+$ Tregs have similar function and characteristic in COPD patients. In conclusion, these data suggest an insufficiency of anti-inflammatory functions of $CD8^+$ Tregs or Tc10 cells in the context of COPD. However, our analysis only included Tregs and Tc10 cells as anti-inflammatory cells, while other IL-10-producing $CD8^+$ T cell subsets also exist (Suzuki et al. 2008). Further studies are required to elucidate the potential autoimmune component of COPD, to determine whether the chronic inflammatory response mainly involves $CD8^+$ T cells and to assess whether this response is similar to delayed-type hypersensitivity such as sarcoidosis.

Some limitations of our study should be acknowledged. Our present study recruits more men volunteers than women donors, which is in line with higher global prevalence of COPD in men than in women. However, recent research has indicated an increased female susceptibility to

smoking-related lung damage, although the findings are controversial (Sorheim et al. 2010). The fact that only a few women volunteers are included limits our ability to assess the gender difference in susceptibility in COPD. Moreover, since aging (Messaoudi et al. 2004) and GOLD stages may be associated with gradual shifts in $CD8^+$ T cell repertoire, larger sample size is required to ascertain the correlation between $CD8^+$ T cells subsets and the age or certain stage of COPD. Lastly, selection bias of participants cannot be excluded, since different study samples could potentially have affected the results.

## Conclusions

Our present study indicates the existence of an imbalance of Tc1/Tc2/Tc17 cells in COPD patients, suggesting that COPD is a chronic inflammatory disease characterized predominantly by a Tc1 outburst. Additionally, the imbalance of pro/anti-inflammatory $CD8^+$ T cell subsets observed in COPD patients may be caused by the lack of Tc10 cells and the impaired anti-inflammatory capacity of $CD8^+$ Tregs. However, this report is limited to data from blood samples and lacks information from lung tissue or bronchoalveolar lavage fluids, and much effort is needed to investigate the net changes in both local and systemic.

## Acknowledgments

The authors thank Liang Shi for the excellent flow cytometric assistance; Zhi-Jian Ye, Ming-Li Yuan and Wen Yin for their helpful suggestions and discussion; Xia Yang and Dan Yang for their assistance in patient recruitment; and Xiao-Nan Tao for the administrative support.

## References

**Barcelo B, Pons J, Fuster A, Sauleda J, Noguera A, Ferrer JM, and Agusti AG. 2006.** Intracellular cytokine profile of T lymphocytes in patients with chronic obstructive pulmonary disease. *Clinical and Experimental Immunology* 145:474-479.

**Barnes PJ, Shapiro SD, and Pauwels RA. 2003.** Chronic obstructive pulmonary disease: molecular and cellular mechanisms. *European Respiratory Journal* 22:672-688.

**Chang Y, Nadigel J, Boulais N, Bourbeau J, Maltais F, Eidelman DH, and Hamid Q. 2011.** CD8 positive T cells express IL-17 in patients with chronic obstructive pulmonary disease. *Respiratory Research* 12:43.

**Dinesh RK, Skaggs BJ, La Cava A, Hahn BH, and Singh RP. 2010.** CD8+ Tregs in lupus, autoimmunity, and beyond. *Autoimmun Rev* 9:560-568.

**Freeman CM, Martinez CH, Todt JC, Martinez FJ, Han MK, Thompson DL, McCloskey L, and Curtis JL. 2015.** Acute exacerbations of chronic obstructive pulmonary disease are associated with decreased CD4+ & CD8+ T cells and increased growth & differentiation factor-15 (GDF-15) in peripheral blood. *Respiratory Research* 16:94.

**Glader P, Moller S, Lilja J, Wieslander E, Lofdahl CG, and von Wachenfeldt K. 2006.** Cigarette smoke extract modulates respiratory defence mechanisms through effects on T-cells and airway epithelial cells. *Respiratory Medicine* 100:818-827.

**Hackett TL, Holloway R, Holgate ST, and Warner JA. 2008.** Dynamics of pro-inflammatory and anti-inflammatory cytokine release during acute inflammation in chronic obstructive pulmonary disease: an ex vivo study. *Respiratory Research* 9:47.

**Henriques A, Ines L, Couto M, Pedreiro S, Santos C, Magalhaes M, Santos P, Velada I, Almeida A, Carvalheiro T, Laranjeira P, Morgado JM, Pais ML, Da SJ, and Paiva A. 2010.** Frequency and functional activity of Th17, Tc17 and other T-cell subsets in Systemic Lupus Erythematosus. *Cellular Immunology* 264:97-103.

**Hogg JC, Chu F, Utokaparch S, Woods R, Elliott WM, Buzatu L, Cherniack RM, Rogers RM, Sciurba FC, Coxson HO, and Pare PD. 2004.** The nature of small-airway obstruction in chronic obstructive pulmonary disease. *New England Journal of Medicine* 350:2645-2653.

**Hou J, Sun Y, Hao Y, Zhuo J, Liu X, Bai P, Han J, Zheng X, and Zeng H. 2013.** Imbalance between subpopulations of regulatory T cells in COPD. *Thorax* 68:1131-1139.

**Lakshmi SP, Reddy AT, Zhang Y, Sciurba FC, Mallampalli RK, Duncan SR, and Reddy RC. 2014.** Down-regulated peroxisome proliferator-activated receptor gamma (PPARgamma) in lung epithelial cells promotes a PPARgamma agonist-reversible proinflammatory phenotype in chronic obstructive pulmonary disease (COPD). *Journal of Biological Chemistry* 289:6383-6393.

**Lambert C, McCue J, Portas M, Ouyang Y, Li J, Rosano TG, Lazis A, and Freed BM. 2005.** Acrolein in cigarette smoke inhibits T-cell responses. *Journal of Allergy and Clinical Immunology* 116:916-922.

**Makris D, Lazarou S, Alexandrakis M, Kourelis TV, Tzanakis N, Kyriakou D, and Gourgoulianis KI. 2008.** Tc2 response at the onset of COPD exacerbations. *Chest* 134:483-488.

**Mathai RT, and Bhat S. 2013.** Peripheral Blood T-Cell Populations in COPD, Asymptomatic Smokers and Healthy Non-Smokers in Indian subpopulation- A Pilot Study. *J Clin Diagn Res* 7:1109-1113.

**Messaoudi I, Lemaoult J, Guevara-Patino JA, Metzner BM, and Nikolich-Zugich J. 2004.** Age-related CD8 T cell clonal expansions constrict CD8 T cell repertoire and have the potential to impair immune defense. *Journal of Experimental Medicine* 200:1347-1358.

**Mortaz E, Redegeld FA, Sarir H, Karimi K, Raats D, Nijkamp FP, and Folkerts G. 2008.** Cigarette smoke stimulates the production of chemokines in mast cells. *Journal of Leukocyte Biology* 83:575-580.

**Mosmann TR, Li L, and Sad S. 1997.** Functions of CD8 T-cell subsets secreting different cytokine patterns. *Seminars in Immunology* 9:87-92.

**O'Shaughnessy TC, Ansari TW, Barnes NC, and Jeffery PK. 1997.** Inflammation in bronchial biopsies of subjects with chronic bronchitis: inverse relationship of CD8+ T lymphocytes with FEV1. *American Journal of Respiratory and Critical Care Medicine*

155:852-857.

**Podolin PL, Foley JP, Carpenter DC, Bolognese BJ, Logan GA, Long ER, Harrison OJ, and Walsh PT. 2013.** T cell depletion protects against alveolar destruction due to chronic cigarette smoke exposure in mice. *American Journal of Physiology: Lung Cellular and Molecular Physiology* 304:L312-323.

**Profita M, Bonanno A, Siena L, Bruno A, Ferraro M, Montalbano AM, Albano GD, Riccobono L, Casarosa P, Pieper MP, and Gjomarkaj M. 2009.** Smoke, choline acetyltransferase, muscarinic receptors, and fibroblast proliferation in chronic obstructive pulmonary disease. *Journal of Pharmacology and Experimental Therapeutics* 329:753-763.

**Rabe KF, Hurd S, Anzueto A, Barnes PJ, Buist SA, Calverley P, Fukuchi Y, Jenkins C, Rodriguez-Roisin R, van Weel C, Zielinski J, and Global Initiative for Chronic Obstructive Lung D. 2007.** Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. *American Journal of Respiratory and Critical Care Medicine* 176:532-555.

**Res PC, Piskin G, de Boer OJ, van der Loos CM, Teeling P, Bos JD, and Teunissen MB. 2010.** Overrepresentation of IL-17A and IL-22 producing CD8 T cells in lesional skin suggests their involvement in the pathogenesis of psoriasis. *PloS One* 5:e14108.

**Saetta M, Baraldo S, Corbino L, Turato G, Braccioni F, Rea F, Cavallesco G, Tropeano G, Mapp CE, Maestrelli P, Ciaccia A, and Fabbri LM. 1999.** CD8+ve cells in the lungs of smokers with chronic obstructive pulmonary disease. *American Journal of Respiratory and Critical Care Medicine* 160:711-717.

**Saetta M, Di Stefano A, Maestrelli P, Ferraresso A, Drigo R, Potena A, Ciaccia A, and Fabbri LM. 1993.** Activated T-lymphocytes and macrophages in bronchial mucosa of subjects with chronic bronchitis. *American Review of Respiratory Disease* 147:301-306.

**Saetta M, Di Stefano A, Turato G, Facchini FM, Corbino L, Mapp CE, Maestrelli P, Ciaccia A, and Fabbri LM. 1998.** CD8+ T-lymphocytes in peripheral airways of smokers with chronic obstructive pulmonary disease. *American Journal of Respiratory and Critical Care Medicine* 157:822-826.

**Sorheim IC, Johannessen A, Gulsvik A, Bakke PS, Silverman EK, and DeMeo DL. 2010.** Gender differences in COPD: are women more susceptible to smoking effects than men? *Thorax* 65:480-485.

**Suzuki M, Konya C, Goronzy JJ, and Weyand CM. 2008.** Inhibitory CD8+ T cells in autoimmune disease. *Human Immunology* 69:781-789.

**Takanashi S, Hasegawa Y, Kanehira Y, Yamamoto K, Fujimoto K, Satoh K, and Okamura K. 1999.** Interleukin-10 level in sputum is reduced in bronchial asthma, COPD and in smokers. *European Respiratory Journal* 14:309-314.

**Wang DW, Zhou RB, Yao YM, Zhu XM, Yin YM, Zhao GJ, Dong N, and Sheng ZY. 2010.** Stimulation of alpha7 nicotinic acetylcholine receptor by nicotine increases suppressive capacity of naturally occurring CD4+CD25+ regulatory T cells in mice in vitro. *Journal of Pharmacology and Experimental Therapeutics* 335:553-561.

**Wang H, Yu M, Ochani M, Amella CA, Tanovic M, Susarla S, Li JH, Wang H, Yang H, Ulloa L, Al-Abed Y, Czura CJ, and Tracey KJ. 2003.** Nicotinic acetylcholine receptor alpha7 subunit is an essential regulator of inflammation. *Nature* 421:384-388.

**Zhang MQ, Wan Y, Jin Y, Xin JB, Zhang JC, Xiong XZ, Chen L, and Chen G. 2014.** Cigarette smoking promotes inflammation in patients with COPD by affecting the polarization and survival of Th/Tregs through up-regulation of muscarinic receptor 3 and 5

expression. *PloS One* 9:e112350.

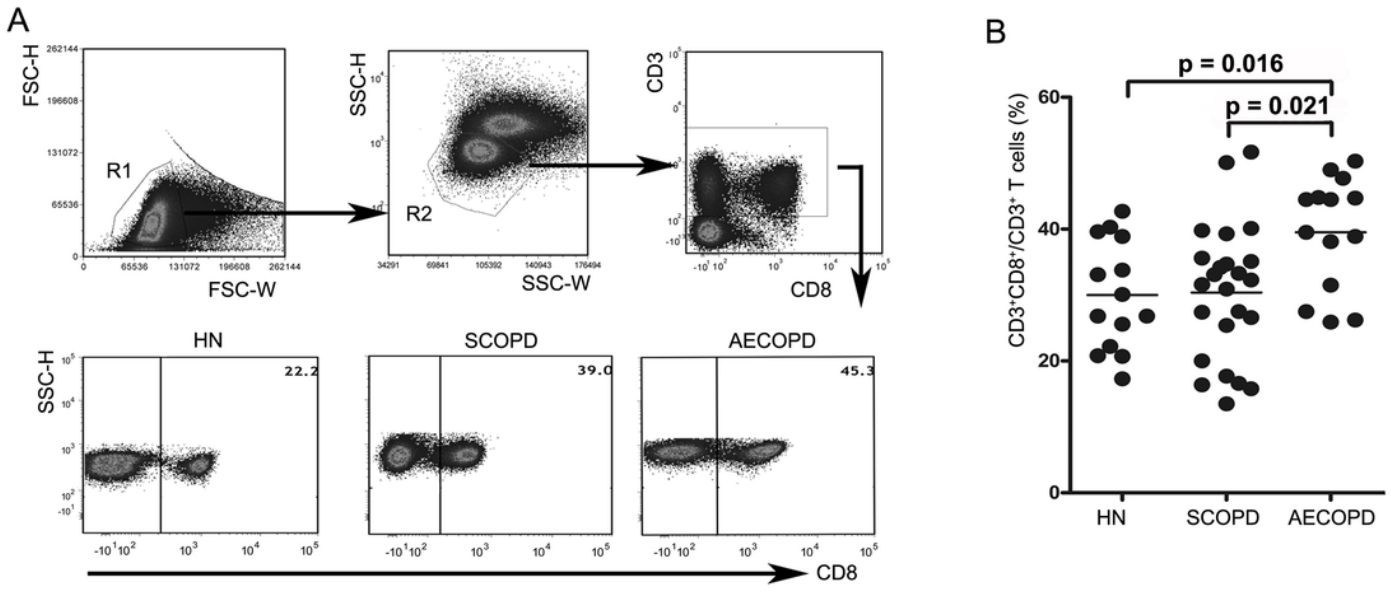
**Zhou H, Hua W, Jin Y, Zhang C, Che L, Xia L, Zhou J, Chen Z, Li W, and Shen H. 2015.** Tc17 cells are associated with cigarette smoke-induced lung inflammation and emphysema. *Respirology* 20:426-433.

**Zhu X, Gadgil AS, Givelber R, George MP, Stoner MW, Sciurba FC, and Duncan SR. 2009.** Peripheral T cell functions correlate with the severity of chronic obstructive pulmonary disease. *Journal of Immunology* 182:3270-3277.

# Figure 1

Acute exacerbations of COPD are accompanied by elevation of circulating $CD8^+$ T cells.

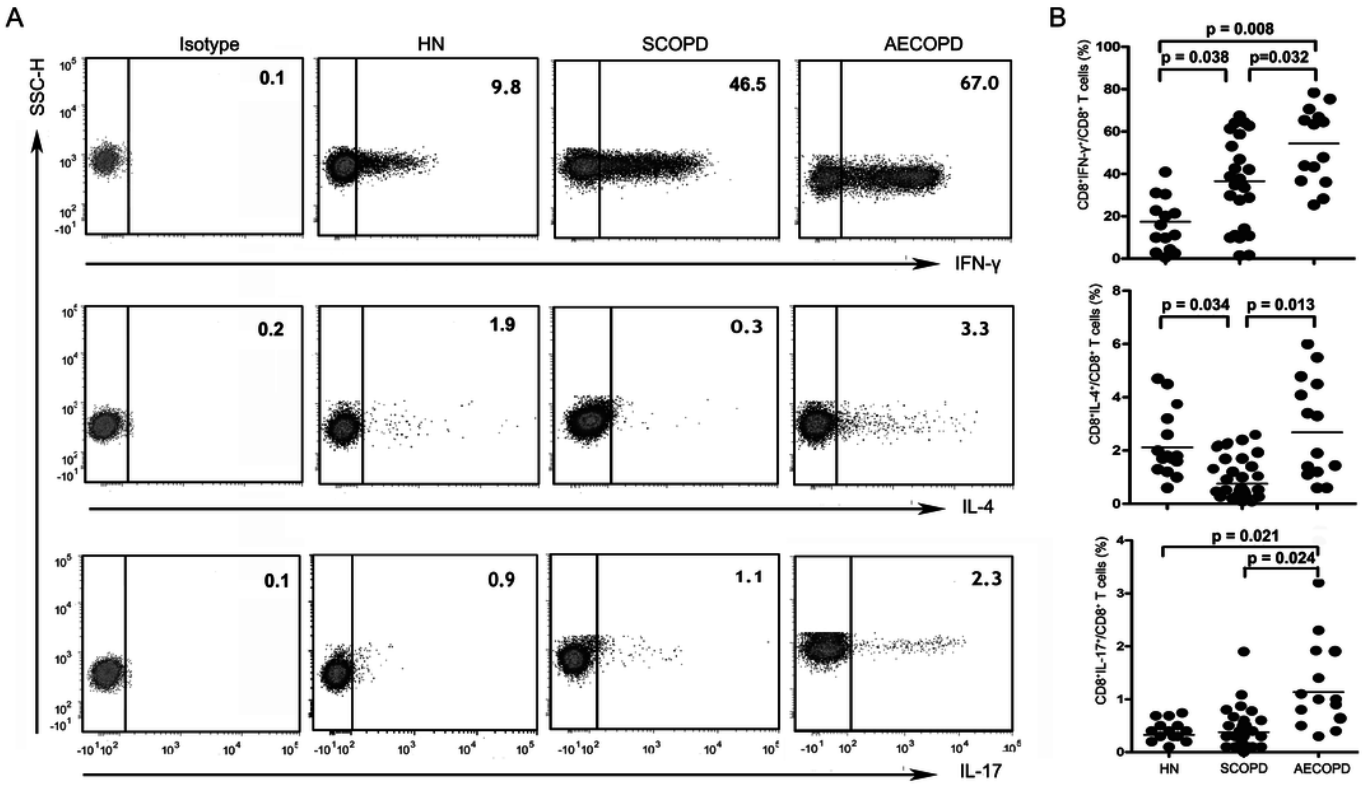
(A) Strategies for gating $CD8^+$ T cells: PBMCs were gated into R1 and R2 gates to exclude monocytes, platelets and debris; $CD8^+$ T cells were then identified based on CD3 and CD8 expression. Representative flow cytometric dot plots are shown. (B) Comparisons of the percentages of $CD8^+$ T cells from HN , SCOPD patients and AECOPD patients (n=14, 24, and 14, respectively) are shown. Horizontal bars indicate the mean values.



# Figure 2

Imbalance of peripheral Tc1, Tc2 and Tc17 cells in COPD.

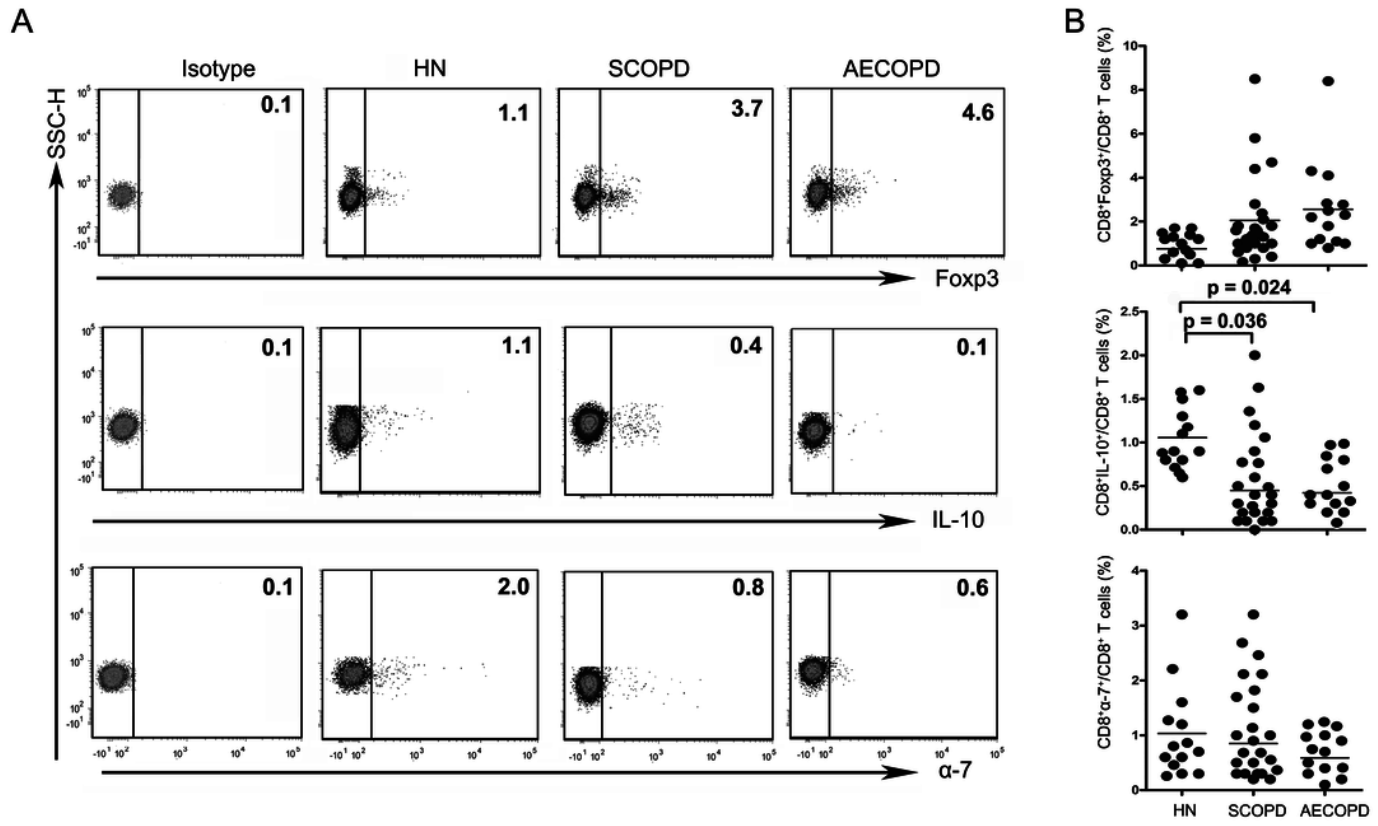
(A) Representative flow cytometric dot plots of Tc1, Tc2 and Tc17 cells (Tc1: $CD3^+CD8^+IFN\text{-}\gamma^+$ T cells, Tc2: $CD3^+CD8^+IL\text{-}4^+$ T cells, Tc17: $CD3^+CD8^+IL\text{-}17A^+$ T cells) in HN, SCOPD patients and AECOPD patients are shown. (B) Comparisons of the percentages of Tc1, Tc2 and Tc17 cells in HN, SCOPD patients and AECOPD patients (n=14, 24, and 14, respectively) are shown. Horizontal bars indicate the mean values.



# Figure 3

The percentage of Tc10 cells is decreased in both the SCOPD and AECOPD groups.

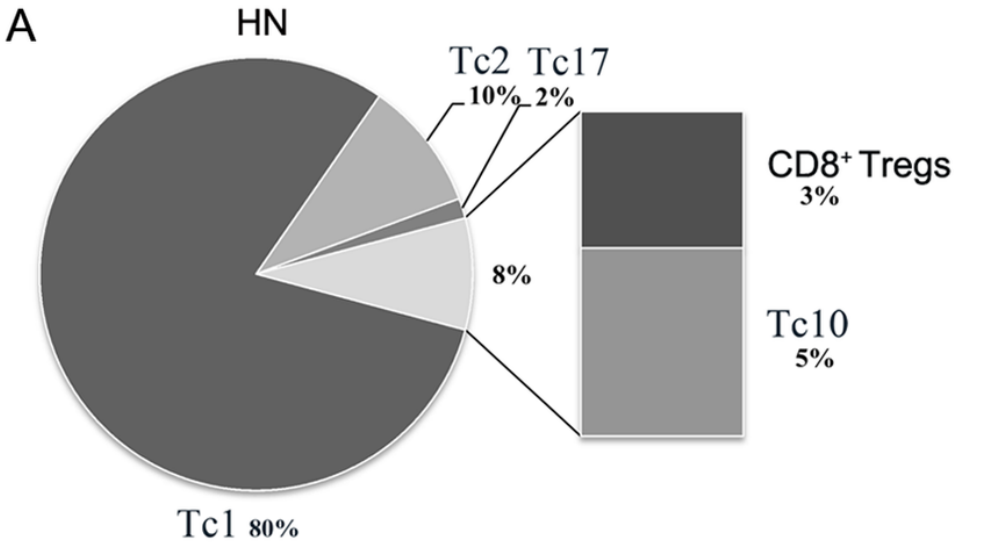
(A) Representative flow cytometric dot plots of $CD8^+$Tregs, Tc10 and $CD8^+\alpha7^+$ T cells ($CD8^+$ Tregs: $CD3^+CD8^+FOXP3^+$T cells; Tc10: $CD3^+CD8^+IL\text{-}10^+$T cells; $CD8^+\alpha7^+$: $CD3^+CD8^+\alpha7^+$ T cells) in HN, SCOPD patients and AECOPD patients are shown. (B) Comparisons of the percentages of $CD8^+$Tregs, Tc10 cells, and $CD8^+\alpha7^+$ T cells in HN, SCOPD patients and AECOPD patients (n=14, 24, and 14, respectively) are shown. Horizontal bars indicate the mean values.

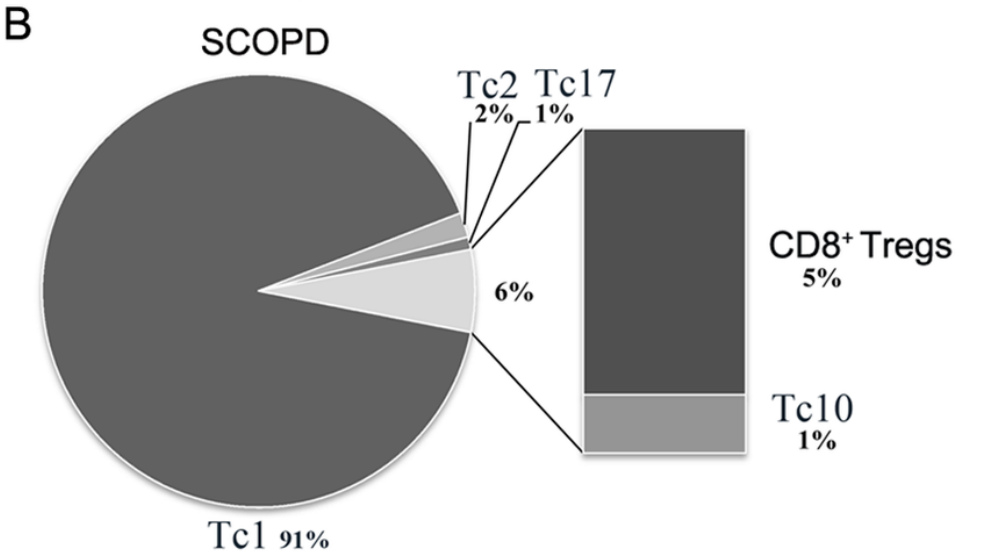


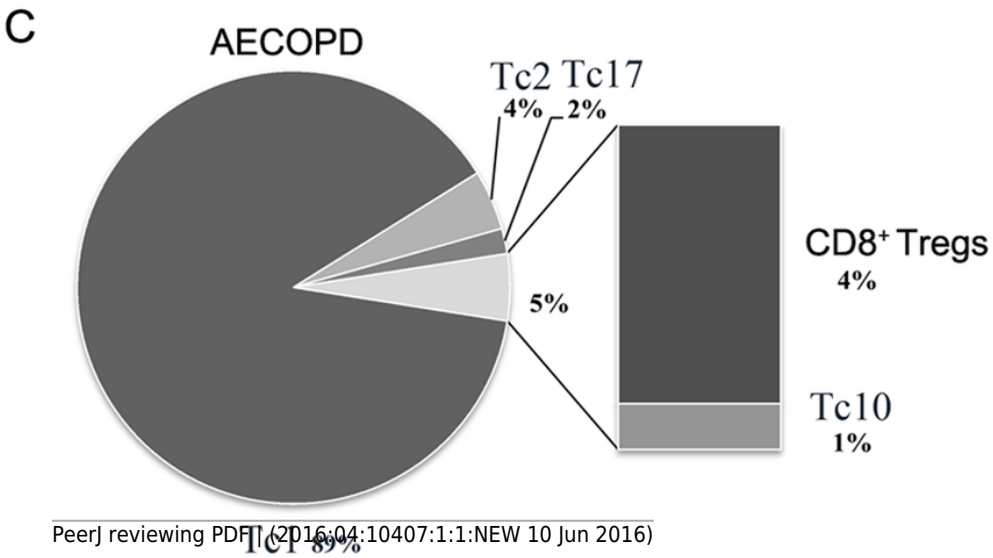
# Figure 4

Comprehensive analysis of the relative percentages of $CD8^+$ T cell subsets.

The relative percentages of Tc1, Tc2, Tc17, $CD8^+$ Tregs and Tc10 cells in HN (A) , SCOPD patients (B) and AECOPD patients (C) were calculated based on their mean values. Anti-inflammatory $CD8^+$ T cells comprised $CD8^+$ Tregs and Tc10 cells. The data are presented in a pie graph.


A
HN
Tc2 Tc17
10% 2%
CD8+ Tregs
3%
8%
Tc10
5%
Tc1 80%


B
SCOPD
Tc2 Tc17
2% 1%
CD8+ Tregs
5%
6%
Tc10
1%
Tc1 91%


C
AECOPD
Tc2 Tc17
4% 2%
CD8+ Tregs
4%
5%
Tc10
1%
Tc1 89%