The Role of Human Leukocyte Antigen-DR in Regulatory T Cells in Patients with Virus-Induced Acute Exacerbation of Chronic Obstructive Pulmonary Disease

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Background: This study assessed the role of different immune phenotypes of T cells in virus-induced acute exacerbation of chronic obstructive pulmonary disease (AECOPD).

Material/Methods: The study involved 103 participants, including individuals with virus-induced AECOPD (n=32), non-virus-induced AECOPD (n=31), and stable COPD (n=20) and individuals who were healthy smokers (n=20). The immune phenotypes of T cells in peripheral blood were evaluated via flow cytometry analysis, and the differences were analyzed.

Results: Patients with virus-induced AECOPD (virus group) had a higher COPD assessment test score on admission than those in the group with non-virus-induced AECOPD (nonvirus group; 25.6±3.8 vs 21.9±4.8, P=0.045). A lower CD4+ human leukocyte antigen-DR (HLA-DR)+ frequency was found in the peripheral blood of the virus group compared with the nonvirus group (2.2 vs 4.2, P=0.015), and the frequency of CD4+ CD25^{high} CD127^{low} HLA-DR+ in CD4+ in the virus group was lower than in the nonvirus group (1.1 vs 3.6, P=0.011). The CD3+, CD4+, CD8+, CD4+ central memory T cell, CD4+ effector memory T cell (Tem), CD4+ end-stage T cell, and CD8+ Tem levels in lymphocytes of peripheral blood were lower in exacerbation groups relative to those in the stable COPD and healthy smoking groups, but similar between exacerbation groups. Similar frequencies and levels of T cells between different stagings of COPD were also identified.

Conclusions: The expression of HLA-DR on the cell surface of CD4+ regulatory T cells (Tregs) was lower in the peripheral blood of patients with virus-induced AECOPD. The expression of HLA-DR in CD4+ Tregs suggested the effect of respiratory viruses on adaptive immunity of patients with AECOPD to some extent.

Keywords: Antigens, CD4 • Disease Progression • Lymphocytes • Pulmonary Disease, Chronic Obstructive • T-Lymphocytes, Regulatory

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Background

Chronic obstructive pulmonary disease (COPD) is a syndrome with progressive lung dysfunction, airway limitation, and inflammation [1]. Acute exacerbation of chronic obstructive pulmonary disease (AECOPD) is a critical event of COPD that leads to the deterioration of physiological functions and lung function [2]. A previous study reported that the 3-month mortality rate of individuals hospitalized due to an exacerbation is more than 15% [3]. Although some progress has been made in AECOPD diagnosis and treatment, there is no effective treatment for the damage of lung function or systemic inflammation, and the survival of patients has not been prolonged [4]. Hence, the Global Initiative on Chronic Obstructive Lung Disease (GOLD) [5] proposed that AECOPD prevention and improved therapies are needed for reducing morbidity and mortality of the disease.

Currently, both the pathology and treatment strategies of AECOPD are diverse [6,7]. The prevalence of viral infection in AECOPD was reported to be 42.7% in a recent trial [8]. A single virus or multiple viruses were detected in AECOPD patients, suggesting that respiratory viral infection is a leading cause for AECOPD [9]. AECOPD caused by viral infection presents more severe clinical symptoms and a longer duration [10,11]. AECOPD is associated with systemic inflammation, along with adaptive immune mechanisms that contribute to it [12]. The CD4+ and CD8+ T cell levels and frequencies in peripheral blood are reported to be decreased in patients with AECOPD relative to those with stable COPD [13]. In another study, the frequency of CD4+ regulatory T cells (Tregs) was elevated in patients with AECOPD compared with stable COPD [14,15].

Human leukocyte antigen-DR (HLA-DR) is a human class II major histocompatibility complex (MHC) antigen that does not occur in resting T cells [16]. It appears in the late-stage activation of T cells and can serve as a marker of activated T cells [16-19]. HLA-DR is expressed on the surface of CD4+ T lymphocytes, especially CD4+ Tregs in the peripheral blood of healthy people [20]. However, the expression of HLA-DR on Tregs in patients with virus-induced AECOPD remains unclear.

Herein, we present the characteristics of adaptive immunity in virus-induced AECOPD and HLA-DR expression on Tregs in patients with virus-induced AECOPD, providing references for selecting clinical treatment strategies for AECOPD.

Material and Methods

Study Population

The prospective observational study involved 63 AECOPD patients admitted to the respiratory ward of Xuanwu Hospital Capital Medical University between October 2018 and September 2019. Twenty patients with stable COPD and no acute exacerbation for almost 3 months were followed in the outpatient clinic. Twenty age-matched healthy smokers with normal lung function were included as a control group (**Table 1**). The study was approved by the Ethics Committee of Xuanwu Hospital Capital Medical University (Approval No. (2020)007), and all subjects signed informed consent.

Inclusion criteria were (1) a previous diagnosis of COPD, (2) age >40 years, and (3) a smoking history of more than 10 packs per year. Exclusion criteria were (1) a previous asthma diagnosis or allergic rhinitis; (2) bacterial pneumonia, bronchiectasis, pulmonary interstitial fibrosis, or pneumothorax identified by chest computed tomography; (3) pulmonary embolism confirmed by pulmonary enhanced computed tomography; (4) acute left heart failure; (5) treatment with systemic glucocorticoids 4 weeks prior to admissions; (6) malignant tumor diagnosis; (7) diabetes; and (8) being unable to cooperate.

Diagnostic Criteria

COPD is defined by having a postbronchodilator ratio of forced expiratory volume in 1 s/forced vital capacity (FEV_1/FVC) <0.70 in accordance with criteria from GOLD [21]. In the current study, patients with COPD were classified into GOLD II, GOLD III, and GOLD IV groups based on GOLD classification [21].

AECOPD is defined as an increase in respiratory symptoms for 2 consecutive days with at least 1 major symptom (dyspnea, purulent sputum, or increased sputum volume) and another major or minor symptom (wheezing, cold symptoms, sore throat, or cough) [2,22].

Sputum Collection

Sputum was collected from patients on admission. Sputum was induced in patients with no spontaneous expectoration according to European Respiratory Society guidelines for sputum induction [23]. In brief, the participants inhaled 400 μg of salbutamol 10 min before induction. After 10 min, they rinsed their mouth with water and then blew their nose. Subsequently, the patients inhaled atomized 3% hypertonic saline for 10 min, and after gargling and again blowing their nose, they expectorated vigorously into petri dishes. If the patients had no sputum or the sputum quantity was insufficient, they repeated the steps until expectorating a sufficient amount of qualified sputum specimens; otherwise, the atomization stopped when the total atomization time reached 30 min. The collected sputum was frozen at -80°C until respiratory virus testing was conducted.

Table 1. Baseline characteristics of 103 subjects in the study.

	Health smokers (n=20)	Stable COPD (n=20)	Virus AECOPD (n=32)	Non-virus AECOPD (n=31)	P
Gender, Males (%)	14 (70)	18 (90)	22 (68.8)	23 (74.2)	0.345
Age (years)	71.3±7.1	70.8±6.5	71.7±10.1	73.1±9.3	0.831
Smoking history (pack-years)	46.3±32.1	50.2±23.4	50.5±31.3	47.6±20.6	0.513
Current smokers (n, %)	12 (60)	11 (55)	17 (53.1)	19 (61.3)	0.912
BMI (kg/m²)	24.6±4.3	24.7±5.9	25.1±4.8	24.6±5.2	0.907
Inhaled corticosteroid (n, %)	–	6 (30)	10 (31.3)	12 (38.7)	0.757
Inhaled bronchodilator (n, %)	–	17 (85)	25 (78.1)	27 (87.1)	0.616
Comorbidities					
Hypertension	6	5	8	5	
Coronary heart disease	0	3	4	4	
Cerebrovascular disease	0	1	2	1	
Frequent exacerbator (n, %)	–	7 (35)	12 (37.5)	15 (48.4)	0.560
FEV_1/FVC*	79.2±5.4	52.7±10.5	49.0±9.4	49.6±13.9	0.000
FEV_1% pred**	102.1±12.8	53.5±13.2	56.8±14.8	51.3±20.1	0.000
RV/TLC (%)	–	54.1±10.2	52.6±11.4	58.1±11.6	0.299
GOLD stage					0.928
II		10	13	12	
III		8	16	15	
IV		2	3	4	
Pre-hospital antibiotic treatment (n,%)	–	–	26 (81.2)	28 (90.3)	0.474
Hospital stay (days)	–	–	13.8±6.7	12.0±4.3	0.789
CAT score on admission	–	–	25.6±3.8	21.9±4.8	0.045
CAT score at discharge	–	–	13.9±3.4	13.4±3.6	0.692
Blood leukocytes cells/mm³	6361±775	6659±1146	7472±3536	7125±3047	0.465
Blood neutrophil cells/mm³	3811±949	4253±1015	5463±3398	5050±3639	0.387
Blood Lymphocyte cells/mm³***	2029±439	1946±464	1347±564	1371±646	0.011
Blood eosinophil cells/mm³	140 (85-178)	130 (98-200)	55 (10-120)	120 (18-225)	0.065
PH	–	–	7.42±0.05	7.40±0.04	0.186
PaO_2	–	–	76.0±12.7	70.6±10.2	0.101
$PaCO_2$	–	–	41.2±8.9	44.8±10.0	0.169

BMI – body mass index; CAT – COPD assessment test; COPD – chronic obstructive pulmonary disease; FEV1 – forced expiratory volume in 1 s; FVC – forced vital capacity; %pred – the percentage predicted; RV – residual volume; TLC – total lung capacity. *, ** Similar data among virus group, nonvirus group, and stable COPD group, but lower than that of healthy smoking group; *** similar data between virus group and nonvirus group, but lower than that of the healthy smoking group and the stable COPD group.

Evaluation of Lung Function and Airway Reversibility

Lung function of inpatients was examined at discharge, while patients with stable COPD were examined at outpatient follow-up. The evaluation of lung function was performed on VmaxEncore6229 via VMAX 21-2B Software (CareFusion Inc, USA) according to American Thoracic Society Guidelines [24]. Lung function indicators including FVC and FEV_1 were calculated as predicted percentages, using the Knudson cotton dust predictive value formula: FVC=maximal volume of air exhaled with maximally forced effort from a position of maximal inspiration (ie, vital capacity performed with a maximally forced expiratory effort); FEV_1=the volume of air exhaled during the first second of FVC.

Respiratory Virus Test

The respiratory virus detection kit was obtained from Beijing Applied Biological Technologies (Beijing, China). DNA/RNA extraction from sputum specimens was performed using the viral DNA/RNA extraction kit (B2090). Parainfluenza virus type I, parainfluenza virus type III, influenza A virus, influenza B virus, adenovirus, respiratory syncytial virus, human rhinovirus, and human metapneumovirus were measured through the molecular detection kit (A2887), real-time polymerase chain reaction (RT-PCR) diagnostic kit for rapid detection of human metapneumovirus (A3881), and RT-PCR diagnostic kit for rapid detection of human rhinovirus (A3891), using ABI 7500 fluorescence quantitative PCR equipment.

Flow Cytometry Analysis

Two milliliters of peripheral blood were collected from participants and placed in an ethylene diamine tetraacetic acid (EDTA) anticoagulant tube for routine blood tests during recruitment. Another 100 µL of peripheral blood was taken to be labeled with antibodies. After antibody labeling, the red blood cells were lysed and washed with phosphate-buffered saline. The antibody labels were then detected. The labeled antibodies were described in previous literature [25] as follows: anti-CD3-allophycocyanin (APC)-cyanine (Cy) 7 (clone SK7), anti-CD4-BB700 (clone SK3), anti-CD8-BV510 (clone SK1), anti-CD25-phycoerythrin (PE) (clone M-A251), anti-CD127-BV421 (clone HIL-7R-M21), anti-CD62L-APC (clone DREG-56), anti-CD45RO-BB515 (clone UCHL1), and anti-HLA-DR-PE-Cy7 (clone G46-6). One hundred microliters of peripheral blood were also collected from the homotypic controls for antibody labeling. Homotypic controls were tested using anti-CD3-APC-Cy7 (clone SK7), anti-CD4-BB700 (clone SK3), anti-CD8-BV510 (clone SK1), immunoglobulin 1 (IgG1) k-PE (clone MOPC-21), IgG1 k-BV421 (clone X40), IgG1 k-APC (clone MOPC-21), IgG2a k-BB515 (clone G155-178), and IgG2a k-PE-Cy7 (clone G155-178) (BD Biosciences).

Flow cytometry BD FACS Canto™ II was applied for data collection and FlowJo V10 software (Tree Star, Inc., Ashland, OR, USA) was utilized for data analysis.

Statistical Analysis

Data analyses were conducted using SPSS 20.0 software (IBM, Armonk, NY, USA). The enumeration data were expressed as number or frequency (%), and comparison of different groups was performed by chi-square test. The normal distributed-measurement data were displayed as mean±standard deviation (SD). Comparisons among multiple groups were performed by ANOVA, while the comparisons between 2 groups were performed by t test. Difference comparisons among multiple groups in the measurement data with nonnormal distribution were subjected to Kruskal-Wallis test. $P<0.05$ was considered as indicating a significant difference. GraphPad Prism 8 (GraphPad Software, San Diego, California, USA) was used to plot the statistical results.

Results

Baseline Data of the Participates

A total of 159 AECOPD patients were recruited in our study. Patients with pneumonia (n=16), pneumothorax (n=9), pulmonary embolism (n=5), acute myocardial infarction (n=4), left heart failure (n=13), asthma or allergic rhinitis (n=5), diabetes (n=4), and other lung diseases (n=14) were excluded. After 18 patients receiving systemic glucocorticoid prior to admission were excluded, 71 patients were finally retained. Among them, 5 failed to produce enough sputum on admission, 1 patient was diagnosed with cancer, and 2 patients withdrew their informed consent on discharge. Finally, 63 cases were involved. The screening process is shown in **Figure 1**.

According to results from the respiratory virus test (**Figure 2**), patients with AECOPD were divided into 2 groups: the virus group (n=32) and the nonvirus group (n=31). The clinical data were not significantly different between the 2 groups in terms of sex, age, body mass index, smoking history, and hemamoeba, neutrophil, and eosinophil counts in peripheral blood. The number of participants with use of an inhaled corticosteroid or bronchodilator and comorbidities including hypertension, coronary heart disease, and cerebrovascular disease and the proportion of frequent exacerbator and lung function indicators (FVC/FEV_1, FEV1%pred) were as similar as possible in all COPD groups. The number of patients in different GOLD stages showed no significant differences. Similar data were found in the use of antibiotics before admission and for the blood gas analysis (pH, PaO_2, and $PaCO_2$) between exacerbation groups. Although the mean hospital stay of the virus group
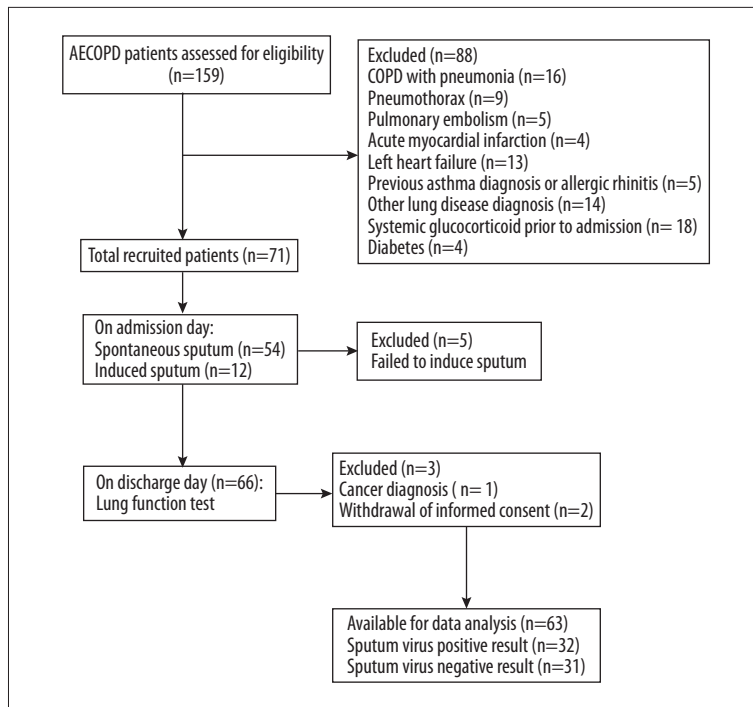
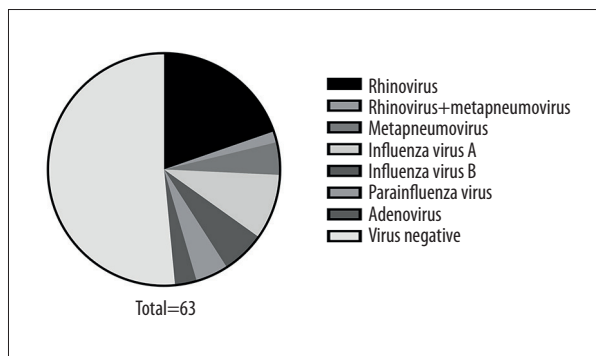**Figure 1.** Patient recruitment and screening process.

Figure 2. Results of the respiratory virus test.

was marginally longer than that of the nonvirus group, the difference was not statistically significant. A higher COPD assessment test score was found in the virus group compared with the nonvirus group (25.6±3.8 vs 21.9±4.8, *P*=0.045) on admission, but the difference disappeared at discharge. The lymphocyte counts in healthy smoking and stable COPD groups were higher than those in exacerbation groups, but the data were similar between exacerbation groups (**Table 1**).

Varied Subpopulation Frequencies of T Cells in Exacerbation Groups, Stable COPD Group, and Healthy Smoking Group

As shown in **Table 2, Figures 3, and 4**, the frequencies of CD3+, CD4+, CD8+, the ratio of CD4+/CD8+, CD4+ Naïve, CD4+ central memory T cell (Tcm), CD4+ effector memory T cell (Tem), CD4+ end-stage T cell (End), CD8+ Naïve, CD8+ Tcm, CD8+ Tem, and

CD8+ End exhibited no significant differences among the 4 groups. The frequencies of CD4+ HLA-DR+ (2.2 vs 4.2, *P*=0.015) and CD4+ Tregs HLA-DR+ in CD4+ T cells (1.1 vs 3.6, *P*=0.011) were decreased in the virus group relative to those in the nonvirus group but were similar among the virus group, stable COPD group, and healthy smoking group. In exacerbation groups, the CD4+ Tregs and CD4+ Tregs HLA-DR− frequencies in CD4+ T cells were elevated compared with those in the stable COPD and healthy smoking groups, but the data were similar between exacerbation groups. The frequency of CD4+ Tregs in the healthy smoking group was higher than that in the stable COPD group. No significant difference was found in CD4+ Tregs HLA-DR− in CD4+ T cells between the stable COPD group and the healthy smoking group. The CD8+ HLA-DR+ frequency presented similar levels among the 4 groups. There was no significant difference of the frequencies of T cells between GOLD II and GOLD III-IV in stable COPD and exacerbation groups (all *P*>0.05).

The levels of CD3+, CD4+, CD4+ Tcm, CD4+ Tem, CD4+ End, CD8+, and CD8+ Tem were reduced in exacerbation groups relative to those in the stable COPD and healthy smoking groups, but were similar between exacerbation groups. The levels of CD4+ Naïve, CD8+ Naïve, and CD8+ Tcm were similar among the 4 groups. CD8+ End level was reduced in the nonvirus group relative to the stable COPD and healthy smoking groups, but compared with that of the virus group, no significant difference was detected. The CD8+ End level in the virus group was lower relative to the healthy smoking group, but it was comparable between the virus group and the stable COPD group.

Table 2. Flow cytometry analysis of frequencies of T cell subsets in the virus group, nonvirus group, stable COPD group, and healthy smoking group.

	Health smokers (n=20)		Stable COPD (n= 20)		Virus AECOPD (n=32)		Non-virus AECOPD (n=31)		P
CD3+	65.2±10.6		60.8±13.8		54.2±16.2		54.0±20.0		0.250
		GOLD II	65.4±11.2		61.5±14.3		57.9±22.2		
		GOLD III-IV	56.2±15.9		48.0±17.2		51.4±20.1		
CD4+	57.5±8.3		56.3±6.6		54.9±11.1		58.5±8.2		0.715
		GOLD II	54.1±6.1		51.7±1.8		55.7±5.2		
		GOLD III-IV	59.0±6.7		57.2±15.1		60.4±9.7		
CD4+Naive	22.4±8.9		22.8±7.9		18.0±9.3		25.1±13.5		0.572
		GOLD II	24.1±17.8		22.3±12.1		21.7±8.1		
		GOLD III-IV	20.8±6.9		14.7±5.7		29.1±16.2		
CD4+Tcm	41.1±4.4		39.3±4.9		42.0±10.1		41.7±10.0		0.641
		GOLD II	40.7±4.0		45.9±10.7		45.9±10.5		
		GOLD III-IV	36.8±5.6		39.1±10.0		38.9±9.4		
CD4+Tem	31.9±8.9		32.7±9.0		37.8±14.4		28.4±10.7		0.330
		GOLD II	34.6±9.5		29.6±10.4		28.3±4.2		
		GOLD III-IV	30.5±8.9		43.9±14.9		28.4±13.9		
CD4+End	3.2 (1.8-7.4)		3.5 (2.1-6.4)		2.4 (1.9-2.7)		3.3 (2.1-5.9)		0.408
		GOLD II	2.2 (1.2-7.0)		2.5 (0.5-2.8)		3.9 (1.1-7.6)		
		GOLD III-IV	4.2 (2.9-5.9)		2.2 (1.9-2.6)		3.3 (2.3-4.3)		
CD4+HLA-DR+	1.6 (1.0-2.3)		1.3 (1.1-2.1)		2.2 (1.9-2.5)		4.2 (2.9-8.5)		0.015
		GOLD II	1.6 (0.9-2.2)		2.4 (1.9-2.6)		4.9 (2.4-9.6)		
		GOLD III-IV	1.2 (1.1-2.0)		2.1 (1.7-2.3)		4.2 (3.2-8.7)		
CD4+Treg	5.8 (5.3-6.8)		4.8 (3.2-5.1)		12.8 (8.8-14.0)		13.1 (12.0-16.4)		0.001
		GOLD II	5.1 (4.6-5.5)		13.3 (12.1-14.8)		12.9 (11.2-15.6)		
		GOLD III-IV	3.9 (3.0-5.1)		9.5 (6.7-13.1)		13.1 (12.5-19.4)		
CD4+Treg-HLA-DR+	0.9 (0.5-1.7)		1.1 (0.6-1.9)		1.1 (0.9-1.7)		3.6 (1.1-4.7)		0.011
		GOLD II	1.1 (0.8-1.4)		1.5 (0.8-1.7)		3.2 (1.3-4.4)		
		GOLD III-IV	0.9 (0.3-2.1)		1.0 (0.9-1.5)		3.6 (1.2-6.9)		
CD4+Treg-HLA-DR-	4.7 (4.2-5.1)		3.1 (2.4-4.8)		10.7 (7.7-12.3)		10.6 (7.0-13.9)		0.001
		GOLD II	3.0 (2.3-4.8)		11.9 (10.7-13.4)		10.1 (7.7-12.9)		
		GOLD III-IV	3.4 (2.3-5.0)		8.5 (5.7-11.5)		11.8 (5.6-17.8)		
CD8+	29.7±8.7		28.8±7.2		37.4±10.9		28.2±7.9		0.179
		GOLD II	31.6±8.4		40.9±3.6		30.6±7.5		
		GOLD III-IV	25.3±3.8		34.8±14.5		26.6±8.4		

Table 2 continued. Flow cytometry analysis of frequencies of T cell subsets in the virus group, nonvirus group, stable COPD group, and healthy smoking group.

	Health smokers (n=20)		Stable COPD (n= 20)		Virus AECOPD (n=32)		Non-virus AECOPD (n=31)		P
CD8+Naive	9.2 (6.3-11.4)		10.1	(6.1-15.0)	11.8	(4.0-23.1)	14.0	(9.7-20.3)	0.354
		GOLD II	6.4	(5.7-20.7)	12.8	(6.0-24.3)	15.4	(9.8-19.6)	
		GOLD III-IV	11.9	(5.0-14.3)	5.9	(2.1-19.3)	13.9	(7.8-23.3)	
CD8+Tcm	10.8±4.7		12.5±4.5		13.9±7.8		14.7±8.3		0.574
		GOLD II	13.3±4.0		16.9±4.7		16.1±4.8		
		GOLD III-IV	11.1±5.6		11.6±9.5		13.9±10.4		
CD8+Tem	42.1±14.2		40.4±12.9		44.5±14.4		40.5±17.7		0.691
		GOLD II	36.0±12.1		35.3±16.7		37.3±17.8		
		GOLD III-IV	45.6±13.2		51.5±8.7		42.5±19.0		
CD8+End	35.5±14.9		30.4±13.9		29.3±14.2		29.9±16.2		0.799
		GOLD II	31.2±10.6		31.2±17.7		31.6±20.8		
		GOLD III-IV	29.5±18.6		27.8±13.6		28.7±15.4		
CD8+HLA-DR+	1.0 (0.8-1.7)		1.2	(0.7-1.9)	2.2	(1.8-2.4)	1.6	(1.1-2.4)	0.284
		GOLD II	1.5	(1.1-2.8)	2.0	(1.8-2.2)	1.5	(0.8-2.3)	
		GOLD III-IV	0.9	(0.5-1.7)	2.3	(1.0-2.4)	1.6	(1.2-2.5)	
CD4+:CD8+ratio	2.1±0.7		1.8±0.6		1.7±1.0		2.3±1.0		0.449
		GOLD II	1.7±0.5		1.3±0.2		1.9±0.4		
		GOLD III-IV	2.0±1.2		2.0±1.3		2.5±1.3		

COPD – chronic obstructive pulmonary disease; End – end-stage T cell (CD45RO⁻CD62L⁻); naïve T cell (CD45RO⁻CD62L⁺); Tcm – central memory T cell (CD45RO⁺CD62L⁺); Tem – effector memory T cell (CD45RO⁺CD62L⁻); Treg – regulatory T cell (CD4⁺CD25ʰⁱᵍʰCD127ˡᵒʷ).

Similar levels of CD4⁺ HLA-DR⁺, CD8⁺ HLA-DR⁺, CD4⁺ Tregs, CD4⁺ Tregs HLA-DR⁻, and CD4⁺ Tregs HLA-DR⁺ were discovered among the 4 groups. Comparable levels of T cells were observed between GOLD II and GOLD III-IV in the COPD groups (all P>0.05) (**Table 3, Figures 3, 4**).

Discussion

AECOPD is an acute event in which a patient's respiratory symptoms deteriorate beyond the normal daily variation [26,27]. The severity and frequency of exacerbations are closely associated with the prognosis of patients [28], and more detailed investigation on AECOPD is required. In the present study, RT-PCR was used to screen AECOPD patients for respiratory viruses. The immune phenotypes of T lymphocytes in peripheral blood were analyzed, showing that the CD25ʰⁱᵍʰ CD127ˡᵒʷ HLA-DR⁺ phenotype was decreased in the CD4⁺ T cell population in the peripheral blood of patients with virus-induced AECOPD.

A previous study showed that significant heterogeneity exists in AECOPD [29], and respiratory viral infection plays a vital role in AECOPD. In recent years, influenza virus and rhinovirus were reported to be the major respiratory viruses leading to AECOPD in China and other Asian countries; other viruses were parainfluenza virus, respiratory syncytial virus, and human metapneumovirus, among others [8,30,31]. Herein, RT-PCR screening revealed 8 respiratory viruses in the sputum of AECOPD patients. Among the 63 patients, the virus positivity rate was 49% and mainly involved rhinoviruses and influenza viruses. Virus-positive patients had higher COPD assessment test scores on admission, indicating more severe clinical symptoms in patients with virus-induced AECOPD; these results were supported by previous studies [10,11]. We hypothesized that the adaptive immune system might play a unique role in virus-induced AECOPD and Tregs may have different characteristics in virus-induced AECOPD. To our knowledge, studies on these factors have rarely been reported.
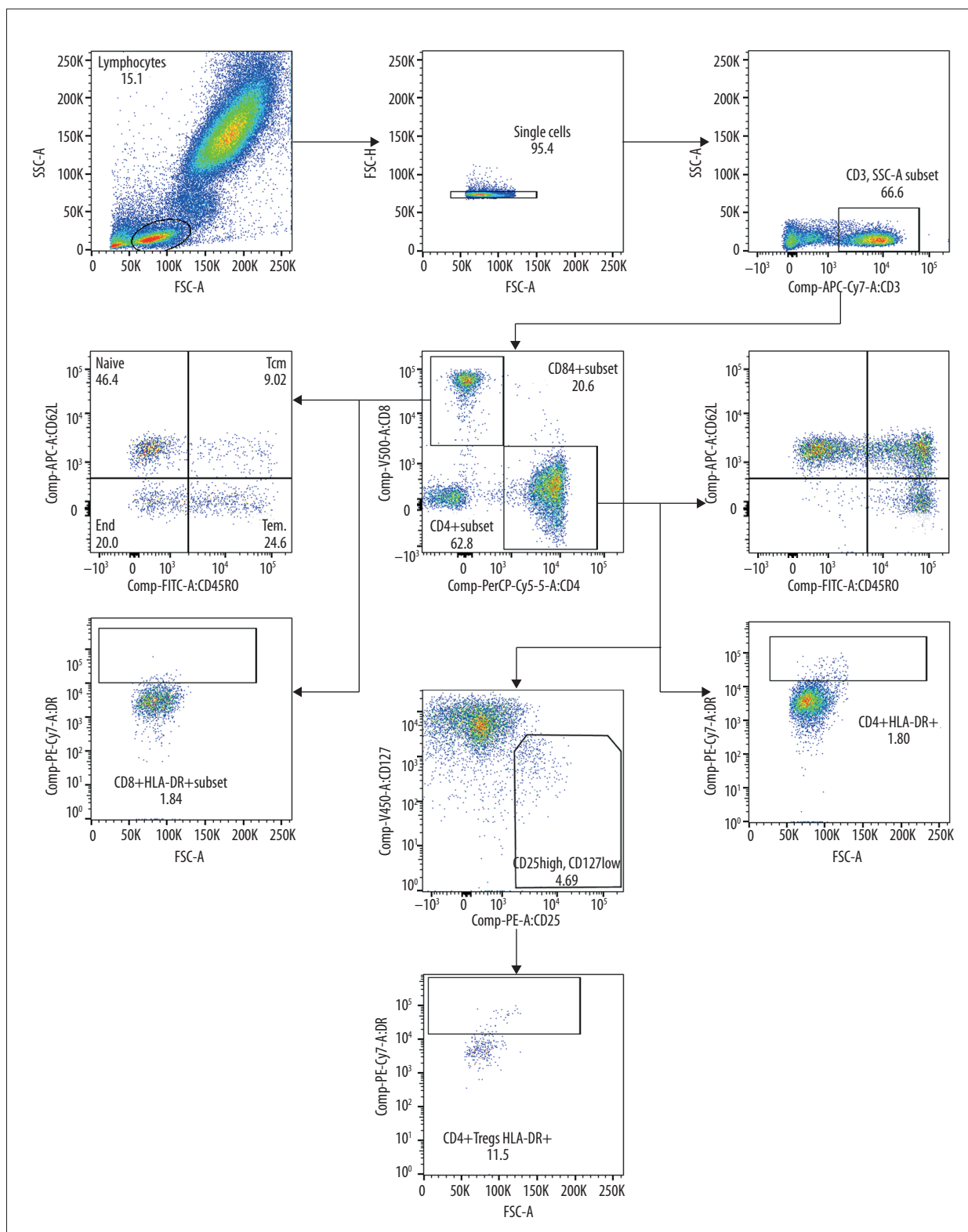
Figure 3. Gating strategy for T cells via flow cytometry analysis.
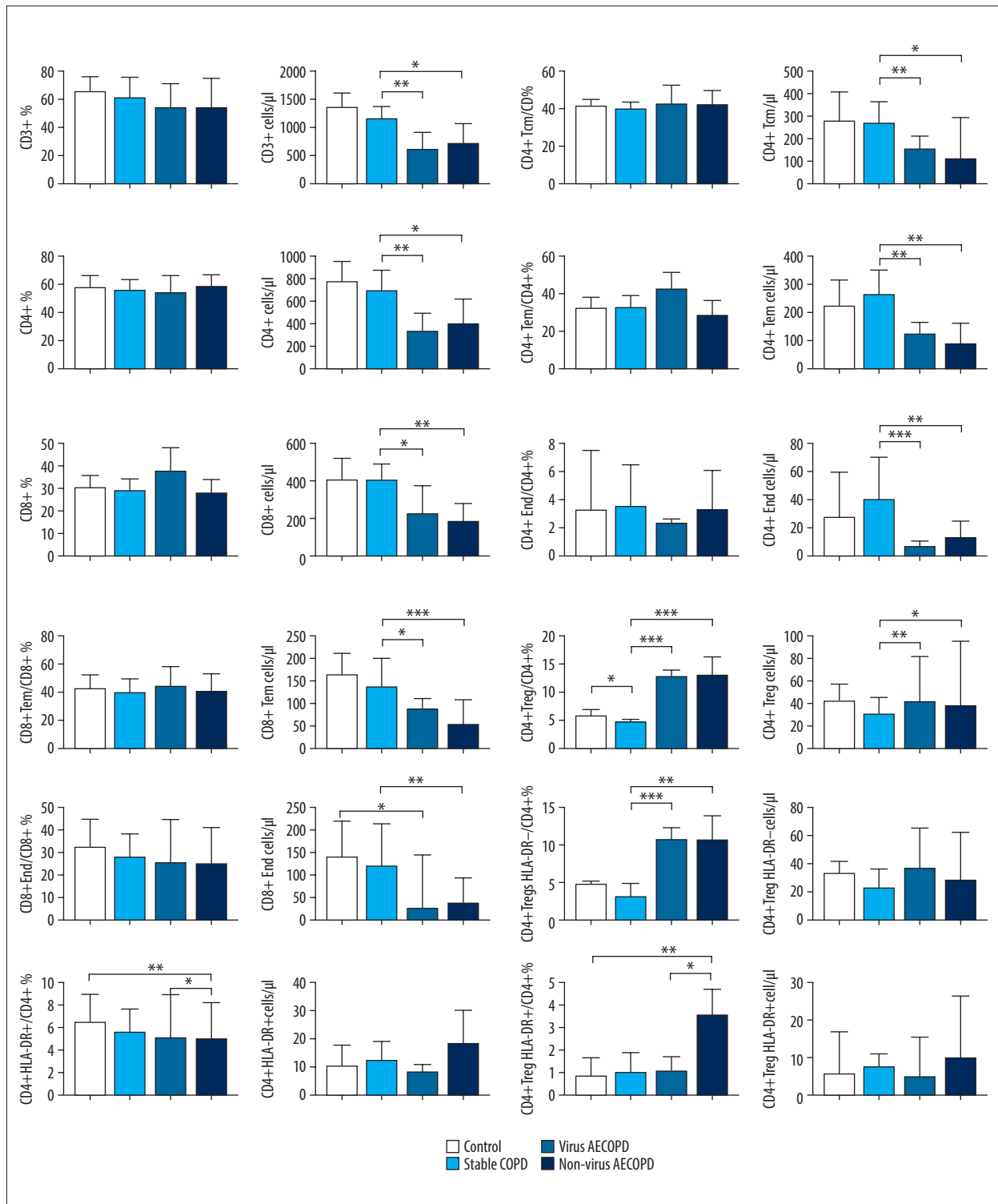
Figure 4. Virus-induced chronic obstructive pulmonary disease (COPD) exacerbations showed lower expression of HLA-DR on CD4+ regulatory T cells. The frequency (**left**) and level (**right**) of T cell subsets in exacerbation groups, stable COPD group, and healthy smoking group are shown. * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

Table 3. Flow cytometry analysis of levels of T cell subsets in exacerbation groups, the stable chronic obstructive pulmonary disease (COPD) group, and the healthy smoking group.

	Health smokers (n=20)		Stable COPD (n=20)	Virus AECOPD (n=32)	Non-virus AECOPD (n=31)	P
CD3+	1336±362		1130±333	598±320	692±510	0.001
		GOLD II	1071±354	768±272	815±473	
		GOLD III-IV	1180±339	471±325	610±561	
CD4+	764±253		688±243	324±173	400±290	0.001
		GOLD II	733±243	400±153	463±297	
		GOLD III-IV	658±261	267±184	357±306	
CD4+Naive	149 (99-184)		126 (72-163)	65 (28-82)	95 (29-182)	0.095
		GOLD II	115 (49-150)	60 (28-80)	95 (34-192)	
		GOLD III-IV	129 (91-182)	47 (11-72)	92 (24-188)	
CD4+Tcm	281 (246-411)		284 (237-393)	156 (74-211)	112 (50-295)	0.004
		GOLD II	340 (263-396)	156 (65-203)	206 (102-293)	
		GOLD III-IV	255 (210-335)	120 (29-200)	77 (41-313)	
CD4+Tem	223 (177-316)		264 (170-353)	105 (72-164)	66 (51-162)	0.010
		GOLD II	268 (170-435)	104 (70-154)	99 (65-233)	
		GOLD III-IV	260 (160-370)	91 (46-168)	56 (36-146)	
CD4+End	28 (17-59)		40 (22-70)	7 (2-10)	12 (2-24)	0.021
		GOLD II	41 (19-61)	5 (1-10)	13 (4-56)	
		GOLD III-IV	39 (26-90)	7 (2-9)	11 (2-24)	
CD4+HLA-DR+	10 (8-17)		12 (8-18)	8 (3-11)	18 (5-30)	0.456
		GOLD II	14 (9-17)	9 (5-11)	27 (10-34)	
		GOLD III-IV	9 (3-20)	6 (2-10)	10 (4-29)	
CD4+Treg	42 (32-58)		31 (22-46)	42 (11-75)	37 (28-95)	0.391
		GOLD II	33 (25-50)	39 (25-72)	49 (27-117)	
		GOLD III-IV	23 (18-45)	26 (7-76)	34 (25-94)	
CD4+Treg-HLA-DR+	6 (4-15)		8 (4-11)	5 (2-7)	10 (3-25)	0.224
		GOLD II	9 (5-16)	5 (2-8)	14 (4-23)	
		GOLD III-IV	6 (2-10)	3 (0.8-7)	8 (3-30)	
CD4+Treg-HLA-DR-	33 (27-41)		22 (17-36)	37 (9-66)	28 (19-62)	0.880
		GOLD II	21 (17-39)	31 (8-65)	31 (22-98)	
		GOLD III-IV	23 (10-34)	23 (6-66)	28 (10-60)	
CD8+	412±163		406±130	232±157	187±137	0.002
		GOLD II	425±113	309±90	238±122	
		GOLD III-IV	390±150	173±183	152±145	
CD8+Naive	31 (25-41)		35 (15-60)	28 (3-41)	16 (7-55)	0.773
		GOLD II	43 (13-72)	29 (3-49)	32 (14-71)	
		GOLD III-IV	27 (18-54)	25 (1-38)	11 (4-58)	
CD8+Tcm	45±27		43±23	34±22	27±23	0.495
		GOLD II	38±26	50±6	39±22	
		GOLD III-IV	45±19	22±21	18±20	

Table 3 continued. Flow cytometry analysis of levels of T cell subsets in exacerbation groups, the stable chronic obstructive pulmonary disease (COPD) group, and the healthy smoking group.

	Health smokers (n=20)		Stable COPD (n=20)		Virus AECOPD (n=32)		Non-virus AECOPD (n=31)		P
CD8+Tem	163 (105-214)		140	(113-201)	88	(44-112)	55	(22-110)	0.001
		GOLD II	142	(90-261)	86	(40-97)	56	(43-112)	
		GOLD III-IV	140	(116-190)	46	(28-210)	54	(21-69)	
CD8+End	140 (86-219)		121	(66-211)	28	(19-145)	38	(16-89)	0.021
		GOLD II	114	(60-204)	25	(18-140)	59	(27-127)	
		GOLD III-IV	133	(70-215)	23	(16-79)	21	(10-95)	
CD8+HLA-DR+	5.2 (3.1-7.3)		4.8	(2.5-8.0)	3.7	(1.1-8.9)	2.5	(1.2-3.9)	0.335
		GOLD II	6.6	(2.9-8.3)	4.1	(1.2-8.2)	3.0	(1.7-4.8)	
		GOLD III-IV	5.0	(2.5-5.0)	2.2	(0.6-9.3)	1.9	(0.6-3.7)	

Herein, the CD3+, CD4+, and CD8+ T-lymphocyte frequencies in the peripheral blood of AECOPD group were similar to those in the stable COPD group, but lower than in the stable COPD group and the healthy smoking group. Previously, similar levels of CD3+, CD4+, and CD8+ T lymphocytes were reported in the peripheral blood of individuals with stable COPD and healthy smokers [32], but the levels of CD4+ and CD8+ were lower in patients with AECOPD than in those with stable COPD [13], which supports our results. Mallia et al [33] established a rhino-virus-induced AECOPD model in vivo and found reduced numbers of CD4+ and CD8+ T lymphocytes in the peripheral blood after infection with a rhinovirus, but the numbers of CD3+ and CD8+ T lymphocytes were increased in bronchoalveolar lavage fluid. These findings implied that T lymphocytes gather from the peripheral blood to areas of inflammation after respiratory infection. In the present study, CD4+ and CD8+ T lymphocytes were divided into 4 groups including Naïve T cell, Tcm, Tem, and End by labeling of the expression of CD45RO and CD62L. From the data, we observed a decrease in the levels of CD4+ Tcm, CD4+ Tem, CD4+ End, CD8+ Tem, and CD8+ End in patients with AECOPD. The trends in variation were similar between the virus group and the nonvirus group, suggesting that viral or bacterial infection activated the lymphocytes of patients with COPD [34]. Naïve T lymphocytes were activated and differentiated into memory cells, and memory lymphocytes were recruited into lymph nodes and inflammatory tissues [35].

Furthermore, HLA-DR, CD25, and CD127 were also labeled. In accordance with recent literature, CD4+ Tregs refer to populations of CD4+ T cells with high CD25 expression and low CD127 expression on the cell surfaces (CD4+ CD25high CD127low) [36-39]. CD4+ CD25high CD127low HLA-DR+ has a stronger in vitro suppressor function on CD4+ CD25– T effector cells than CD4+ CD25high CD127low HLA-DR– [40], but it is also highly apoptotic [41]. The results for CD4+ Tregs in the peripheral blood from stable COPD patients were not consistent. CD4+ Tregs were

defined as CD4+ CD25high CD127low in the study of Chiappori et al [42], who found a lower frequency of these cells in the peripheral blood of patients with stable COPD relative to healthy smokers, with a positive association between these cells and FEV1% predicted. Additionally, the reduced frequency of CD4+ Tregs in the peripheral blood of patients with stable COPD compared with healthy nonsmokers was illustrated in other studies [42,43]. CD4+ Tregs referred to CD4+ CD25+ FoxP3+ in the study by Li et al [44], and these authors reported that CD4+ CD25+ FoxP3+ frequencies in the peripheral blood were similar in patients with stable COPD and healthy smokers. In contrast, Zheng et al [45] reported a lower frequency of CD4+ CD25+ FoxP3+ in the peripheral blood of stable COPD patients relative to healthy smokers. In the study of Bruzzaniti et al [46], CD4+ Tregs referred to CD4+ FoxP3+, and the CD4+ FoxP3+ frequency was increased in patients with stable COPD at GOLD II relative to that of healthy smokers and patients with stable COPD at GOLD III and IV. Kalathil et al [47] stated that the frequency of CD4+ Tregs in patients with no acute exacerbation at 4 weeks was increased compared with that in healthy nonsmokers. The inconsistent results from the studies may be due to differing selection criteria for stable COPD patients. In addition, CD4+ CD25+ FoxP3+ frequency in the peripheral blood of AECOPD patients was elevated compared with that of patients with stable COPD [14,15]. Another study reported contrary results [45], which may be due to the different selection criteria for AECOPD patients. The current study showed that higher CD4+ CD25high CD127low frequency was found in AECOPD compared with stable COPD. The frequency of CD4+ CD25high CD127low in stable COPD was lower than that in healthy smokers and the exacerbation groups presented a consistent trend. In addition, the levels of CD4+ Tregs between GOLD II and GOLD III-IV were similar in the stable COPD and exacerbation groups. The CD4+ Tregs frequency was slightly higher in the peripheral blood of patients with stable COPD at GOLD II compared with GOLD II-IV, but the difference was not statistically significant,

which was inconsistent with the conclusions of Chiappori et al [42] and Bruzzaniti et al [46]. This may be a result of the small sample size in each group in our study.

The study of Majori et al [48] delineated that the frequencies of HLA-DR+ in CD4+ and CD8+ T cells in the peripheral blood of patients with stable COPD were similar to a control group. Nevertheless, no previous studies have reported the expression of HLA-DR in T cells or CD4+ Tregs being a characteristic of virus-induced AECOPD. The current study depicted a lower frequency of CD4+ HLA-DR+ in the peripheral blood of patients in the virus-induced AECOPD group compared with the nonvirus group. The frequency of CD4+ Tregs HLA-DR+ was lower in CD4+ T cells, hinting that CD4+ Tregs in the virus group had a weaker and longer suppressor function on effector T cells in vivo, which was consistent with the more severe clinical symptoms of patients with virus-induced AECOPD.

This study had some limitations. First, the sample size was small, which may have decreased the statistical power. Another limitation was that we only analyzed T cells in peripheral blood; no immune cells were analyzed in sputum or alveolar lavage fluid, and the results revealed the systemic immune state rather than the local immune state of the lungs. In addition, we only compared the number of T cell phenotypes instead of

conducting further investigation of cell function. These limitations indicate that the results of our study should be interpreted cautiously.

Conclusions

The frequency of HLA-DR+ phenotypes of CD4+ Tregs was lower in the peripheral blood of patients with virus-induced AECOPD. The study of phenotypes of CD4+ Tregs in patients with AECOPD is helpful to better understand the effect of viruses on AECOPD, and it may provide a basis for individualized treatment of AECOPD in the future.

Acknowledgments

Conflict of Interests

None.

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