The role of eosinophils and corresponding cytokines and chemokines asthma-COPD overlap (ACO) patients

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Research

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Abstract

Background: ACO has been characterized as a kind of clinical disease with overlap symptoms of asthma and COPD. However, little is known of the role of eosinophils and corresponding cytokines and chemokines in ACO patients with different treatment responses.

Methods: To evaluate factors which associates with different treatment responses in patients with ACO. In the present study, we investigated the eosinophils proportion of peripheral blood from ACO patients with acute exacerbation (AE) after treatment, ACO patients with clinical response (CR) after treatment, and healthy volunteers (HV) by using flow cytometry analysis. The plasma levels of corresponding cytokines and chemokines from the three groups were evaluated by ELISA.

Results: The results showed that ACO patients that had acute exacerbation have relatively lower eosinophils proportions compared to healthy volunteers but have higher eosinophils inflammation compared with patients with clinical response. The percentage of NK1R+ expression population eosinophils was also decreased. Further analysis revealed ACO patients that had acute exacerbation also have relatively higher plasma levels of cytokines and chemokines compared to patients with clinical response after treatments and healthy volunteers.

Conclusion: ACO patients from AE group have relatively lower eosinophil proportion and NK1R+ expression population eosinophil proportion, but higher plasma levels of cytokines and chemokines. Inhibitors of cytokines and chemokines are likely useful agents for treatment of ACO patients which had acute exacerbations.

Background

Asthma and chronic obstructive pulmonary disease (COPD) are major public health problems around the world due to the high incidence and healthcare costs [1]. It is reported that a subpopulation of COPD patients shows clinical characteristics of asthma, which makes a great challenge for diagnosis and treatments [2]. Asthma-COPD overlap (ACO) has been considered as a kind of clinical disease with overlap symptoms of asthma and COPD. The incidence of ACO varied from 15 to 60%, with variation depending on gender and age. The patients with ACO also have worse prognosis than that of patients with COPD or asthma alone [1, 3, 4]. However, the pathogenesis of ACO remained elusive and the treatment of ACO is also very limited.

Immune system plays important roles in pathogenesis of ACO. Cytokines are kinds of small, secreted proteins that play important role in immune response. Eosinophils, basophils as well as type 2 helper T cells are found to be predominantly infiltrated in early-onset asthma [5, 6]. In contrast, studies from adult-onset asthma revealed that it was characterized predominantly by inflammation involving type 1 helper T lymphocytes [7]. Type 17 helper T cells were involved in asthma by secreting IL-17 to recruit neutrophils to the airway, which aggravates the asthma [8, 9]. COPD is also characterized as serve inflammation. Studies

from COPD indicated that Th1 cells, macrophages, and neutrophils promoted COPD progression by secreting classical pro-inflammatory cytokines such as TNF-α, IFN-γ, IL1, IL6 and IL-84 [10, 11]. Moreover, Th2 inflammation-related genes were found to be significantly up-regulated in airway walls of patients with COPD, which was similar in asthma patients [12].

Currently, Omalizumab, a recombinant humanized monoclonal anti-immunoglobulin E, is often used for treatments for patients with ACO [13–15]. However, the potential roles of eosinophils and corresponding cytokines and chemokines in ACO are still unclear. In this study, we investigated the roles of eosinophils and corresponding cytokines and chemokines including IL-5, CCL5, CCL7, CCL11, and CCL13 in blood of ACO patients with acute exacerbations or clinical response after treatment, aiming to provide more clinical evidence for ACO treatments.

Methods

Patients

The blood samples were obtained from patients who were diagnosed as ACO according to the recent guidelines of GINA and GOLD at the First Affiliated Hospital of Jinzhou

Medical University hospital from 2018 to 2019 [16]. The exclusion criteria included tumor, severe liver, kidney or heart diseases. Patients with cognitive and communication disorders caused by neurological diseases were also excluded from this study. The normal blood samples were obtained from healthy volunteers. The ethics committee of the First Affiliated Hospital of Jinzhou Medical University approved the study and all the patients were informed consent. Detailed information of patients is in Table 1.

Table 1
Clinical features of the patients included in this study

Variables	Acute Exacerbation	Clinical Response	Healthy Volunteers
No. of samples	34	34	8
Median age, y (range)	53 (39–78)	56 (45–82)	53 (35–77)
Gender			
Male (%)	15 (44.1%)	14 (41.2%)	3 (37.5%)
Female (%)	19 (55.9%)	20 (58.8%)	5 (62.5%)
Weight (kg)	61.0	60.3	60.9
Height (cm)	163.4	163.0	164.8
BMI	22.7	22.8	22.4

ELISA assay

Blood samples were taken from ACO patients after treatments. 5 ml fasting venous blood samples were collected in the morning between7 and 8 am, centrifuged under 4 ℃ at 3000 rpm for 30 min and subsequently stored at 80 ℃ until used. The IL-5, CCL5, CCL7, CCL11, and CCL13 levels in serum of ACO patients or healthy volunteers were detected by ELISA assay via BD OptEIA™ Human ELISA kits according to the manufacturer's instructions (Cat. 555202, Cat. 555034, Cat. 555175, Cat. 564754, and Cat. 3010104. BD, USA).

Flow cytometry assay

5 ml fasting venous blood samples were collected in EDTA-containing tubes (100 mM), centrifuged under 4 ℃ at 3000 rpm for 30 min, and resuspended using PBS. Red blood cells were lysed with red blood cell lysis buffer. The blood was then incubated with 2% Fc block (Cat. 564219. BD, USA) at 4 ℃ for 15 min to reduce nonspecific binding. FSC-W and FSC-A discrimination was adopted to exclude doublet cells, and Hochest (Cat. C0021. Solarbio, China) dye was used to exclude dead cells. Blood eosinophils were identified as SSC^{hi} $CD123^{-}$ $HLA\text{-}DR^{-}$ $CCR3^{+}$ cells (Cat. 565928, Cat. 564516, Cat. 562570. BD, USA) [17]. To detect SP and NK1R expression in eosinophilic granulocytes, FITC-conjugated mouse anti-human SP (Cat. IC007A. R&D, USA) and APC-conjugated mouse anti-human NK1R antibodies (Cat. FAB66871A. R&D, USA) were added for 30 min at 4 °C. After washing, the cells were analyzed on FACSCANTO II (BD Biosciences). FlowJo was used to analyze the results.

Statistical analysis

Levels of different proteins were calculated according to the standard curve of the ELISA assay. The differences of proteins in blood of ASO patients and healthy were assessed by the Student *t* test. The representative data shown are means ± SEM. Statistical analyses were performed with Prism 6 (GraphPad Software). P values under 0.05 were considered as significant.

Results

The peripheral blood lymphocytes proportion in ACO acute exacerbation or clinical response groups.

The clinical characteristics of all participants are shown in Table 1. The three groups of patients had similar age distribution and sex ratios, which provide robustness of subsequent analyses. We performed FACS analyses of blood from ACO patients with acute exacerbation (AE) after treatment, ACO patients with clinical response (CR) after treatment, and healthy volunteers (HV). The eosinophils were characterized as SSC^{hi} $CD123^{-}$ $HLA\text{-}DR^{-}$ $CCR3^{+}$ cells (Figure. 1a-c). As the results showed, ACO patients that had acute exacerbation after treatments had lower counts of eosinophils compared to healthy volunteers, further analysis revealed that ACO patients that had acute exacerbation after treatments had higher percentage of eosinophils compared with patients with clinical response (Figure. 1d). Together, these results indicate that ACO patients that had acute exacerbation have relatively lower eosinophils

proportions compared to healthy volunteers but have higher eosinophils inflammation compared with patients with clinical response.

Expression of SP and NK1R in peripheral blood leukocytes of ACO patients with clinical response or acute exacerbation

Substance P (SP) and its receptor NK1R were reported to be important pro-inflammatory mediators of eosinophils [18, 19]. SP/NK-1 complex also played important roles in many human diseases including eczema, intestinal fibrogenesis after chronic colitis, and chronic spontaneous urticarial [20]. In order to investigate the roles of SP and NK1R in ACO, we examined the expression of SP and NK1R in eosinophils identified from AE, CR and HV peripheral blood. According to the gating strategy, the results showed that there was no significant difference of percentages of SP positive eosinophils (SSC^{hi} $CD123^{-}$ $HLA\text{-}DR^{-}$ $CCR3^{+}$) in AE, CR and HV groups (Figure. 2a). On the other hand, the results showed that the NK1R expressing eosinophils were dramatically decreased in both CR and AE groups compared with HV blood (Figure. 2b).

Levels of cytokines and chemokines in ACO acute exacerbation or clinical response groups.

In order to investigate the potential role of cytokines and chemokines in ACO, we examined the change of their levels in the plasma of ACO patients from the following three groups by ELISA assay. The result revealed that plasma CCL5, CCL7, CCL11, and CCL14 levels were significantly increased in patients that had acute exacerbation after treatments (Figure. 3a-d). However, there were no significant changes of levels of CCL5, CCL7, CCL11, and CCL14 in plasma of ACO patients with clinical response after treatments and healthy volunteers (Figure. 3a-d). Moreover, plasma IL5 showed slighted increased in AE group compared with CR group, but no significant change of plasma IL5 level was observed between AE and HV groups (Figure. 3e). Taken together, the following results indicate that ACO patients that had acute exacerbation have relatively higher plasma levels of cytokines and chemokines compared to patients with clinical response after treatments and healthy volunteers.

Discussion

In recent times, even though several studies have focused on the symptoms and clinical features of ACO, the pathogenesis and treatment response of this disease remained poorly studied [2, 4, 21]. In this study, we examined the proportions of eosinophilic granulocytes in the peripheral blood obtained from ACO patients with acute exacerbation after treatment, ACO patients with clinical response after treatment, or healthy volunteers. Interestingly, ACO patients that had acute exacerbation have relatively lower eosinophils proportions compared to healthy volunteers, but have higher eosinophils inflammation compared with patients with clinical response. The plasma levels of cytokines and chemokines including CCL5, CCL7, CCL11, CCL14, and IL-5 were also elevated, while all of these cytokines and chemokines were increased in ACO patients with acute exacerbation after treatment compared to those with clinical response or healthy controls. We demonstrate that plasma levels of cytokines and chemokines, as well as

peripheral blood eosinophilic granulocytes proportion can be biomarkers for develop appropriate treatment strategies for ACO patients, which also indicated the essential roles of immune response in ACO pathogenesis.

Eosinophilic granulocytes which are driven by CD4 positive T cells often lead to the chronic inflammation asthma [22]. Eosinophilic inflammation has been also observed in some population of COPD patients and is associated with greater reversibility of obstruction when using steroids [23]. Moreover, there is evidence that inflammation is it has been already proven from histopathological and other studies that inflammation concerns both small and large airways in asthma and COPD patients [24–26]. SP/ NK1R complex has been recognized as important regulators of eosinophilic inflammation, and also been involved in the molecular bases of asthma and COPD [27–29]. It is reported that SP could activate eosinophils through degranulation [30]. Furthermore, the SP/NK1R axis is also the potential targets for treating asthma and COPD, inhibiting the pro-inflammatory effects of SP using tachykinin receptor antagonists may relieve the inflammation diseases such as asthma and COPD [31, 32]. However, the role of SP/NK1R in ACO remains unclear, we find that there are no significant differences percentages of SP + eosinophils between AE, CR and HV groups. In contrast, AE and CR groups show dramatically decrease of the percentage of NK1R + eosinophils, which may be the potential therapeutic targets in the furfure managements of ACO.

Type two cytokines including interleukin-4 (IL-4), IL-5, and IL-13 are associated with asthma and chronic obstructive pulmonary disease through promoting promote airway eosinophilia, mucus overproduction, bronchial hyperresponsiveness (BHR), and immunogloubulin E (IgE) synthesis [6]. IL-5 plays an essential role in eosinophilic inflammation by stimulating the differentiation of eosinophils and prolonging their survival in the airways [33, 34]. Antibodies target IL-5, such as Mepolizumab and Reslizumab, are often used to decrease the exacerbation rates of asthma patients. Moreover, Mepolizumab could be also used for COPD managements to reduce small exacerbations [35, 36]. In this study, increased IL-5 was also observed in AE groups, which imply the potential application of anti-IL-5 antibodies in ACO treatments.

The chemokines are closed correlated with chronic asthmatics through the accumulation of eosinophils in and around the airways. When these eosinophils are activated, they would degranulate and damage the surrounding tissue, which lead to exacerbate the asthmatic condition [37, 38]. Chemokines include CCL5, CCL7, CCL11 and CCL13. Early studies revealed that CCL5 was a potential eosinophil chemoattractant, and it could elicit an eosinophil-rich exudate when injected in the mice [39]. Moreover, all of the CCL5, CCL7, CCL11 and CCL13 could bind to CCR3 to induce degranulation of eosinophils, which indicated that CCR3 could be a potential therapeutic target. CCR3 deletion in mice significantly decreased the accumulating of eosinophils to the airways, which decrease the exacerbation rates at some extent [40, 41]. Our data revealed that all of the four chemokines were dramatically increased in AE groups, which indicate CCR3 may paly vital roles in ACO pathogenesis, remained further analysis.

Conclusions

ACO patients that had acute exacerbation have relatively lower eosinophils proportions compared to healthy volunteers but have higher eosinophils inflammation compared with patients with clinical response. Furthermore, ACO patients that had acute exacerbation also have relatively higher plasma levels of cytokines and chemokines compared to patients with clinical response after treatments and healthy volunteers. Inhibitors of cytokines and chemokines are likely useful agents for treatment of ACO patients which had acute exacerbations.

List Of Abbreviations

ACO: asthma-COPD overlap

IL: interleukin

SP: Substance P

COPD: Asthma and chronic obstructive pulmonary disease

AE: acute exacerbation

CR: clinical response

HV: healthy volunteers

Declarations

Ethics approval and consent to participate

The ethics committee of the First Affiliated Hospital of Jinzhou Medical University approved the study and all the patients were informed consent.

Availability of data and materials

Data are available following submission of a valid research proposal to the corresponding author.

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Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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Authors' contributions

QL and SL conceived and designed the study; QL, SG, LL collected samples and whole data; QL, HZ, LL, and XL prepared the samples; QL conducted statistical analysis; QL wrote the first manuscript; HZ, XL, and SL edited and finalized the manuscript. All authors read and approved the final manuscript.

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Figures

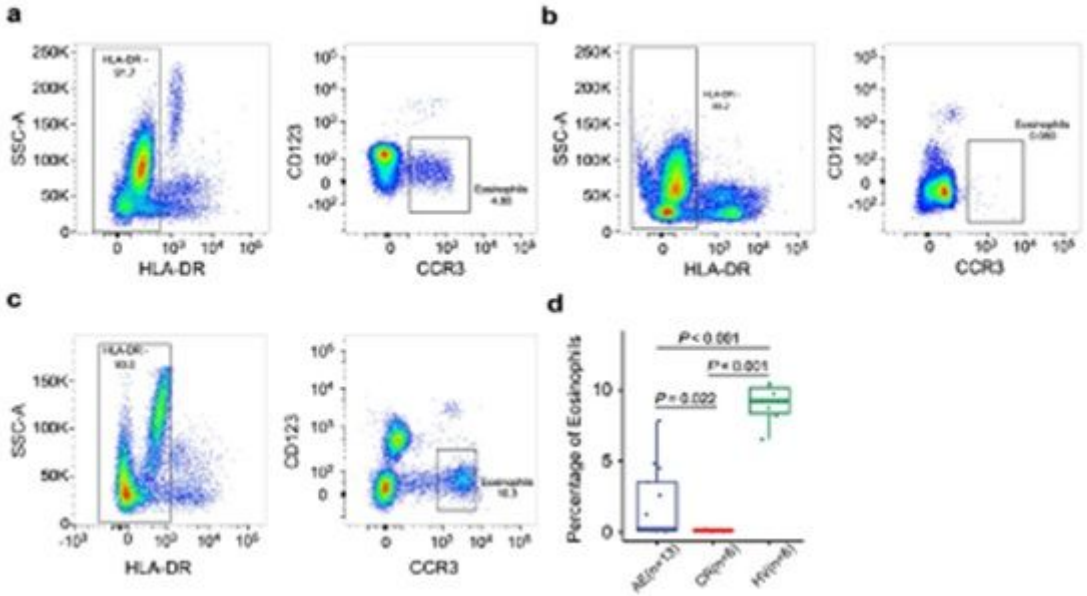

Figure 1

Flow cytometry analysis of eosinophils in peripheral blood leukocytes of ASO patients with acute exacerbation (AE), with clinical response (CR), or healthy volunteers (HV). a-c: Representative graphs of percentages of eosinophils out of whole peripheral blood leukocytes in AE, CR and HV groups. d: Represents median values of percentage of eosinophils from AE, CR and HV groups. The lines in the boxes represent the median values, the boxes represent 25–75%. The p value between two groups was determined using student t test.

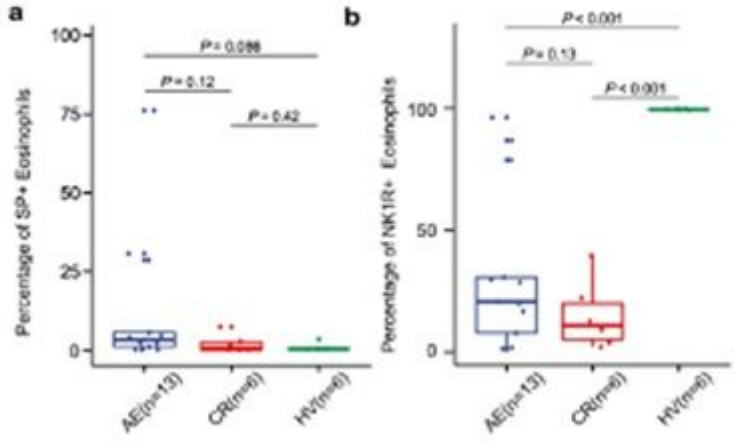

Figure 2

Flow cytometry analysis of expression of substance P (SP) and NK1R in eosinophils of ASO patients with acute exacerbation (AE), with clinical response (CR), or healthy volunteers (HV). a: Represents median values of percentage of SP+ cells out of eosinophils in AE, CR and HV groups. b: Represents median values of percentage of NK1R+ cells out of eosinophils in AE, CR and HV groups. The lines in the boxes represent the median values, the boxes represent 25–75%. The p value between two groups was determined using student t test.

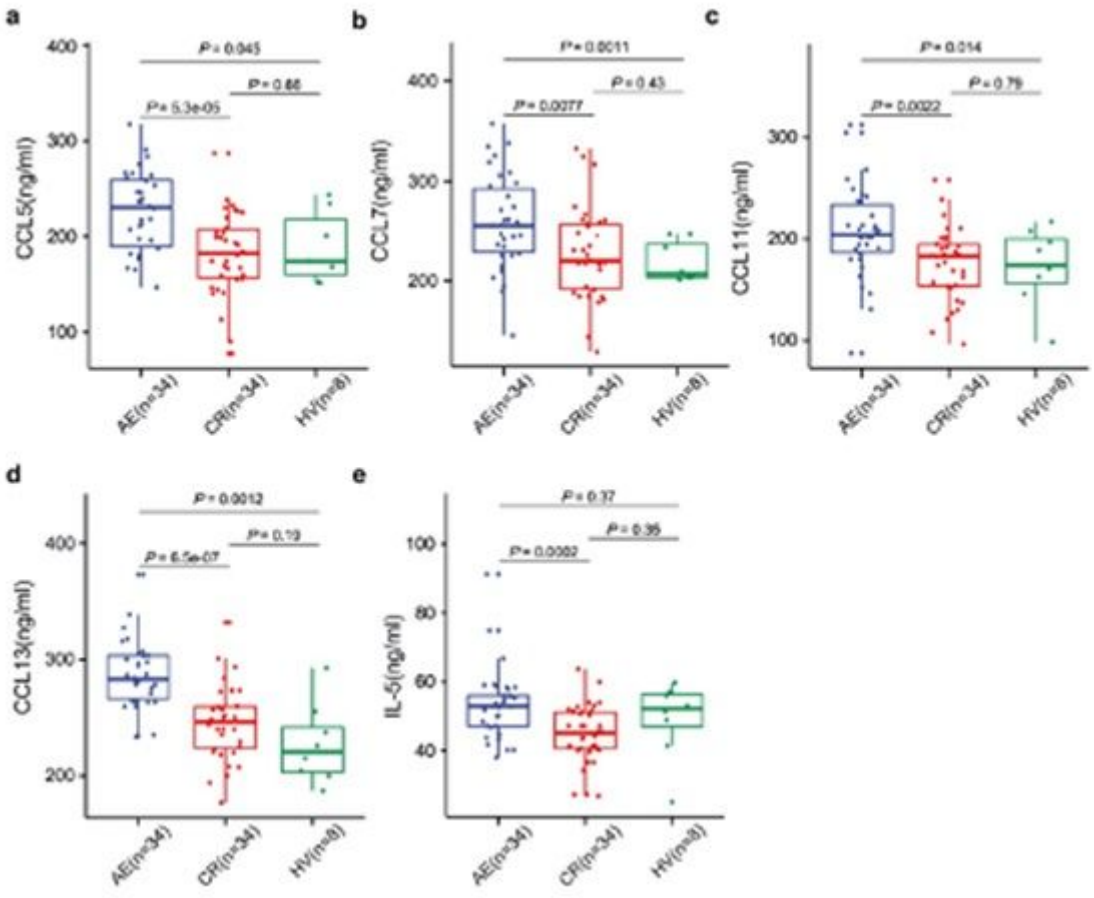

Figure 3

The levels of plasma cytokines and chemokines in ASO patients with acute exacerbation (AE), with clinical response (CR), or healthy volunteers (HV). a-e: Represents median values of CCL5 (a), CCL7 (b), CCL11 (c), CCL13 (d), and IL-5 (e) in AE, CR and HV groups. The lines in the boxes represent the median values, the boxes represent 25–75%. The p value between two groups was determined using student t test.