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An evaluation of CD39 as a novel immunoregulatory mechanism invoked by COPD

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ABSTRACT

Acute exacerbations of chronic obstructive pulmonary disease (AECOPD) are characterized by increased pulmonary and systemic inflammation and commonly caused by bacterial and/or viral infection. Little is known about the T-cell dysregulation in AECOPD that promotes these outcomes. CD39 is an ectonucleotidase able to hydrolyse adenosine triphosphate to create adenosine that may inhibit T-cell responses in patients with AECOPD. Here T-cell expression of CD39 measured by flow cytometry was higher in AECOPD patients than stable COPD patients or healthy controls. Higher expression of CD39 was associated with higher levels of plasma soluble tumor necrosis factor receptor but lower interferon-γ (IFNγ) levels in supernatants from staphylococcal enterotoxin-B stimulated peripheral blood mononuclear cells. This links increased expression of CD39 with systemic inflammation and impaired T-cell responses (e.g. IFNγ). The blockade of CD39 pathways may be a novel approach to the control of AECOPD, reducing the dependency on antibiotics.

1. Introduction

Chronic obstructive pulmonary disease (COPD) is amongst the top five causes of global morbidity and mortality. COPD is characterized by chronic airway and systemic inflammation and so predisposes patients to ischemic heart disease, vascular disease, muscle wasting and cachexia [1,2]. COPD patients may also experience acute exacerbations (AECOPD), commonly caused by infections [3]. AECOPD can accelerate the decline in lung function [4], but the associated immune dysregulation is not well understood. AECOPD may be an immune-deficient state reflecting an impaired T-helper type I (Th1) responses to infectious agents (e.g. decreased production of IFNγ) [5–9]. There are several anti-inflammatory molecules, including CD39 that may be highly expressed in AECOPD that may inhibit IFNγ responses.

CD39 is an ectonucleotidase which generates adenosine monophosphate (AMP) from adenosine triphosphate/adenosine diphosphate (ATP/ADP). CD73 (another ectonucleotidase) then converts AMP to adenosine, an anti-inflammatory molecule that can inhibit the function of $CD4^+$ and $CD8^+$ T-cells and natural killer cells [10]. CD39 is highly expressed by regulatory T-cells (Tregs) and is important for their immune-suppressive function [11,12]. Inflammation usually results in the release of ATP that is converted to adenosine by Tregs via the CD39/CD73 pathway. The binding of adenosine to A_{2A} receptors downregulates the activity of effector T-cells but promotes the expansion of Tregs and their suppression of effector T-cell proliferation [13].

Therefore, CD39 and CD73 are important in the regulation of immune responses by inhibiting the ATP-driven pro-inflammatory immune cell activity and promote an anti-inflammatory state mediated by adenosine [10]. Since AECOPD may be an immunodeficient state, we hypothesized that chronic inflammation induces the expression of CD39 that then inhibits protective effector T-cell responses (e.g. IFNγ production) against bacteria in AECOPD.

2. Material and methods

2.1. Study subjects

AECOPD patients (n = 21) admitted to the Royal Perth Hospital Emergency Department (Western Australia) were recruited. Stable

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COPD (sCOPD) patients who were previous smokers (>15 pack-years and ceased smoking >5 years earlier) from a dedicated Royal Perth Hospital COPD clinic (n = 33) and healthy age-matched, non-smoking subjects with no clinical evidence of COPD were also included as controls (n = 33). The diagnosis and severity of COPD was categorised by a respiratory physician according to the GOLD criteria (Stages 2–4). All patients had been treated with anticholinergic drugs, long-acting beta agonists and inhaled corticosteroids but none were receiving systemic corticosteroids or had diabetes, neuromuscular, allergic or rheumatological disease at the time of sampling. The study was approved by the Ethics Committee at the Royal Perth Hospital and all participants gave informed consent.

2.2. Sample and data collection

Peripheral blood mononuclear cells (PBMC) were isolated from blood collected in lithium heparin tubes by Ficoll gradient centrifugation and cryopreserved in 10% dimethyl sulfoxide/fetal calf serum. Plasma was stored at −80 °C. Plasma levels of IL-6, C-reactive protein (CRP) and sTNFR were measured by ELISA (R&D Systems, Minneapolis, MN). Proportions of $CD39^+$ T-cells were quantified by flow cytometry after staining PBMC with anti-CD3-APC-H7, CD4-V500, CD8-PerCP-Cy5.5, CD39-FITC, CD28-PE-Cy7 and PD-1-APC antibodies (BD Biosciences, San Jose, CA). Intracytoplasmic staining was performed using the BD Pharmingen™ FoxP3 buffer set and FoxP3-PE antibody (BD Biosciences). 200,000 events were acquired using a BD FACSCanto II cytometer and analyses were done with FlowJo v5.7.2 software (Tree Star, Ashland, OR). Gating for T-cell subsets and expression of CD39 is shown in Fig. 1. Concentrations of IFNγ, TNFα, IL-6, IL-10 (BD Biosciences) and IL-17 (eBioscience, San Diego, CA) were measured by ELISA in supernatants from PBMC (2×10^6 cells/mL) cultured with SEB (1 μg/mL; Sigma-Aldrich, St. Louis, MO) for 24 hr.

2.3. Data analysis

Non-parametric Mann-Whitney tests were used to compare groups. Correlations were assessed using Spearman's rank correlation coefficient. Statistical analyses were performed with Graphpad Prism 5.04 software (La Jolla, CA). Statistically significant differences ($p < 0.05$) are indicated in the figures and $p = 0.05$–0.10 is noted as marking a trend.

3. Results

3.1. AECOPD patients exhibited systemic inflammation but decreased SEB-induced IFNγ production

Stable COPD (sCOPD) and AECOPD patients had elevated plasma levels of CRP ($p = 0.004$ & $p < 0.001$, respectively), IL-6 ($p = 0.001$ & $p = 0.003$, resp.) and sTNFR ($p = 0.007$ & $p < 0.001$, resp.) when compared to healthy controls (Fig. 2A-C). AECOPD patients had marginally higher plasma CRP ($p = 0.07$), IL-6 ($p = 0.09$) and sTNFR ($p = 0.04$) than sCOPD patients.

Following stimulation with SEB, levels of IFNγ, TNFα and IL-6 was lower in PBMC from AECOPD patients than sCOPD patients ($p = 0.0001$–0.002) and healthy controls ($p = 0.0004$–0.005) (Fig. 2D-F). The production of IL-17 and IL-10 was lower in AECOPD

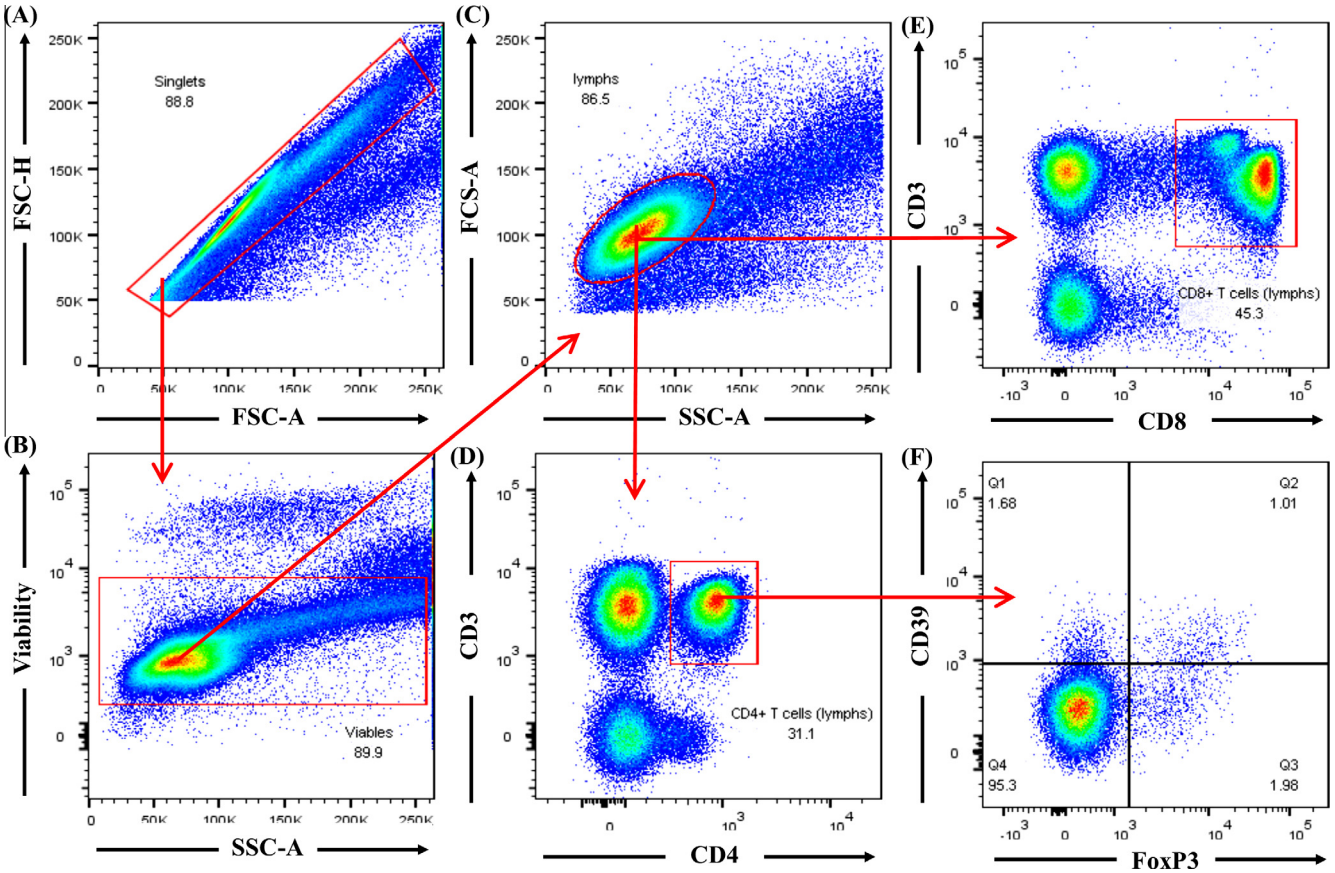

Fig. 1. Gating strategies used to identify CD39+ T-cell subsets: (A) Singlets were gated based on the expression of forward scatter-area (FCS-A) and FCS-Height. (B) Dead cells were then excluded and (C) lymphocytes were gated based on the expression of side scatter (SSC)-A and FSC-A. (D) $CD4^+$ or (E) $CD8^+$ T-cells were identified by co-expression of CD3 with CD4 or CD8 respectively. (F) Co-expression of CD39 and Foxp3 from $CD4^+$ T-cells.