Subsets of airway myeloid-derived regulatory cells distinguish mild asthma from chronic obstructive pulmonary disease

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Background: Subsets of myeloid-derived regulatory cells (MDRCs), which are phenotypically similar to the myeloid-derived suppressor cells found in patients with cancer, have recently been appreciated as critical regulators of airway inflammation in mouse models of asthma.

Objective: We test the hypothesis that subsets of airway MDRCs contribute differentially to the inflammatory milieu in human asthma and chronic obstructive pulmonary disease (COPD).

Methods: We used bronchoalveolar lavage to identify and characterize human airway MDRCs from 10 healthy subjects, 9 patients with mild asthma, and 8 patients with COPD, none of whom were treated with inhaled or systemic corticosteroids. We defined subsets of airway MDRCs using flow cytometry, the molecular mediators they produce, and their abilities to regulate proliferation of polyclonally activated autologous T lymphocytes.

Results: We found substantial differences in the functional potential of MDRC subsets in healthy subjects, patients with asthma, and patients with COPD, with these differences regulated by the nitrosative and oxidative free radicals and cytokines they produced. Nitric oxide–producing MDRCs suppressed and superoxide-producing MDRCs enhanced proliferation of polyclonally activated autologous CD4 T cells. HLA-DR$^+$CD11$^+$CD11c$^+$CD163$^-$ superoxide-producing MDRCs, which stimulated proliferation of autologous T cells, comprised a high fraction of MDRCs in the airways of patients with mild asthma or COPD but not those of healthy control subjects. CD11b$^+$CD14$^+$CD16$^-$HLA-DR$^-$ nitric oxide–producing MDRCs, which suppressed T-cell proliferation, were present in high numbers in airways of patients with mild asthma but not patients with COPD or healthy control subjects.

Conclusion: Subsets of airway MDRCs conclusively discriminate patients with mild asthma, patients with COPD, and healthy subjects from each other. The distinctive activities of these MDRCs in patients with asthma or COPD might provide novel targets for new therapeutics for these common disorders. (J Allergy Clin Immunol 2015;135:413-24.)

Key words: *Myeloid cell, macrophage, nitric oxide, superoxide, regulatory T cell*

Myeloid-derived suppressor cells (MDSCs) are a heterogeneous group of immature myeloid cells that inhibit lymphocyte function through a range of mechanisms. These include production of reactive oxygen species (ROS) and reactive

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Abbreviations used

BAL: Bronchoalveolar lavage
BMI: Body mass index
COPD: Chronic obstructive pulmonary disease
DAF-FM-DA: 4-Amino-5-methylamino-2′,7′-difluorofluorescein diacetate
DHE: Dihydroxyethidium
DPI: Diphenyleneiodonium
FACS: Fluorescence-activated cell sorting
iNOS: Inducible nitric oxide synthase
MDRC: Myeloid-derived regulatory cell
MDSC: Myeloid-derived suppressor cell
NADPH: Nicotinamide adenine dinucleotide phosphate
NK: Natural killer
NO: Nitric oxide
nor-NOHA: N(omega)-hydroxy-nor-L-arginine
PCA: Principal component analysis
PMA: Phorbol 12-myristate 13-acetate
RNS: Reactive nitrogen species
ROS: Reactive oxygen species
SOD: Superoxide dismutase
UAB: University of Alabama at Birmingham

nitrogen species (RNS), which are generated by the inducible nitric oxide synthase (iNOS) and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase enzymes, and depletion of key nutrients required for normal function of T cells, especially arginine through activation of arginase and tryptophan and cysteine through sequestration in tumor-specific T cells.[1-6] Additionally, activation of T cells can be impaired by nitration of their antigen or chemokine receptors[7] or suppressed by induction of regulatory T cells through TGF-β produced by MDSCs.[8]

We and others have shown that the iNOS, NADPH oxidase, and arginase pathways are critical for the ability of these myeloid lineage cells to control T-cell responses.[2,6,9-14] MDSCs are significant sources of nitric oxide (NO) and ROS in patients with cancer, as well as in those with other conditions characterized by chronic inflammation.[2-4,9,10] In a mouse model of allergic airway inflammation, we demonstrated that distinct subsets of NO-producing anti-inflammatory MDSCs and $O_2^{\cdot-}$-producing proinflammatory myeloid cells are major sources of free radicals and critical regulators of the inflammatory response.[10] NO-producing myeloid cells suppressed airway hyperresponsiveness in mice through iNOS-derived NO, arguing for a protective function of NO in attenuation of the inflammatory response in asthmatic patients.[10] Superoxide generated by a subpopulation of cells with phenotypic characteristics of MDSCs contributed to increased T-cell inflammatory responses and increased airway hyperresponsiveness in an NADPH oxidase–dependent fashion.[10] We referred to these NO- and $O_2^{\cdot-}$-producing cell subsets as myeloid-derived regulatory cells (MDRCs) because of their broad functions as both upregulators and downregulators of the inflammatory response. An imbalance in the ratio of these anti-inflammatory and proinflammatory myeloid cell subsets might contribute to many chronic airway inflammatory disorders.

Increased levels of RNS, including NO and its metabolites, and ROS, especially $O_2^{\cdot-}$, are prevalent in human subjects with inflammatory disorders of the lung.[15-18] In asthmatic patients levels of NO produced by iNOS and levels of urea produced by arginase are correlated with the degree of inflammation and with clinical exacerbations.[19-22] The NO synthase/arginase ratio can also contribute to bronchial tone in patients with chronic obstructive pulmonary disease (COPD).[23,24] Although levels of exhaled NO are much lower in patients with stable COPD than in asthmatic patients, cross-talk between ROS and RNS and the role of RNS, particularly peroxynitrite, in the inflammatory mechanisms underlying COPD are well appreciated.[22,25,26] Despite the fact that there might be differences in the inflammatory patterns and contributions of nitrosative and oxidative stress between patients with bronchial asthma and those with COPD, the iNOS, NADPH oxidase, and arginase pathways are likely to contribute to the inflammatory milieu in both of these common airway diseases.

We and others have shown that increased concentrations of the metabolites of iNOS are localized to the smaller distal airway in human asthmatic patients.[15,27] This suggests that in these patients the primary cellular sources of iNOS-derived NO might be localized in the bronchiolar and/or alveolar compartments. In contrast, we found that ROS were present in both the proximal and distal airway compartments.[15] We hypothesize that MDRC subsets contribute importantly to the inflammatory milieu in the airways of patients with asthma and those with COPD. We hypothesize further that the production of RNS and ROS by individual MDRC subsets contributes to their abilities to regulate the inflammatory and immune responses. Although peripheral blood MDSC subsets have recently been shown to be increased in asthmatic patients,[28,29] the phenotypic and functional relationships of these cells in the airways of these patients have not been investigated.

In this study we have analyzed free radical–producing myeloid cells recovered by means of bronchoalveolar lavage (BAL) from 10 healthy subjects, 9 patients with mild asthma, and 8 patients with moderate COPD. We identified distinct subsets of NO- and $O_2^{\cdot-}$-producing MDRCs in the airways of patients with asthma and those with COPD and showed that these cells can modulate T-cell responses through free radical–dependent mechanisms. Importantly, we found that the proportions of HLA-DR$^+$CD11c$^+$CD163$^-$ $O_2^{\cdot-}$-producing MDRCs were high in the airways of patients with mild asthma or COPD but low in healthy subjects and that proportions of CD11b$^+$CD14$^+$CD16$^-$HLA-DR$^-$ NO-producing MDRCs were high only in patients with mild asthma but not in patients with COPD or healthy control subjects. Although additional airway MDRC subsets with distinct free radical profiles and T cell–modulating functions were identified and showed some degree of association with the 3 clinical phenotypes studied here, the MDRC subsets described above contributed prominently to the ability to completely discriminate the asthmatic, COPD, and healthy populations we analyzed. The tight association of MDRC phenotypes with the discrete inflammatory phenotypes that characterize asthma and COPD suggests that MDRCs and their associated regulatory free radical and immunoregulatory cytokine mechanisms are central participants in these disorders. An improved understanding of the nature of these MDRC populations and their mechanisms of action might lead to identification of new therapeutic targets that focus on unique disease features in patients with asthma and those with COPD.