Myeloid-derived suppressor cell function is diminished in aspirin-triggered allergic airway hyperresponsiveness in mice

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Background: Myeloid-derived suppressor cells (MDSCs) have recently been implicated in the pathogenesis of asthma, but their regulation in patients with aspirin-intolerant asthma (AIA) remains unclear.

Objective: We sought to characterize MDSC accumulation and pathogenic functions in allergic airway inflammation mediated by COX-1 deficiency or aspirin treatment in mice.

Methods: Allergic airway inflammation was induced in mice by means of ovalbumin challenge. The distribution and function of MDSCs in mice were analyzed by using flow cytometry and pharmacologic/gene manipulation approaches.

Results: $CD11b^{+}Gr1^{high}Ly6G^{+}Ly6C^{int}$ MDSCs (polymorphonuclear MDSCs [PMN-MDSCs]) recruited to the lungs are negatively correlated with airway inflammation in allergen-challenged mice. Aspirin-treated and COX-1 knockout (KO) mice showed significantly lower accumulation of PMN-MDSCs in the inflamed lung and immune organs accompanied by increased T_H2 airway responses. The T_H2-suppressive function of PMN-MDSCs was notably impaired by COX-1 deletion or inhibition, predominantly through downregulation of arginase-1. COX-1–derived prostaglandin E_2 promoted PMN-MDSC generation in bone marrow through E prostanoid 2 and 4 receptors (EP2 and EP4), whereas the impaired arginase-1 expression in PMN-MDSCs in COX-1 KO mice was mediated by dysregulation of the prostaglandin E_2/EP4/cyclic AMP/protein kinase A pathway. EP4 agonist administration alleviated allergy-induced airway hyperresponsiveness in COX-1 KO mice. Moreover, the immunosuppressive function of PMN-MDSCs from patients with AIA was dramatically decreased compared with that from patients with aspirin-tolerant asthma.

Conclusion: The immunosuppressive activity of PMN-MDSCs was diminished in both allergen-challenged COX-1 KO mice and patients with AIA, probably through an EP4-mediated signaling pathway, indicating that activation of PMN-MDSCs might be a promising therapeutic strategy for asthma, particularly AIA. (J Allergy Clin Immunol 2014;134:1163-74.)

Key words: *Myeloid-derived suppressor cells, aspirin-intolerant asthma, T_H2, COX, prostaglandin, arginase*

Aspirin-intolerant asthma (AIA) is a distinctive condition involving severe bronchospasm in an asthmatic patient caused by ingestion of aspirin or other COX-1–inhibiting nonsteroidal anti-inflammatory drug (NSAIDs).[1] Although there are 2 different COX isoforms (ie, COX-1 and COX-2), aspirin is more than 170-fold selective in inhibiting COX-1 than COX-2.[2] Despite the presence of COX-2, its expression is limited and enzymatic activity is diminished in nasal polyps and bronchial wall epithelial cells of patients with AIA.[1] Moreover, the majority of asthmatic patients with aspirin hypersensitivity are able to tolerate selective COX-2 inhibitors, indicating that COX-1 inhibition is implicated in the exacerbated response in patients with AIA. In addition, a positive correlation exists between prostaglandin (PG) inhibition by NSAIDs and the occurrence of AIA in asthmatic patients,[3] suggesting that blockage of COX-1–derived PG biosynthesis is particularly involved in the pathogenesis of AIA.

PGE_2, a dominant COX product, mediates the proinflammatory response in many diseases, such as arthritis and cancer.[4] However, PGE_2 displays some beneficial anti-inflammatory properties in the airways, including antifibrotic, bronchodilating, and inhibitory activities against allergic inflammation–induced pulmonary vascular smooth muscle remodeling.[5] In asthmatic patients inhalation of PGE_2 significantly attenuates allergen-induced airway responses and pulmonary inflammation.[6] In contrast, PGE_2

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Abbreviations used

AHR: Airway hyperresponsiveness
AIA: Aspirin-intolerant asthma
APC: Allophycocyanin
Arg1: Arginase-1
ATA: Aspirin-tolerant asthma
BALF: Bronchoalveolar lavage fluid
BM: Bone marrow
cAMP: Cyclic AMP
CFSE: 5(6)-Carboxyfluorescein diacetate succinimidyl ester
CREB: cAMP response element-binding protein
Ef-OVA: Endotoxin-free ovalbumin
Ef-OVA_{LPS}/Ef-OVA mice: Mice sensitized with Ef-OVA and low-dose LPS (0.1 μg) and challenged with Ef-OVA
EP: E prostanoid receptor
KO: Knockout
L-NMMA: L-N^G-monomethyl arginine
MDSC: Myeloid-derived suppressor cell
Mo-MDSC: Monocytic MDSC
NO: Nitric oxide
NSAID: Nonsteroidal anti-inflammatory drug
OVA: Ovalbumin
PE: Phycoerythrin
PG: Prostaglandin
PKA: Protein kinase A
PMN-MDSC: Polymorphonuclear MDSC
ROS: Reactive oxygen species
TX: Thromboxane
WT: Wild-type

generation is markedly reduced in pulmonary tissues from patients with AIA compared with those from control subjects,[7] whereas treatment with PGE_2 administered by means of inhalation can prevent aspirin-triggered bronchoconstriction in patients with AIA.[1] These findings indicate that PGE_2 produced primarily from COX-1 plays a crucial role in the pathogenesis of AIA; however, its exact mechanism in patients with AIA has yet to be fully elucidated.

Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of cells that consist of 2 distinct subsets: $Ly6G^+Ly6C^{int}$ polymorphonuclear MDSCs (PMN-MDSCs) and $Ly6G^-Ly6C^{high}$ monocytic MDSCs (Mo-MDSCs).[8] In general, MDSCs can differentiate into mature granulocytes, dendritic cells, and macrophages in the bone marrow (BM), and their expansion was discovered in a range of pathologic conditions, including tumors, infections, trauma, and chronic inflammatory diseases, such as diabetes and inflammatory bowel disease.[8,9] Recently, LPS was shown to induce the expansion of $CD11b^+Gr1^{int}F4/80^+$ immature myeloid cells in patients with allergic airway inflammation.[10] These cells alleviated asthma by suppressing the dendritic cell–mediated reaction of primed T_H2 cells,[10,11] suggesting a potential protective effect of MDSCs in the development of asthma. Both clinical observations and laboratory experiments demonstrated that COX and its product, PGE_2, were involved in the regulation of activation and accumulation of MDSCs.[12] We hypothesized that the suppressive function of MDSCs might be impaired by COX-1 inhibition in an allergen-induced airway hyperresponsiveness (AHR) model with increased T_H2 activation.

METHODS

See the Methods section in this article's Online Repository at www.jacionline.org for a detailed explanation of the methods and materials used in this study.

RESULTS

PMN-MDSCs are negatively correlated with allergen-induced AHR phenotype in mice with COX-1 inhibition

Allergic asthma is an inflammatory disease initiated and driven by T_H2 cytokines. LPS is often present with ovalbumin (OVA) allergen during sensitization in mouse models and is responsible for $CD4^+$ T-cell differentiation. Low-dose LPS induces T_H2 responses, whereas high-dose LPS induces T_H1 responses.[13,14] Indeed, a considerable amount of endotoxin (49.7 ± 2.0 EU/mg, data not shown) could be detected in the OVA preparation, as previously reported.[15] Both endotoxin-free ovalbumin (Ef-OVA) plus low-dose LPS (Ef-OVA_{LPS}) and traditional OVA sensitization protocols were used to induce airway inflammation in mice to explore whether MDSCs are involved in COX-1 inhibition–mediated AHR. As shown in Fig 1, *A*, and Fig E1, *A*, in this article's Online Repository at www.jacionline.org, a population of $CD11b^+Gr1^{high}$ cells was significantly increased in lungs from OVA-challenged mice when compared with PBS-challenged mice (1.40- to 1.65-fold, $P < .05$). Strikingly, the $CD11b^+Gr1^{high}$ population was much lower in aspirin-treated or COX-1 knockout (KO) mice than in wild-type (WT) mice both before and after OVA challenge (Fig 1, *B*, and see Fig E1, *B*), suggesting that the reduction in $CD11b^+Gr1^{high}$ cell counts might contribute to COX-1 inhibition–mediated AHR. Immunofluorescence staining further confirmed that $CD11b^+Gr1^+$ MDSCs accumulated in the peribronchial areas of the lungs of these mice (Fig 1, *C*, and see Fig E1, *C*). A reduction in MDSC counts was also observed in other immune tissues and organs, such as the BM and spleen (see Fig E2, *A*, and Fig E3, *A*, in this article's Online Repository at www.jacionline.org). As expected, COX-1 deficiency or aspirin administration aggravated airway inflammatory responses to OVA in mice, as evidenced by increased bronchoalveolar lavage fluid (BALF) protein levels, increased eosinophil counts in BALF, more inflammatory cells surrounding bronchioles and blood vessels, and increased T_H2 cytokine levels and airway resistance (see Fig E2, *B-F*, and Fig E3, *B-G*). Flow cytometric analysis revealed that the $CD11b^+Gr1^{high}$ cells were predominantly $Ly6G^+Ly6C^{int}$ (>92%) in airway inflammation status and thus were named PMN-MDSCs, whereas $Ly6G^-Ly6C^+$ cells/Mo-MDSCs (Fig 1, *D*, and see Fig E1, *D*) could be clearly separated as gated in $CD11b^+Gr1^{int}$, as previously described.[16] Again, the number of PMN-MDSCs, but not Mo-MDSCs, was significantly decreased in the BM, spleens, and lungs of WT/aspirin and COX-1 KO AHR mice compared with that seen in control animals (Fig 1, *E*, and see Fig E1, *E*; Fig E2, *A*; and Fig E3, *A*). Moreover, the PMN-MDSC population was negatively correlated with protein secretion in BALF in OVA-treated mice (Fig 1, *F*, and see Fig E1, *F*), indicating that PMN-MDSCs alleviate the airway inflammation induced by OVA.

We next investigated whether infusion of additional PMN-MDSCs could inhibit the airway response to OVA (Fig 2, *A*, and see Fig E4, *A*, in this article's Online Repository at www.jacionline.org). PMN-MDSC infusion in WT mice resulted in