Cyclooxygenase inhibition abrogates aeroallergen-induced immune tolerance by suppressing prostaglandin I_2 receptor signaling

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Background: The prevalence of allergic diseases has doubled in developed countries in the past several decades. Cyclooxygenase (COX)-inhibiting drugs augmented allergic diseases in mice by increasing allergic sensitization and memory immune responses. However, whether COX inhibition can promote allergic airway diseases by inhibiting immune tolerance is not known.
Objective: To determine the role of the COX pathway and prostaglandin I_2 (PGI_2) signaling through the PGI_2 receptor (IP) in aeroallergen-induced immune tolerance.
Methods: Wild-type (WT) BALB/c mice and IP knockout mice were aerosolized with ovalbumin (OVA) to induce immune tolerance prior to immune sensitization with an intraperitoneal injection of OVA/alum. The COX inhibitor indomethacin or vehicle was administered in drinking water to inhibit enzyme activity during the sensitization phase. Two weeks after sensitization, the mice were challenged with OVA aerosols. Mouse bronchoalveolar lavage fluid was harvested for cell counts and T_H2 cytokine measurements.
Results: WT mice treated with indomethacin had greater numbers of total cells, eosinophils, and lymphocytes, and increased IL-5 and IL-13 protein expression in BAL fluid compared to vehicle-treated mice. Similarly, IP knockout mice had augmented inflammation and T_H2 cytokine responses compared to WT mice. In contrast, the PGI_2 analog cicaprost attenuated the anti-tolerance effect of COX inhibition.
Conclusion: COX inhibition abrogated immune tolerance by suppressing PGI_2 IP signaling, suggesting that PGI_2 signaling promotes immune tolerance and that clinical use of COX-inhibiting drugs may increase the risk of developing allergic diseases. (J Allergy Clin Immunol 2014;134:698-705.)

Key words: *PGI_2, allergy, immune tolerance*

Allergic disease is one of the most common causes of chronic illness and affects 40 to 50 million Americans. In the past several decades, the reported prevalence of allergic rhinitis, asthma, and atopic eczema increased markedly in developed countries.[1,2] Mounting evidence suggests that drugs inhibiting cyclooxygenase (COX) enzymes in the arachidonic acid metabolic pathway may be contributing to the increased allergy prevalence. Epidemiological studies reveal a correlation between frequent use of COX-inhibiting medications and increased risk of developing allergic disorders and asthma.[3] Recently, a positive association was reported between the intake of non-aspirin nonsteroidal anti-inflammatory drugs (NSAIDs) and current asthma in adult survivors of childhood asthma.[4] In addition, there was a higher prevalence of new-onset asthma in subjects who regularly used NSAIDs other than aspirin compared to nonusers.[5] In animal studies, COX inhibition with indomethacin increased allergic sensitization and allergen-specific immune memory response, and augmented allergic airway inflammation and T_H2 immune responses,[6-10] supporting a role for the COX pathway in the development of allergic diseases. Similarly, mice deficient in COX enzymes have increased allergic inflammation compared to wild-type (WT) mice.[11]

People without allergic diseases have antigen-specific immune tolerance to common aeroallergens. Some studies support the possibility that regulatory T (Treg) cells suppress allergic diseases and contribute to allergen-specific immune tolerance.[12,13] Treg cells are a subtype of CD4 T cells that are critical for the maintenance of self-tolerance and tolerance to foreign antigens by inhibiting effector T cell responses.[14] The establishment and maintenance of tolerance induced by repeated airway exposures to low-dose ovalbumin (OVA) were dependent on Treg cells that express both cell surface and soluble TGF-β.[13] Therefore, Treg cells may be a mechanism by which immune tolerance prevents the immune system from responding to innocuous environmental antigens. While we and others have reported that COX inhibition increased allergic airway inflammatory responses,[6-10] the effect of COX products and COX inhibition on allergen-induced immune tolerance in the airways is not known.

Prostaglandin I_2 (PGI_2) is one of the lipid products formed in the COX pathway of arachidonic metabolism. The other lipid molecules produced in the COX pathway are prostaglandin E_2

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Abbreviations used
BAL: Bronchoalveolar lavage
COX: Cyclooxygenase
IP: PGI_2 receptor
KO: Knockout
LTE_4: Leukotriene E_4
5-LO: 5-Lipoxygenase
MLN: Mediastinal lymph nodes
OVA: Ovalbumin
PGE_2: Prostaglandin E_2
PGI_2: Prostaglandin I_2
Treg: Regulatory T
WT: Wild-type

(PGE_2), prostaglandin D_2, prostaglandin $F_{2\alpha}$, and thromboxane A_2. PGI_2 binds to the G protein–coupled receptor IP and exerts its biological activities through autocrine and paracrine mechanisms. We have previously described the immune suppressive function of PGI_2, and PGI_2 analogs inhibited T_H1 and T_H2 effector cytokine production *in vitro*.[15,16] Jaffar et al[17] reported that endothelial cell-derived PGI_2 inhibited the recruitment of T_H2 cells in the lung in an adoptive T-cell transfer model. In contrast, IP knockout (KO) mice have augmented T_H2 immune responses, increased airway inflammation and hyper-responsiveness to OVA.[18] IP KO mice also had increased T_H1 responses in a mouse model of respiratory syncytial virus infection.[19] Therefore, PGI_2 modulates immune responses and possibly immune tolerance as well.

Studies have shown that the COX pathway regulates the generation and maintenance of immune tolerance.[20,21] COX-2 products produced by mucosal dendritic cells were required for the development of functional Treg cells and the maintenance of immune tolerance induced by oral ingestion of OVA.[20] Inhibition of COX-2 in dendritic cells derived from mesenteric lymph nodes caused increased T_H2 differentiation and IL-4 production, which was directly related to impaired Treg cell differentiation and immune tolerance.[20] Treg cells suppressed antitumor immune activity in a COX-2 dependent manner in colorectal cancer patients,[21] also suggesting a role for the COX pathway in Treg function and immune tolerance. However, COX products may also modulate immune tolerance independent of Treg cells. For instance, PGE_2 promoted immune tolerance in the gut that was not related to Treg cell number or function.[22] In this study, we found that COX inhibition ablated immune tolerance by suppressing IP signaling, and the effect was not associated with Treg cell numbers. This has important clinical implications, because medications that inhibit PGI_2 production may adversely affect immune tolerance.

METHODS

Mice

Wild type BALB/c mice were obtained from the Jackson Laboratory (Bar Harbor, Me). IP KO mice were generated by homologous recombination in embryonic stem cells and were backcrossed to a BALB/c background for 10 generations.[23] Age-matched WT BALB/c mice and IP KO mice were used at 8 to 12 weeks old. 5-Lipoxygenase (5-LO) KO mice on a mixed 129-C57BL/6 background were purchased from the Jackson Laboratory and then backcrossed 10 generations to BALB/c in our lab. Animal experiments were reviewed and approved by the Institutional Animal Care and Use Committee at Vanderbilt University and were conducted according to the guidelines for the Care and Use of Laboratory Animals prepared by the Institute of Laboratory Animal Resources, National Research Council.

Induction of immune tolerance and allergic inflammation

The mouse immune tolerance protocol (Fig 1) was modified from the method reported by Ostroukhova and colleagues.[24] The "tolerance protocol" consisted of 3 phases: tolerance induction, OVA sensitization, and OVA challenge. For this protocol, wild type BALB/c, IP KO, and 5-LO KO mice were exposed to aerosols of 1% OVA/PBS solution using an ultrasonic nebulizer for 40 minutes per day from day −14 to day −10 (5 days) to induce immune tolerance. At day 0, mice were OVA sensitized by an intraperitoneal injection of 0.1 mL OVA/alum solution (10 μg OVA formulated with 20 mg of aluminum hydroxide). Starting at day 14, the mice were challenged with an OVA aerosol for 40 minutes per day for 4 days. The "allergy protocol" consisted of sensitization with an intraperitoneal injection of OVA/alum at day 0 and OVA challenge with 4 days of OVA aerosols from days 14 to 17.

In some experiments, WT BALB/c and 5-LO KO mice were treated with the COX inhibitor indomethacin (30 μg/mL) or vehicle (1% ethanol) in drinking water during sensitization (Fig 1) phase from day −2 to day 2. Some groups of mice were also treated with intraperitoneal injections of cicaprost (a gift from Dr M. Huebner, Bayer Pharma AG, Berlin, Germany). The mice were injected twice daily from day −2 to day 2 with 300 ng cicaprost in 100 μl of PBS per mouse per injection.

BAL cell analysis

On day 18, bronchoalveolar lavage (BAL) fluid was collected,[9] and leukocytes in the BAL were immediately counted for total number of cells. The BAL cells were also used for cytospin preparation. The cytospin slides were stained using the Diff-Quik kit (American Scientific Products, Charlotte, NC) for differential cell counts to enumerate macrophages, eosinophils, lymphocytes, and neutrophils.

Histological analyses of lung sections

The mice were sacrificed on day 18, and the lung block was removed. Lung tissue was fixed in 10% formalin solution, paraffin-embedded, cut in 6 μm sections, mounted, and stained with hematoxylin and eosin for routine histology to evaluate inflammation. Slides were examined by a pathologist blinded to experimental groups to score interstitial inflammation using a 0 to 3 scoring system: 0 indicated no inflammatory cells; 1, a few inflammatory cells; 2, increased accumulation of inflammatory cells; and 3, abundant accumulation of inflammatory cells.

Cytokine and IgE measurements by ELISA

The levels of IL-5 and IL-13 in BAL fluid were measured by Quantikine and DuoSet ELISA kits (R&D Systems, Minneapolis, Minn) according to the manufacturer's instructions. Total IgE was determined by Clonotyping ELISA kit (Southern Biotech, Birmingham, Ala). OVA-specific IgE in mouse sera was quantified as following. Immulon 2 HB plates were coated with 2 μg OVA in bicarbonate buffer (pH 9.6) per well overnight at 4°C and blocked with 1% BSA. Serum samples were diluted in 0.1% Tween 20 PBS and incubated for 2 hours at room temperature. OVA-specific IgE was detected by using goat-anti-mouse IgE conjugated with horseradish peroxidase (HRP) (Southern Biotech, Birmingham, Ala). Plates were developed with 3,3′,5,5′-Tetramethylbenzidine substrate solution (R&D Systems), and reactions were stopped with 1N HCl. Absorbance values at OD 450 were measured. Concentrations of OVA-specific IgE were determined using a standard curve made with serum from hyper-immunized mice.

Measurement of leukotriene E_4 (LTE_4)

The urine of WT BALB/c mice was collected on the last day of indomethacin treatment (Fig 1, *A*). The amounts of LTE_4 were quantitated