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To the Editor

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Thank you for the opportunity of responding to the reviewer's comments regarding our manuscript "The biology of prostaglandins and its role as a target for allergic airway disease therapy", Lee et al.

Reviewer: 2

We thank the reviewer for his/her helpful suggestions, which led us to revise the manuscript. A point-by-point response is below.

Blue: reviewer's comment

Black: author's response

Red: text in the revised manuscript

General comments:

The scope of this review is very broad and this makes it challenging to find a logical structure and stay focused. The authors decided to integrate information of prostaglandin biosynthesis with its effector functions and with preclinical and clinical data. Unfortunately, the authors did not manage to make a consistent review of broad interest and this is mainly due to a pure summary of the literature, without any critical discussions of results or methodological problems. The mix between human and mouse data is partially confusing and sometimes it is not even clear whether the authors describe human or mouse data. In order to educate the reader and make this review a useful piece of reference I would recommend following: Decide on the audience you wish to address. The single chapters (biosynthesis, effects on different immune cells, clinical studies) are of diverse interest for the different groups of readers (scientists, clinician scientists, medical doctors, etc.). As an allergologist/immunologist I am mostly interested in a really short, but comprehensive introduction to the biosynthesis of prostaglandins and its receptors followed by a detailed review of the effects on the single immune cells and an overview of current clinical studies in this field. Include the most important information into a table. This can be done as in Table 1 (prostaglandin receptors), but here you should distinguish between ligand and receptor and you could add the main producers of the respective prostaglandin.

We totally agree with your opinion. Since we (authors) are clinical physicians, we are focusing on whether targeting prostaglandins could be beneficial treatment strategy for allergic airway disease. We thought that before discussing the effects of prostaglandin-targeting agents, overall information of prostaglandins including their biosynthesis and their function in the pathogenesis of allergic airway inflammation should be provided, since these parts might be unfamiliar to most of the clinicians. Following your advices, we revised the manuscript of introduction as below. We also revised Table 1 with additional information of the producers of individual prostaglandins.

(Page 2, Line 44)

Although the discovery of PGs and their role in allergic reactions are known for a long time, the development of novel drugs targeting their signaling and metabolism is currently in progress, and clinical studies applying these to allergic airway diseases are being undertaken. In this review, we will discuss the potential of PGs to serve as therapeutic targets in allergic airway inflammation of the upper and lower airways. Based on an introduction of the pathways of PG generation, and the mechanisms of individual PGs in important cellular allergic reactions, we will present the regulation of PGs in clinical settings and the effects of pharmacological agents that modulate PG activation in various airway diseases such as asthma, allergic rhinitis, AERD, and nasal polyposis.

(Page 3, line 88)

Table 1. Prostaglandins and their specific receptors, downstream signaling, and region of expression.

Ligands	Production	Receptor	Downstream signaling	Receptor expression
PGD ₂	mast cells, eosinophils, T cells, dendritic cells, macrophages, endothelial cells, platelets, lung parenchyma	DP1	↑ cAMP	mucus-secreting goblet cells, nasal serous glands, vascular endothelial cells, T cells, dendritic cells, eosinophils
		DP2 (CRTH2)	↓ cAMP, ↑ Ca ²⁺	T cells, basophils, eosinophils, ILC2
		EP1	↑ Ca ²⁺	T cells, dendritic cells, B cells, smooth muscle cells
PGE ₂	epithelial cells, fibroblasts, macrophage, smooth muscle cells, platelets	EP2	↑ cAMP	T cells, dendritic cells, B cells, ILC2, mast cells, basophils, smooth muscle cells
		EP3	↓ cAMP	T cells, B cells, dendritic cells, smooth muscle cells
		EP4	↑ cAMP	T cells, B cells, dendritic cells, smooth muscle cells
PGF _{2α}	lung parenchyma, vascular smooth muscle cells, peripheral blood lymphocytes	FP	↑ IP3/DAG/ Ca ²⁺	none
PGI ₂	endothelial cells, vascular smooth muscle cells, lung parenchyma	IP	↑ cAMP	T cells, dendritic cells, B cells, ILC2, endothelial cells, platelets
TXA ₂	platelets, vascular smooth muscle cells, macrophages	TP	↑ IP3/DAG/Ca ²⁺ , ↑ ↓ cAMP	megakaryocytes, monocytes

A table would be also very helpful to summarize the most important clinical studies, here I clearly miss information regarding subject number, key results, etc. This table should include drug, dosing, mechanism of action and references.

We agree with your comments and therefore added Table 2 which describes summary of clinical trials including the name of pharmacologic agents, dose, the duration of treatment, and key results according to the mechanism of actions.

(Page 11, line 364)

Table 2. Summary of clinical studies on pharmacologic agents targeting PGs in asthma and allergic rhinitis.

Drug and dose	Indication (sample size)	Key results	Ref.
DP2 antagonist			
Fevipirant (QAW039), oral administration, 500 mg daily for 28 days	Mild to moderate uncontrolled allergic asthma (n=170)	Improvement of lung function in patients with FEV ₁ <70%	[132]
Fevipirant (QAW039), oral administration, 1-450 mg daily or 2-150 mg twice daily, with inhaled budesonide 200 µg twice a day, for 12 weeks	Allergic asthma uncontrolled by a low-dose inhaled corticosteroid (n=2598)	Total daily dose of 150 mg (150 mg once or 75 mg twice per a day) showed an improvement in forced expiratory volume	[133]
Fevipirant (QAW039), oral administration, 225 mg twice daily for 12 weeks	Moderate to severe asthma with serum eosinophil ≥ 2% (n=61)	Decreased sputum eosinophil count	[134]
ARRY-502, oral administration, 200 mg twice daily for 4 weeks	Mild allergic asthma (n=184)	Reduction of FeNO level and decreased serum markers of Th2 inflammation	[135]
AZD1981, oral administration, 100 mg twice daily	Stable asthma withdrawn from inhaled corticosteroid (n=209)	No efficacy on morning peak expiratory flow	[136]
AZD1981, oral administration, 50-1000 mg twice daily, for 4 weeks, with an inhaled corticosteroid	Asthma uncontrolled by inhaled corticosteroid (n=510)	400 mg group showed improved FEV ₁ , significant improvement in questionnaire score and FEV ₁ in atopic subgroup	[136]
OC000549, oral administration, 25 mg daily/200 mg daily/100 mg twice daily, for 12 weeks, with use of short-acting β ₂ agonist	Mild to moderate asthma (n=460)	Improved FEV ₁ (prominent in eosinophilic subjects), lower incidence of symptom exacerbation and respiratory infection	[137]
OC000549, oral administration, 200 mg twice daily for 8 days	Seasonal allergic rhinitis (n=35)	Reduced grass-pollen induced nasal and ocular symptoms	[138]
GB001, oral administration, 30 mg daily for 28 days, with use of low dose inhaled fluticasone propionate	Mild to moderate atopic asthma (n=36)	Improved FEV ₁ (prominent in patients with high FeNO or high blood eosinophil)	[139]

Drug and dose	Indication (sample size)	Key results	Ref.
BI671800, oral administration, 50/200/400 mg twice daily for 6 weeks	Mild to moderate asthma (n=389)	Greater improvement in FEV ₁ compared to moderate doses of fluticasone	[140]
BI671800, oral administration, 400 mg twice daily with inhaled fluticasone (88 µg)	Mild to moderate asthma with inhaled corticosteroid (n=243)	Improvement in FEV ₁ compared to placebo, however not significantly improved over montelukast	[140]
Setipiprant (ACT-129968), oral administration, 1000 mg twice daily for 5 days, washout period for 3 weeks	Stable allergic asthma (n=15)	Reduction in both allergen-induced late asthmatic responses and airway hyper-responsiveness	[141]
Setipiprant (ACT-129968), oral administration, 100/500/1000 mg twice daily or 1000 mg daily for 2 weeks	Seasonal allergic rhinitis (n=557)	Dose-related improvements in both nasal and ocular symptom scores	[142]
Setipiprant (ACT-129968), oral administration, 1000 mg twice daily for 2 weeks	Seasonal allergic rhinitis (n=604)	No significant effect on either nasal or ocular symptom scores	[142]
Dual antagonist for DP2 and TP			
Ramatroban (BYAu3405), oral administration, 150 mg twice daily for 4 weeks	Perianal allergic rhinitis (n=10)	Inhibitory effect on allergen challenge-induced nasal mucosal swelling	[143]
Ramatroban (BYAu3405), oral administration, 150 mg twice daily for 4 weeks	Perianal allergic rhinitis (n=11)	Inhibitory effect on histamine-induced nasal reactivity, decreased eosinophil counts in nasal lavage fluid	[144]
TP antagonist			
Seratrodastr, oral administration, 80 mg daily for 4 weeks	Asthma (n=14)	Decreased airway hyper-responsiveness, no definite effect on exhaled nitric oxide and sputum eosinophils	[145]
AA02414, oral administration, 80 mg daily for 4 months	Asthma (n=31)	Improved symptom score, peak expiratory flow, and bronchial responsiveness to metacholine, decreased activated eosinophil infiltration	[146]
DP1 antagonist			
ONO-4053, oral administration, 300 mg daily for 2 weeks	Seasonal allergic rhinitis (Japanese cedar pollen) (n=200)	Greater improvement in nasal symptoms compared to either placebo or pranlukast	[147]
PGI₂ analogue			
OP-41483, oral administration, 200 µg 4 times daily for 4 weeks	Stable asthma (n=8)	No direct effect on bronchial responsiveness	[148]

FEV₁ — one second-forced expiratory volume; FeNO — fractional exhaled nitric oxide

The clinical studies chapter is very inconsistent. It is only partly about clinical studies dealing with molecules targeting prostaglandin signaling and additionally it describes the role of prostaglandins in AERD and patients with nasal polyps.

We appreciated your suggestion. In order to make consistency in the clinical studies chapter, the manuscript was modified to give a brief description of the role of prostaglandins in patients, and then to introduce clinical trials conducted up to date in each section of the chapter (including asthma and allergic rhinitis).

(Page 11, line 358)

4. Clinical studies of prostaglandins in allergic airway diseases

The expression and role of PGs in allergic airway diseases have been studied for a long time. Recently, clinical phase II and III studies were conducted to evaluate the therapeutic effects of PGs, especially for asthma and allergic rhinitis (Table 2). This section briefly introduces the role of PGs in each disease (asthma, allergic rhinitis, AERD, and nasal polyps) in clinical settings, and provides an overview of the clinical studies that have used pharmacologic agents targeting PGs.

(Page13, line 370)

4.1. Asthma

Numerous studies have focused on the clinical relevance of prostaglandins in asthma. In a study that examined concentrations of PG mediators from BAL fluid, both PGD₂ and PGF₂α showed 12- and 20-fold higher levels in asthmatic patients than non-allergic controls, respectively [149]. Another study reported that concentrations of PGD₂, TXB₂, and PGI₂ metabolites was much higher (up to 208-fold) in BAL fluid 5 minutes after an allergen challenge in allergic asthmatic patients compared to the controls [150]. In addition, epithelial cells obtained from severely asthmatic patients showed higher levels of H-PGDS compared to controls, correlating with PGD₂ levels. Furthermore, BAL fluid collected from severely asthmatic patients showed stronger expression of DP2 receptors than that from mild asthmatics or healthy controls; however, there was no difference in DP1 receptor expression among groups [151]. As for PGE₂ levels, studies regarding their correlation with eosinophils reported that a lower sputum PGE₂ level is associated with eosinophilia in asthmatic patients [152].

(Page 14, line 429)

4.2. Allergic rhinitis

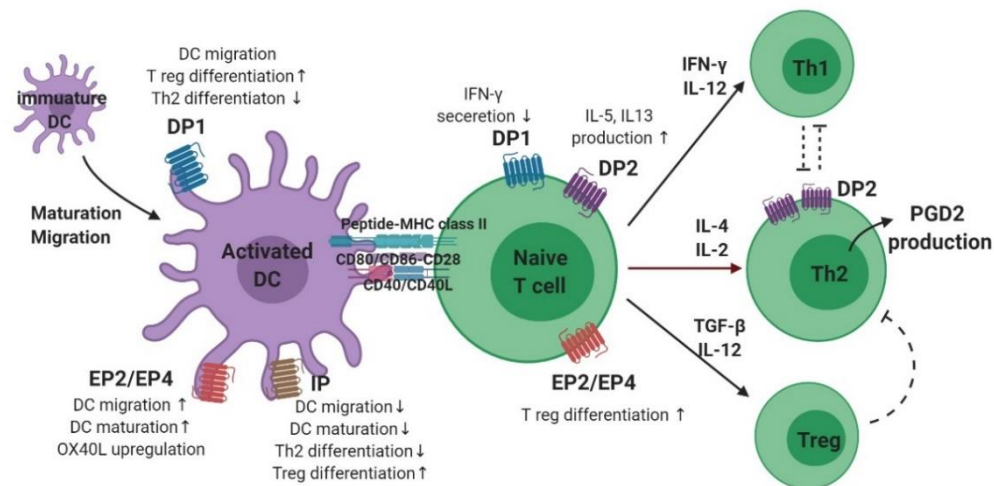
Clinical studies of allergic rhinitis have also suggested the importance of PGs in the disease. In nasal lavage fluid (NALF), the PGD₂ level was higher in patients with allergic rhinitis compared to those with non-allergic rhinitis [175]. Nasal mucosa samples obtained from the turbinates of patients with allergic rhinitis revealed stronger DP2 expression in eosinophils, mast cells, T lymphocytes, and epithelial cells compared to the non-allergic nasal mucosa [176]. In patients with seasonal allergic rhinitis and sensitization to grass pollen, the PGE₂ level in NALF decreased significantly during the pollen-exposure season, which was negatively correlated with nasal eosinophilia [177].

Again, here you have to decide on your focus and your audience. Please revise the figures carefully. They are very confusing, especially Fig. 2. Maybe you should focus on the single cell types and make a figure for each. Or leave the general overview but then make additionally a graphical summary for each single cell type.

Thank you for the comments. We created Figure 2 to represent the effect of prostaglandins on overall allergic pathophysiology. And we added figures summarizing the role of prostaglandins on (1) the interaction between dendritic cells and T cells , (2) innate lymphoid cell 2s, and (3) eosinophils.

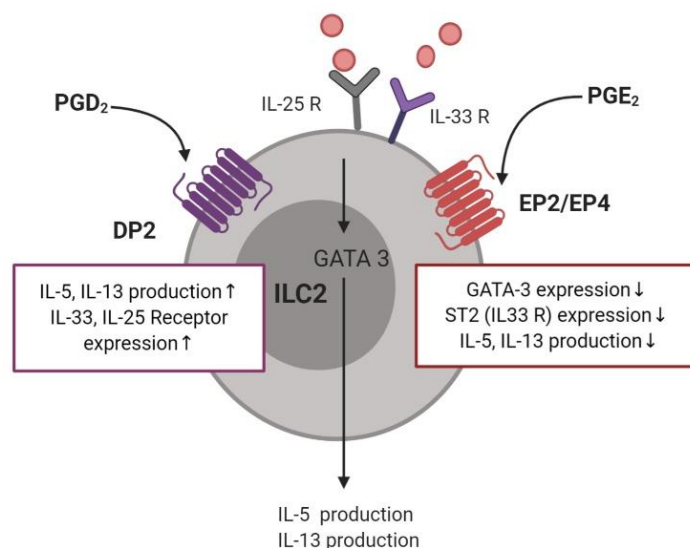
(Page 6, line 191)

Figure 3. The role of prostaglandins and their receptors on dendritic cell-T cell interaction and Th2 differentiation



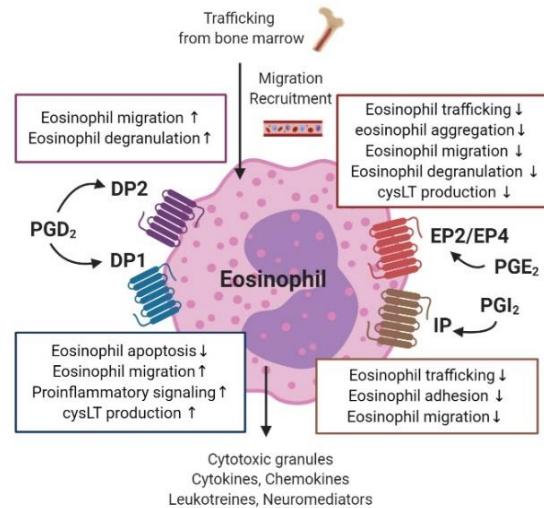
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Figure 4. The role of prostaglandins and their receptors on type 2 innate lymphoid cells



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Figure 5. The role of prostaglandins and their receptors on eosinophil function including bone marrow trafficking, migration and degranulation



Minor comments:

Add all abbreviations to the list in alphabetical order.

According to your comments, we re-ordered the abbreviations according to alphabetical order as below.

(Page 16, line 525)

Abbreviations

AA	arachidonic acid
AERD	aspirin exacerbated respiratory disease
cAMP	cyclic adenosine 3',5'-monophosphate
BAL	bronchoalveolar lavage
COX	cyclooxygenase
cPGES	cytosolic prostaglandin E synthase
cysLT	Cysteinyl leukotriene
DC	dendritic cell
FEV ₁	forced expiratory volume 1
H-PGDS	hematopoietic-prostaglandin D synthase
IgE	immunoglobulin E
IL	interleukin
ILC	innate lymphoid cell
INF- γ	interferon γ
L-PGDS	lipocalin-prostaglandin D synthase
LPS	lipopolysaccharide
LT	leukotriene
LTC ₄	leukotrieneC ₄
LTD ₄	leukotrieneD ₄
LTE ₄	leukotrieneE ₄
mPGES	microsomal prostaglandin E synthase
NALF	nasal lavage fluid
NSAID	non-steroidal anti-inflammatory drug
PG	prostaglandin
PGD ₂	prostaglandin D ₂
PGE ₂	prostaglandin E ₂
PGF _{2α}	prostaglandin F _{2α}
PGFS	prostaglandin F synthase
PGG ₂	prostaglandin G ₂
PGH ₂	prostaglandin H ₂
PGI ₂	prostaglandin I ₂
PLA ₂	phospholipase A2
TNF- α	tumor necrosis factor α
Treg	regulatory T cell
TSLP	thymic stromal lymphopoietin
TXA ₂	thromboxane A ₂
TXB ₂	thromboxane B ₂

In conclusion, we've attempted to respond to the reviewers' comments in what we feel to be a reasonable way, and we hope that our responses are deemed suitable. We look forward to a decision regarding acceptability of the revised manuscript for publication in the *International Journal of Molecular Sciences*

Sincerely,

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