Involvement of EP2 and EP4 Receptors in Eosinophilic Esophagitis: A Pilot Study

Franziska Durchschein1 · Andreas Eherer1 · Magdalena Grill2 · Eva M. Sturm2 · Veronika Pommer2 · Cord Langner3 · Christoph Högenauer1,4 · Rudolf Schicho2,4

Received: 11 January 2019 / Accepted: 8 April 2019 / Published online: 15 April 2019 © The Author(s) 2019

Abstract

Background  The prostaglandin D2 receptor DP2 has been implicated in eosinophil infiltration and the development of eosinophilic esophagitis (EoE).

Aims and Methods  In this study, we investigated an involvement of PGE2 (EP1–EP4) and PGD2 (DP1) receptors in EoE by measuring their expression in peripheral blood eosinophils and esophageal mucosal biopsies of EoE patients and by performing migration and adhesion assays with eosinophils from healthy donors.

Results  Expression of EP2 and EP4, but not EP1 and EP3, was decreased in blood eosinophils of patients with EoE vs. control subjects. Adhesion of eosinophils to esophageal epithelial cells was decreased by EP2 receptor agonist butaprost and EP4 agonist ONO-AE1-329, whereas DP1 agonist BW245C increased adhesion. In chemotaxis assays with supernatant from human esophageal epithelial cells, only ONO-AE1-329 but not butaprost or BW245C inhibited the migration of eosinophils. Expression of EP and DP receptors in epithelial cells and eosinophils was detected in sections of esophageal biopsies from EoE patients by immunohistochemistry. qPCR of biopsies from EoE patients revealed that gene expression of EP4 and DP1 was the highest among PGE2 and PGD2 receptors. Esophageal epithelial cells in culture showed high gene expression for EP2 and EP4. Activation of EP2 and EP4 receptors decreased barrier integrity of esophageal epithelial cells in impedance assays.

Conclusions  Activation of EP2 and EP4 receptors may inhibit eosinophil recruitment to the esophageal mucosa. However, their activation could negatively affect esophageal barrier integrity suggesting that eosinophilic rather than epithelial EP2 and EP4 have a protective role in EoE.

Keywords  Eosinophils · Esophageal epithelial cells · Prostaglandin receptors · EP receptors · DP1

Introduction

EoE is an inflammatory and antigen-driven disorder of the esophagus [1]. Histologically, it is characterized by eosinophil infiltration into the esophageal mucosa and diagnosed when more than 15 eosinophils per high powered field are present in biopsies [2, 3]. Clinically, EoE is accompanied by dysphagia, food impaction, and intractable dyspepsia that is partially responsive to treatment with proton pump inhibitors [2–4]. In the long term, fibrosis of the lamina propria and remodeling of the esophagus occurs, leading to a narrowing of the esophageal lumen and stricture formation [5]. The pathophysiology of many features of EoE, including the infiltration of eosinophils into the esophageal mucosa, is still unclear.

Prostaglandin (PG) D2 and E2 receptors have been linked to eosinophilic airway disorders such as asthma, non-asthmatic eosinophilic bronchitis [6, 7], and allergic rhinitis [8] indicating a potential role in the development of eosinophil-driven diseases. In addition, treatment with a PGD2 receptor antagonist previously revealed a benefit in a small group of EoE patients [9] suggesting that PGD2 and PGE2 receptors
may be also involved in the development of EoE. PGD₂ is an important mediator in inflammatory reactions with either pro- or anti-inflammatory effects [7]. Which of the effects occurs depends on the type, activation state, interaction, tissue, and cellular presence of the PGD₂ receptors involved [10]. PGD₂ receptors comprise two G protein-coupled receptors, namely D-type prostanoid receptor 1 (DP1) and 2 (DP2) (formerly CRTH2). DP1 is located on many types of leukocytes including dendritic cells, lymphocytes, neutrophils, and eosinophils [7, 11], and it conveys anti-inflammatory actions in models of gastrointestinal (GI) inflammation [12, 13], while DP2 is mainly located on Th2 cells, eosinophils, basophils, and monocytes [7]. Opposite to DP1, DP2 plays a proinflammatory role in GI inflammation [13]. The two receptors have been shown to cooperate in trafficking and chemotaxis of eosinophils [11].

In contrast to PGD₂, PGE₂ acts via four G protein-coupled receptors (EP1-EP4) with different signal transduction pathways, tissue localization, and regulation of expression [14]. Different receptors and divers signaling options for one mediator explain why PGE₂, similar to PGD₂, is able to exert proinflammatory as well as anti-inflammatory effects [15]. Flow cytometric data suggest that all types of EP receptors are present in human eosinophils [16–18]; however, EP receptors have also been found in esophageal epithelial cells [19]. In GI inflammation, PGE₂ can either increase [20] or diminish inflammatory processes [21]. EP2 and, in particular, EP4 have been shown to mediate PGE₂-induced anti-migratory effects in eosinophils [16–18].

Based on the recent findings that a DP2 antagonist inhibited eosinophil infiltration into the esophageal mucosa in animals and humans and ameliorated disease symptoms [9, 22], we explored whether EP1, EP2, EP3, and EP4 as well as DP1 play a role in EoE by assessing the presence of these receptors in blood eosinophils of EoE patients in comparison with controls and by measuring gene expression of the receptors in esophageal mucosal biopsies of EoE patients. Since infiltration of eosinophils into esophageal mucosa represents a major feature of EoE, we performed chemotaxis and adhesion assays with eosinophils from healthy donors and primary esophageal epithelial cells.

Materials and Methods

EoE Patients

Ten patients with clinically, endoscopically, and histologically confirmed EoE (46.7 ± 13.7 [mean age ± SD]; 7 males/3 females), and eight healthy control subjects (33 ± 13; 3 males/5 females) were included in the study. Diagnosis of EoE was established by clinical, endoscopic, and histological criteria. Conditions and criteria of diagnosis followed the recommendations according to the current published guidelines [2]. From EoE patients, blood and esophageal mucosal biopsies (from the proximal and distal part of the esophagus) were collected (see Table 1 for patient characteristics). From healthy control subjects, only blood was collected. EoE patients were recruited from the Department of Internal Medicine, and healthy volunteers were recruited from the Division of Pharmacology at the Medical University of Graz. Eosinophils isolated from healthy volunteers served as controls for flow cytometry experiments and for migration and adhesion assays. Subjects with significant comorbidities, intercurrent illness (such as infections), and pregnant women were excluded from the study. Blood was collected in Vacuette® EDTA blood tubes (Greiner-Bio-One, Austria) and immediately processed for flow cytometric experiments. Collected biopsies were immediately frozen (two/patient) or fixed (two/patient) in 10% phosphate-buffered formalin for histochemical analysis. Two subjects of the EoE cohort were on proton pump inhibitors at the time of blood collection, while subjects who had biopsies taken were on no therapy at the time of collection (see EoE patient characteristics in Table 1).

Esophageal Epithelial Cells

Human primary esophageal epithelial cells were purchased from Cell Biologics (Chicago, IL, USA; Cat.# H-6046), cultivated in complete human epithelial cell medium provided by the vendor and used until passage seven.

Flow Cytometric Analysis of Prostaglandin D₂ and E₂ Receptor Expression in Whole Blood

Whole blood from EoE patients or healthy individuals was lysed with 1x BD FACS lysis solution to remove erythrocytes. Samples were washed once in PBS, resuspended in

| Table 1 EoE patient characteristics |
|-----------------------------------|
| Gender | Age | Eosinophils for flow cytometry | Biopsies for qPCR | Therapy |
|-------|-----|-------------------------------|------------------|--------|
| f | 56 | + | − | − |
| m | 48 | + | − | PPI |
| m | 54 | + | − | − |
| m | 44 | + | − | − |
| m | 39 | + | − | − |
| f | 22 | + | − | PPI |
| m | 52 | + | + | − |
| f | 59 | + | + | − |
| m | 65 | − | + | − |
| m | 28 | − | + | − |

m male, f female, PPI proton pump inhibitors
fixative solution, and kept on ice for 10 min. After washing with PBS, cells were blocked with Ultra-V Block (Lonza; Allendale, NJ, USA) for 30 min at 4 °C. For flow cytometric visualization of the DP1 receptor, cells were incubated with mouse polyclonal antibody against DP1 (20 μg/ml; Sigma-Aldrich, St. Louis, MO, USA), or normal mouse IgG (20 μg/ml; Sigma-Aldrich) for 30 min at 4 °C. For flow cytometric visualization of the DP2 receptor, cells were stained with Alexa Fluor 647-conjugated rat anti-human DP2 antibody or Alexa Fluor 647-conjugated rat IgG2a isotype control (10 μg/ml; BD Biosciences; San Jose, CA, USA) for 30 min at 4 °C. Cells were washed in PBS, resuspended in antibody Diluent (Dako, Glostrup, Denmark) and incubated for 30 min at 4 °C with the secondary antibody (1:2000; Alexa Fluor 488-conjugated rabbit anti-mouse secondary antibody; Life Technologies, Carlsbad, CA, USA). For flow cytometric visualization of the EP receptors, cells were incubated with rabbit polyclonal antibodies against EP1, EP2, and EP3 (20 μg/ml; Alomone Labs, Jerusalem, Israel) and mouse polyclonal antibody against EP4 (20 μg/ml; Alomone Labs, Jerusalem, Israel), or normal rabbit or mouse IgG (20 μg/ml; Santa Cruz Biotechnology, Dallas, TX, USA) for 30 min at 4 °C. Cells were washed in PBS, resuspended in antibody Diluent (Dako, Glostrup, Denmark), and incubated for 30 min at 4 °C with the secondary antibody (1:2000; PE-conjugated goat anti-rabbit or rabbit anti-mouse secondary antibody; Life Technologies, Carlsbad, CA, USA). Finally, all samples were washed with PBS, fixative solution was added, and samples were kept on ice until analyzed on a FACSCanto II flow cytometer. Eosinophils were identified on the basis of forward versus side scatter parameters and autofluorescence. Receptor expression was recorded as mean fluorescence intensity and expressed as fold increase over isotype control signals.

Evaluation of Eosinophil Chemotaxis by Transwell Migration Assay

Migration assays were performed with peripheral blood eosinophils in 96-well Transwell plates with 5-μm membrane inserts (Corning Inc., Lowell, MA, USA). Eosinophils from healthy donors were incubated with the following compounds: EP4 agonist ONO-AE1-329 (100 nM) or EP4 antagonist ONO-AE3-208 (100 nM; ONO compounds were a generous gift from ONO Pharmaceutical, Osaka, Japan), EP2 agonist butaprost (100 and 300 nM; Cayman Chemicals, Ann Arbor, MI, USA), DP1 agonist BW245C (100 nM), and DP1 antagonist MK0524 (1 μM; Cayman Chemicals). Thereafter, a suspension of 5 × 10⁴ eosinophils was placed in the upper well. Supernatant from the esophageal epithelial cell culture was added into the bottom well as a chemoattractant. Eosinophils were allowed to migrate for 1 h at 37 °C and 5% CO2 in a humidified incubator and evaluated by flow cytometry. Each migration experiment was performed in triplicates. The average number of migrated cells was determined from at least five independent experiments.

Eosinophil Adhesion Assay

Human esophageal epithelial cells were seeded in black wall clear bottom 96-well plates and allowed to adhere for 48 h prior to each experiment. The cell monolayers were then starved in incomplete human epithelial cell medium + 2% FBS for 1 h and subsequently treated with TNF-α (10 ng/ml) for 4 h. Eosinophils were incubated with 100 and 300 nM of the EP4 agonist ONO AE1-329, the EP2 agonist butaprost, and with 100 nM of the DP1 agonist BW245 for 1 h. Eosinophils were afterward washed once and loaded with calcein-AM (1:1000) in incomplete human epithelial cell medium + 2% FBS for 30 min at 37 °C in the dark. After two additional washing steps, eosinophils were added to the esophageal epithelial cell monolayer and incubated for 30 min at 37 °C. After a wash with HBSS, signals of adherent eosinophils were measured on a FLEX station II. Data were measured as relative fluorescence units (RFUs) and expressed as % of adhesion of control (untreated eosinophils).

ECIS® Impedance Assay

Electric cell-substrate impedance sensing (ECIS®, Applied Biophysics, Troy, NY, USA) with gold electrode arrays of the type 8W10E + was used to monitor the integrity of esophageal epithelial cell monolayers at real time in vitro as previously described [23]. ECIS® array wells were activated with l-cysteine (10 mM) and precoated with 1% gelatine for 30 min at 37 °C. Then, wells were allowed to equilibrate with 200 μL cell medium for 15 min at 37 °C before esophageal epithelial cells were added in additional 200 μL medium to each well (1.5 × 10⁵ cells/well). Monolayer formation was followed by impedance measurements. Cells were treated with either 100 or 300 nM of EP4 agonist ONO-AE1-329 or EP2 agonist butaprost. Data are expressed as normalized resistance over time.

qPCR

RNA isolation, reverse transcription (RT-PCR) and quantitative real-time PCR (qPCR) were performed as previously described [24] using primers for EP1, EP2, EP3, EP4, DP1, DP2, and β-actin (all from Bio-Rad; listed in Table 2). Relative gene expression was assessed according to the ΔΔCq method.
Histology and Immunohistochemistry

Paraffin-embedded sections of biopsies from EoE patients (n = 3) were cut (5 µm) and deparaffinized. For immunohistochemistry, sections were microwaved for 2×5 min cycles in 10 mM citrate buffer, and processed by ABC method according to the manufacturer’s protocol (Vectastain ABC kit; Vector Laboratories, Burlingame, CA, USA). Sections were incubated with rabbit polyclonal antibodies against EP2 (1:500) (Alomone Labs, Jerusalem, Israel), EP4 (1:500), and DP1 (1:100) (Cayman Chemicals), visualized with 3-3′-diaminobenzidine (DAB) or Vector® ImmPact™ NovaRED HRP substrate kit according to the manufacturer’s protocol (Vector Laboratories), and counterstained with hematoxylin. As a negative control, primary antibodies were omitted during the staining procedure. Stainings were evaluated by two investigators. Images were taken with a high-resolution digital camera (Olympus UC 90) and analyzed by CellSense® standard 1.17 imaging software (Olympus, Vienna, Austria). Only contrast, brightness and color balance of images were adjusted. Sirius Red (Direct Red 80®, Sigma) was used to stain eosinophils in deparaffinized sections.

Statistical Analysis

Data are described as mean ± SEM (or otherwise stated) of at least n = 5 observations or otherwise stated. Statistical analyses were performed using one-way ANOVA followed by Tukey’s post hoc test, or by Student’s t test run on GraphPad Prism® software. p values of < 0.05 were considered significant.

Results

Expression of EP and DP Receptors in Human Blood Eosinophils

We first evaluated EP and DP receptor expression in peripheral blood eosinophils of EoE patients and healthy control subjects. We observed significant differences in EP2 (p = 0.0055) and EP4 (p = 0.0206) expression between EoE patients and control subjects but no changes in the expression levels of EP1 and EP3 (Fig. 1). Also, no differences were observed for DP1 and DP2 expression between EoE patients and control subjects (Fig. 1).

Next, we tested whether EP2 and EP4 receptors influence the migration and adhesion capacity of eosinophils to human primary esophageal epithelial cells and their supernatant. To this end, eosinophils were incubated with 100 or 300 nM of the EP4 agonist ONO-AE1-329 or the EP4 antagonist ONO-AE3-208, with 100 and 300 nM of the EP2 agonist butaprost, and, since flow cytometry data suggested a slight increase in DP1 expression in the eosinophils (Fig. 1), with 100 nM of the DP1 agonist BW245C or 1 µM of DP1 antagonist MK0524. Using supernatant from human primary esophageal epithelial cells as a chemoattractant, only the EP4 agonist (Fig. 2a), but not the EP2 and DP1 agonists (Fig. 2b, c), had an effect on the chemotactic behavior of eosinophils. In adhesion assays, however, both the EP4 agonist ONO-AE1-329 (Fig. 2d) and EP2 agonist butaprost (Fig. 2e) dose-dependently decreased the adhesion of eosinophils to primary esophageal epithelial cells, whereas the DP1 agonist BW245 clearly enhanced eosinophil adhesion (Fig. 2f).

Expression of EP and DP Receptors in Biopsies from EoE Patients

Expression levels of EP and DP receptors were investigated by qPCR in esophageal mucosal biopsies and by immunohistochemistry in histological sections of esophageal mucosa from EoE patients. Among EP receptors, highest gene expression was observed for EP4, while expression levels of EP2 and EP3 were very low (Fig. 3a). qPCR in esophageal squamous epithelial cell cultures showed that gene expression of EP4 was the highest among EP receptors, while levels of DP1 and DP2 were extremely low (Fig. 3b), indicating that infiltrated rather than esophageal epithelial cells are responsible for the DP1 expression measured in mucosal biopsies from EoE patients (Fig. 3a).

In accordance with these findings, we observed DP1 immunostaining in eosinophils (arrows) but hardly any in epithelial cells (Fig. 4a). EP4 immunostaining was present in eosinophils (Fig. 4b, arrow) but also in epithelial cells (Fig. 4b, arrowhead), confirming our qPCR data. Although EP2 transcripts are present in esophageal epithelial cells (Fig. 3b), they were barely detectable in the qPCR of EoE biopsies (Fig. 3a). However, EP2 immunostaining could be
observed in eosinophils (Fig. 4c, arrows) and epithelial cells of EoE biopsies (Fig. 4c, arrowhead).

**ECIS® Impedance (Epithelial Barrier) Assay**

Since EP receptors were also observed in esophageal epithelial cells (Figs. 3b, 4b and c), we investigated their functional implication by assessing their role in barrier integrity. We conducted ECIS® impedance assays in esophageal epithelial cells incubated with EP4 agonist ONO-AE1-329 (Fig. 5a; 10 and 300 nM) and EP2 agonist butaprost (Fig. 5b; 10 and 300 nM). A decrease in resistance correlates with a decrease in the monolayer’s integrity. As a result of the treatments, the EP4 agonist dose-dependently decreased resistance in comparison with vehicle (control) (Fig. 5a), while a decrease in resistance after incubation with the EP2 agonist was marginal though significant (Fig. 5b).

**Discussion**

EoE is an allergen-mediated eosinophilic inflammatory disease, and the increased number of eosinophils found in EoE biopsies is a determinant for the disease process [25]. Receptors that regulate migration of eosinophils may, therefore, play an important role in EoE pathophysiology. EP and DP receptors represent such regulators, and they have been implicated in eosinophil trafficking in allergic lung disease [8, 26, 27] and skin inflammation [28]. Here, we provide a first insight as to how EP receptors may impact...
the pathogenesis of EoE. The study has several limitations though. Since it is a pilot study, a low number of EoE patients was only included. Secondly, for the evaluations of biopsies, we only used material from EoE patients but not from healthy subjects. Finally, for migration and adhesion assays, eosinophils from EoE patients would have been desirable.

EP receptors have not yet been investigated in EoE peripheral blood eosinophils; however, two studies described increased expression of DP2 in EoE [29, 30]. Our findings indicate that surface expression of EP2 and EP4 was decreased in peripheral blood eosinophils of EoE patients, whereas expression of DP1 and DP2 was not different from control subjects, suggesting that, because of the anti-migratory capacity of EP4 and EP2 [16–18], eosinophils from EoE patients could have been more prone to infiltrate tissue. The activation of EP2 and EP4 diminished adhesion to epithelial cells and reduced, at least after EP4 agonism, chemotaxis toward esophageal epithelial cell supernatant, suggesting that particularly EP4 receptors may control the influx of eosinophils into esophageal mucosa. It is not quite clear why the EP2 agonist butaprost was not effective in inhibiting eosinophil migration toward supernatant although agonists of EP2 are able to inhibit eosinophil migration at similar concentrations [17]. A possible explanation may be that the EP2 receptors became desensitized by PGE2 (or by a metabolite) that was present in the supernatant of esophageal epithelial cells which are known to express Cox-2 enzymes [19] and are, therefore, capable of producing prostaglandins. Nishigaki et al. already showed that EP2 and EP4

---

**Fig. 2** Chemotaxis and adhesion assays with human blood eosinophils. Eosinophils from healthy donors were incubated with 100 nM of EP4 agonist ONO-AE1-329 or with 100 nM of the EP4 antagonist ONO-AE3-208 (a), with 100 and 300 nM of EP2 agonist butaprost (b), or with 100 nM of DP1 agonist BW245C and 1 µM of DP1 antagonist MK0524 (c), and chemotaxis was assessed using esophageal epithelial cell supernatant for chemotraction. n = 6–12 (for a, and b) and n = 3 (c); values are mean ± SEM; one-way ANOVA; Tukey’s post hoc test. ***p values < 0.001. Adhesion assays were performed with human blood eosinophils and esophageal epithelial cells after incubation of eosinophils with EP4 agonist ONO-AE1-329 (d), EP2 agonist butaprost (e), and with DP1 agonist BW245C (f) vs. untreated eosinophils (control; set at 100%). n = 5–7; values are mean ± SEM; one-way ANOVA; Tukey’s post hoc test. *p < 0.05; **p < 0.01
have different susceptibilities to PGE2 and its first metabo-
lite 15-keto-PGE2 [31]. EP4 and EP2 could have, therefore,
exhibited agonist-induced desensitization of different mag-
nitude in our experiments. Since the activation/blockade of
DP1 did not change chemotactic behavior of eosinophils
(despite the fact that DP1 agonism inhibits DP2-induced
migration of eosinophils [11, 32]), a mechanism similar to
EP2 may also apply for DP1.

The histological hallmark of EoE diagnosis is charac-
terized by eosinophils diffusely infiltrating the esophageal
squamous epithelium (see Fig. 3a) [3]. We were interested
whether EP and DP receptors may influence adhesion of
eosinophils to esophageal epithelial cells, as this process is
crucial for inducing cell death or releasing soluble factors
in the epithelium. For instance, CD11a/CD18-dependent
adhesion of eosinophils to colon cancer cells is required for
releasing cytotoxic mediators to induce apoptosis [33]. In
our experiments, agonists of EP4, EP2, and DP1 affected
adhesion of eosinophils to esophageal mucosal cells in a
differential manner. Therefore, endogenous PGs produced
by epithelial cells, or by eosinophils and other infiltrated
cells of the esophagus mucosa [34], may activate EP and DP
receptors depending on time and site of release.

The decreased adhesion of eosinophils by EP4 activation
is in line with earlier work from our laboratory [35]. In that
study, EP4 agonists decreased the adhesion of eosinophils
to vascular endothelium preventing the activation and cell-
surface clustering of β2 integrins, and the L-selectin shed-
ding of eosinophils [35]. As our present study also shows
a decreased adhesion after incubation with the EP2 agonist
butaprost, different/additional adhesion molecules than those
found on endothelial cells (such as E-selectin) are probably
present on the esophageal epithelial cells [36].

Molecular events in the esophageal epithelium, such as an
imbalance expression of proteases and protease inhibitors
(e.g., SPINK7), have been recently reported to be critical for
EoE pathophysiology [37, 38]. Thus, we performed barrier
assays with esophageal epithelial cells. In agreement with
another study [9, 19], we detected EP but hardly any DP
expression in esophageal mucosal biopsies pointing at a role for
EP rather than DP receptors in esophageal epithelial homeo-
stasis. The role of EP receptors in the esophageal mucosa is
still unknown, but, in general, eicosanoids and their receptors

---

**Fig. 3** Relative EP and DP mRNA expression in esophageal mucosal biopsies from EoE patients, n=4 (a) and cultivated esophageal epithelial cells (b) (from 3 different passages; EP1 expression was set at 1). The inset in a shows the esophageal squamous epithelium from an EoE patient stained with Sirius Red to identify eosinophils present (size bar: 100 µm). The inset in b shows cultured esophageal squamous epithelial cells

---

**Fig. 4** Immunohistochemistry shows strong DP1 staining in eosinophils (a, arrows), while epithelial cells in esophageal mucosal biopsies are practically devoid of staining. EP4 (b) and EP2 (c) immunostaining is prominent in eosinophils (arrows) but also visible in epithelial cells (arrowheads). 3-3′-Diaminobenzidine (DAB) was used as visualization substrate for DP1 and EP4 staining (brown precipitates), while for EP2, ImmPact™ NovaRED substrate was used (red precipitate). Calibration bar: 50 µm
may not play the same protective role in the esophageal than the gastric mucosa [39]. A biphasic effect for PGE_2 that depends on the applied dosage has been described in a model of acid reflux esophagitis, suggesting a more complex function of EP receptors in the esophageal epithelium [40]. We found that EP4 and EP2 activation may be even harmful to the epithelial barrier despite the finding that EP4 may be protective for endothelial barriers [41]. Although PGE_2 induces cell growth in CaCo-2 cells, PGE_2 has been also shown to cause disruption of a CaCo-2 cell barrier that involves EP1/EP4 activity supporting our observation that EP4 agonism decreases esophageal epithelial cell barrier [42, 43].

In summary, we show that surface expression of EP2 and EP4 receptors is decreased in blood eosinophils of EoE patients relative to healthy subjects. Among EP receptors, EP4 is the most expressed in esophageal mucosal biopsies of EoE patients. Since EP4 agonists inhibit cell migration, and EP4 and EP2 agonists decrease adhesion of eosinophils to the squamous esophageal epithelial cells, the low surface expression of eosinophilic EP4 and EP2 receptors could have an impact on the influx of eosinophils into the mucosa. However, EP4 and EP2 in esophageal epithelial cells could lower the epithelial barrier integrity which is counterproductive in EoE.

Acknowledgments Open access funding provided by Medical University of Graz. The authors wish to thank Iris Red for excellent technical support.

Author's contribution RS, CH, FD, AE, and EMS designed the study and supervised the work. FD, AE, and CH collected the patient samples and provided the clinical data. EMS performed flow cytometric experiments. VP performed all other assays (qPCR, migration, adhesion, barrier assay). CL made the histological diagnosis of EOE and provided the histological tissue. MG performed the immunohistochemical studies. EMS, VP, and RS analyzed all the results and performed the statistical analyses. RS, EMS, VP, MG, FD, CH, and AE wrote the manuscript. All authors were involved in data discussion and critical reviewing of the manuscript.

Funding This work was supported by the Austrian Science Fund (FWF Grants P30144 and KLI521 to RS).

Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

Ethical approval Ethical approval was granted by the ethics committee of the Medical University of Graz (protocol numbers EK 28-492 ex 15/16 and EK 17-291 ex 05/06. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

Open Access This article is distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (http://creativecommons.org/licenses/by-nc/4.0/), which permits any noncommercial use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

References

1. Rothenberg ME. Biology and treatment of eosinophilic esophagitis. Gastroenterology. 2009;137:1238–1249.
2. Lucendo AJ, Molina-Infante J, Arias A, et al. Guidelines on eosinophilic esophagitis: Evidence-based statements and recommendations for diagnosis and management in children and adults. United Eur Gastroenterol J. 2017;5:335–358.
3. Dellon ES, Liacouras CA, Molina-Infante J, et al. Updated international consensus diagnostic criteria for eosinophilic esophagitis: Proceedings of the AGREE conference. Gastroenterology. 2018;155:1022–1033.
4. Chehade M, Jones SM, Peked RD, et al. Phenotypic characterization of eosinophilic esophagitis in a large multicenter patient population from the consortium for food allergy research. J Allergy Clin Immunol Pract. 2018;6:1534–1544.
5. Warners MJ, Oude Nijhuis RAB, de Wijkerslooth LRH, Smout AJPM, Bredenoord AJ. The natural course of eosinophilic esophagitis and long-term consequences of undiagnosed disease in a large cohort. Am J Gastroenterol. 2018;113:836–844.

6. Sastre B, Fernandez-Nieto M, Lopez E, et al. PGE(2) decreases muscle cell proliferation in patients with non-asthmatic eosinophilic bronchitis. Prostaglandins Other Lipid Mediat. 2011;95:11–18.

7. Schuligoi R, Sturm E, Luschp N, et al. CRTH2 and D-type prostanoid receptor antagonists as novel therapeutic agents for inflammatory diseases. Pharmacology. 2010;85:372–382.

8. Kupczyk M, Kuna P. Targeting the PGD2/CRTH2/DP1 signaling pathway in asthma and allergic disease: Current status and future perspectives. Drugs. 2017;77:1281–1294.

9. Straumann A, Hoelsl S, Bassmann C, et al. Anti-eosinophil activity and clinical efficacy of the CRTH2 antagonist OCS00459 in eosinophilic esophagitis. Allergy. 2013;68:375–385.

10. Jandl K, Heinemann A. The therapeutic potential of CRTH2/DP2 beyond allergy and asthma. Prostaglandins Other Lipid Mediat. 2017;133:42–48.

11. Schratl P, Royer JF, Kostenis E, et al. The role of the prostaglandin D2 receptor, DP, in eosinophil trafficking. J Immunol. 2007;179:4792–4799.

12. Ajuebor MN, Singh A, Wallace JL. Cyclooxygenase-2-derived prostaglandin D(2) is an early anti-inflammatory signal in experimental colitis. Am J Physiol Gastrointest Liver Physiol. 2000;279:G2238–G2442.

13. Sturm EM, Radnai B, Jandl K, et al. Opposing roles of prostaglandin D2 receptors in ulcerative colitis. J Immunol. 2014;193:827–839.

14. Sugimoto Y, Narumiya S. Prostaglandin E receptors. J Biol Chem. 2007;282:11613–11617.

15. Kawahara K, Hohjoh H, Inazumi T, Tsuchiya S, Sugimoto Y. Prostanoid receptor agonists as therapeutic agents for the treatment of asthma. Sci STKE. 2005;2005:pe47.

16. Hohjoh H, Inazumi T, Tsuchiya S, Sugimoto Y. Prostanoid receptors and acute inflammation in skin. Biochimie. 2014;107:78–81.

17. Johnson M, Bove M, Bergquist H, et al. Distinctive blood eosinophilic phenotypes and cytokine patterns in eosinophilic esophagitis, inflammatory bowel disease and airway allergy. J Innate Immun. 2011;3:594–604.

18. Sturm EM, Parzmair GP, Radnai B, et al. Phosphoinositide-dependent protein kinase 1 (PDK1) mediates potent inhibitory effects on eosinophil trafficking through E-prostanoid 2 receptors. J Immunol. 2011;68:3573–3587.

19. Jimenez P, Piazuelo E, Cebrian C, et al. Prostaglandin EP2 receptor agonists and acute inflammation in skin. J Biol Chem. 2014;193:827–839.

20. Sheibanie AF, Yen J, Khayrullina T, et al. The proinflammatory effect of prostaglandin E2 in experimental inflammatory bowel disease is mediated through the IL-23–> IL-17 axis. J Immunol. 2010;181:7273–7283.

21. Sturm EM, Parzmair GP, Radnai B, et al. Phosphoinositide-dependent protein kinase 1 (PDK1) mediates potent inhibitory effects on eosinophils. Eur J Immunol. 2015;45:1548–1559.

22. Jimenez P, Piazuelo E, Cebrian C, et al. Prostaglandin EP2 receptor expression is increased in Barrett’s oesophagus and oesophageal adenocarcinoma. Aliment Pharmacol Ther. 2010;31:440–451.

23. Scheibani AF, Yen JH, Khayrullina T, et al. The proinflammatory effect of prostaglandin E2 in experimental inflammatory bowel disease is mediated through the IL-23–> IL-17 axis. J Immunol. 2007;178:8138–8147.

24. Jiang GL, Nieves A, Im WB, Old DW, Dinh DT, Wheeler L. The effect of prostaglandin of eosinophilic esophagitis. Dis Esophagus. 2014;27:601–606.

25. Hasenohrl C, Feuersinger D, Sturm EM, et al. G protein-coupled receptor GPR55 promotes migration and adhesion of colon cancer cells indicating a role in metastasis. Br J Pharmacol. 2016;173:142–154.

26. Kargl J, Andersen L, Hasenohrl C, et al. GPR55 promotes migration and adhesion of colon cancer cells indicating a role in metastasis. Br J Pharmacol. 2016;173:142–154.

27. Sridhara S, Ravi K, Smyrk TC, et al. Increased numbers of eosinophils, rather than only etiology, predict histologic changes in patients with esophageal eosinophilia. Clin Gastroenterol Hepatol. 2012;10:735–741.

28. Birrell MA, Maher SA, Dekkak B, et al. Anti-inflammatory effects of PGE2 in the lung: Role of the EP4 receptor subtype. Thorax. 2015;70:740–747.

29. Chung KF. Evaluation of selective prostaglandin E2 (PGE2) receptor agonists as therapeutic agents for the treatment of asthma. Sci STKE. 2005;2005:pe47.

30. Longblom C, Kappi T, Bergquist H, et al. Differences in eosinophil molecular profiles between children and adults with eosinophilic esophagitis. Allergy. 2017;72:1406–1414.

31. Nishigaki N, Negishi M, Ichikawa A. Two gs-coupled prostaglandin E receptor subtypes, EP2 and EP4, differ in desensitization and sensitivity to the metabolic inactivation of the agonist. Mol Pharmacol. 1996;50:1031–1037.

32. Chiba T, Ueki S, Ito W, et al. The opposing role of two prostaglandin D2 receptors, DP and CRTH2, in human eosinophil migration. Ann Allergy Asthma Immunol. 2011;106:511–517.

33. Legrand F, Driss V, Delbeke M, et al. Human eosinophils exert TNF-α and granzyme A-mediated tumoricidal activity toward colon carcinoma cells. J Immunol. 2010;185:7443–7451.

34. Rosenberg HF, Dyer KD, Foster PS. Eosinophils: Changing perspectives in health and disease. Nat Rev Immunol. 2013;13:9–22.

35. Konya V, Philispose S, Balint Z, et al. Interaction of eosinophils with endothelial cells is modulated by prostaglandin EP4 receptors. Eur J Immunol. 2011;41:2379–2389.

36. Bloom S, Simmons D, Jewell DP. Adhesion molecules intercellular adhesion molecule-1 (ICAM-1), ICAM-3 and B7 are not expressed by epithelium in normal or inflamed colon. Clin Exp Immunol. 1995;101:157–163.

37. Simon D, Page B, Vogel M, et al. Evidence of an abnormal epithelial barrier in active, untreated and corticosteroid-treated eosinophilic esophagitis. Allergy. 2018;73:239–247.

38. Rohman M, Azouz NF, Rothenberg ME. Epithelial origin of eosinophilic esophagitis. J Allergy Clin Immunol. 2018;142:10–23.

39. Long JD, Orlando RC. Eicosanoids and the esophagus. Prostaglandins Other Lipid Mediat. 2000;61:91–104.

40. Takeuchi K, Kato S, Amagase K. Prostaglandin EP receptors involved in modulating gastrointestinal mucosal integrity. J Pharmacol Sci. 2010;114:248–261.

41. Oskolkova O, Gawlik G, Tian Y, et al. Prostaglandin E receptor-4 receptor mediates endothelial barrier-enhancing and anti-inflammatory effects of oxidized phospholipids. FASEB J. 2017;31:4187–4202.

42. Cabral M, Martin-Venegas R, Moreno JJ. Role of arachidonic acid metabolites on the control of non-differentiated intestinal epithelial cell growth. Int J Biochem Cell Biol. 2013;45:1620–1628.

43. Rodriguez-Lagunas MJ, Martin-Venegas R, Moreno JJ, Ferrer R. PGE2 promotes Ca2 + -mediated epithelial barrier disruption through EP1 and EP4 receptors in caco-2 cell monolayers. Am J Physiol Cell Physiol. 2010;299:C324–C334.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.