

Bronchial Epithelial IgA Secretion Is Impaired in Asthma

Role of IL-4/IL-13

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Abstract

Rationale: Asthma is associated with increased lung IgE production, but whether the secretory IgA system is affected in this disease remains unknown.

Objectives: We explored mucosal IgA transport in human asthma and its potential regulation by T-helper cell type 2 inflammation.

Methods: Bronchial biopsies from asthma and control subjects were assayed for bronchial epithelial polymeric immunoglobulin receptor (pIgR) expression and correlated to T-helper cell type 2 biomarkers. Bronchial epithelium reconstituted *in vitro* from these subjects, on culture in air-liquid interface, was assayed for pIgR expression and regulation by IL-4/IL-13.

Measurements and Main Results: Downregulation of pIgR protein was observed in the bronchial epithelium from patients with asthma ($P = 0.0002$ vs. control subjects). This epithelial defect was not observed *ex vivo* in the cultured epithelium from patients with asthma. Exogenous IL-13 and IL-4 could inhibit pIgR expression and IgA transcytosis. Mechanistic experiments showed that autocrine transforming growth factor- β mediates the IL-4/IL-13 effect on the pIgR, with a partial contribution of upregulated transforming growth factor- α /epidermal growth factor receptor.

Conclusions: This study shows impaired bronchial epithelial pIgR expression in asthma, presumably affecting secretory IgA-mediated frontline defense as a result of type 2 immune activation of the transforming growth factor pathway.

Keywords: polymeric immunoglobulin receptor; IL-13; IgA; asthma

Chronic airway diseases, such as asthma or chronic obstructive pulmonary disease (COPD), represent major public health problems, associated with abnormal lung

responses to environmental antigens or irritants. Asthma is a frequent and complex heterogeneous disease (1) that involves most frequently T-helper cell type 2 (Th2)-

mediated eosinophilic inflammation. Severe refractory asthma is characterized by clinical features (persistent airflow obstruction and recurrent exacerbations)

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At a Glance Commentary

Scientific Knowledge on the

Subject: Lung IgE production and sensitization to perennial allergens (e.g., house dust mite) have long been associated with asthma. Several studies indicated impaired front-line mechanisms of antiviral defense and that the epithelium is probably playing a key instructive role in this disease. In contrast, little is known about IgA mucosal immunity in asthma. IgA is the main humoral defense factor in mucosal tissues, including the airways, where it is produced as dimeric IgA, which is able to bind to the polymeric immunoglobulin receptor expressed at the basolateral side of epithelial cells.

What This Study Adds to the

Field: This study reveals that asthma is characterized by impaired bronchial epithelial expression of the polymeric immunoglobulin receptor, recapitulated *in vitro* through IL-4 receptor α activation, which alters not only the epithelial junctional barrier but also IgA-mediated lung defense.

that are contrasting with milder forms of the disease (2).

Lung IgE production and sensitization to perennial allergens (e.g., house dust mite) have long been associated with asthma (3, 4). In addition, several studies indicated impaired front-line mechanisms of antiviral defense (5) and that the epithelium probably plays a key instructive role in this disease (6–8). In contrast, little is known about IgA mucosal immunity in asthma. IgA is the main humoral defense factor in mucosal tissues, including the airways. It is produced as dimeric (d-) IgA, which is able to bind to the polymeric immunoglobulin receptor (pIgR) expressed at the basolateral side of epithelial cells. The pIgR translocates IgA up to the apical pole, where dimeric IgA (d-IgA) is released after proteolytic cleavage of pIgR. d-IgA remains bound to the extracellular part of the pIgR named secretory component (SC), to generate secretory (S-) IgA.

Epidemiologic studies showed that selective IgA deficiency is associated with an increased risk for mite allergic sensitization and chronic bronchitis (9). Whereas some

studies suggested a role for antigen-specific IgA for eosinophil degranulation in the lung after allergen challenge (10), decreased IgA content was originally described in bronchoalveolar lavage from patients with severe asthma (11). In COPD, the S-IgA system has been more extensively studied and includes impaired pIgR-mediated transepithelial transport of IgA (12, 13), probably related to a proteolytic degradation by neutrophil-derived proteinases (14) and to an intrinsic dysprogramming of the epithelium that is partly mediated by TGF (transforming growth factor)- β 1 (12), while IgA synthesis is upregulated (15).

Although asthma is associated with increased lung IgE production, whether the S-IgA system is affected in this disease remains unknown. The aim of this study was to explore mucosal immunity in human asthma through an integrated approach of endobronchial biopsies and *ex vivo* bronchial epithelial cultures, addressing the hypothesis that the S-IgA system could be altered as a consequence of Th2 inflammation. Because alterations of the airway epithelium are probably key early events in asthma, this study first aimed to assess bronchial epithelial pIgR expression, which is the limiting factor for lung IgA immunity (16). In addition, the “memory” of putatively abnormal pIgR expression was evaluated by studying its persistence *ex vivo*, in the epithelium cultured on air–liquid interface (ALI). The relationship between pIgR expression and Th2/eosinophilic inflammation was then explored as a putative mechanism of dysregulated IgA immunity, considering that this inflammation is usually prominent in asthma and that a negative correlation between pIgR expression and tissue eosinophilia was previously observed in chronic rhinosinusitis (17). The working hypothesis was that S-IgA immunity is dysregulated in asthma through an inflammatory type 2-mediated aberrant regulation of the bronchial epithelium. Some of the results of this study have been previously reported in the form of abstracts (18, 19).

Methods

Patients and Lung Tissue Sampling

A total of 115 patients ($n = 29$ control subjects, $n = 26$ patients with mild to moderate asthma, and $n = 42$ patients with severe asthma) undergoing endobronchial biopsies ($n = 97$) or lung resection surgery

for a solitary tumor ($n = 18$) were included in this study (Table 1). Of note, no difference was observed between endoscopic and surgical specimens from a same group of subjects in terms of pIgR immunostaining (data not shown). Bronchial biopsies were processed for immunohistochemistry (immediately immersed in 4% formaldehyde in phosphate-buffered saline at pH 7.4), for qRT-PCR (immediately immersed in RNA later), and for primary bronchial epithelial cell culture (put in culture medium), according to random selection of patients and to availability of high-quality samples (Table 1, last row).

Primary Human Bronchial Epithelial Cell Culture

Bronchial epithelial cells (BEC) were derived from bronchial biopsies from these well-characterized patients with asthma, compared with control subjects ($n = 8$ control subjects, $n = 10$ mild-to-moderate asthma, $n = 13$ severe asthma) (Table 2), with four additional control subjects selectively used for neutralization experiments. BEC were cultured in polarized and ALI conditions, to reconstitute a pseudostratified mucociliary epithelium (15, 20, 21), and in the presence or not of IL-13 (10 ng/ml; R&D systems), IL-4 (10 ng/ml; Miltenyi Biotec), lipoxin-A4 (LXA4) (10^{-7} M; Sigma Aldrich), TGF- β 1, TGF- β 2, or TGF- α (10 ng/ml; R&D Systems) since the beginning of the ALI phase. Specific antibodies/inhibitors were used in neutralization experiments: AS1517499 as STAT6 (signal transducer and activator of transcription 6) inhibitor (0.5 μ M; Axon Medchem BV), goat antihuman TGF- β R2 antibody (Ab) (10 μ g/ml; R&D Systems), goat antihuman IL-4R α (IL-4 receptor α) Ab (10 μ g/ml; R&D Systems) and erlotinib as epidermal growth factor receptor (EGFR)–tyrosine kinase inhibitor (0.5 μ M; Axon Medchem BV). The medium was replaced every 2 days and analysis performed at 2, 3, or 4 weeks of culture. Please refer to the online supplement for more details.

Gene Expression by qRT-PCR

Expression levels of pIgR, eotaxin, GATA-3 (Trans-acting T-cell-specific transcription factor), proteoglycan-2 encoding the eosinophil granule major basic protein, and pIgR-related transcription factors (upstream stimulatory factor [USF]1, USF2, AP2 [activating enhancer binding protein 2])

Table 1. Patient Characteristics of the Study Population

	Control Subjects	Asthma	
		Mild	Severe
Subjects, <i>N</i>	29	26	42
Age, yr	49.6 ± 15.4	52.9 ± 12.8	54.0 ± 12.5
Sex, M/F, <i>n</i>	16/13	9/17	21/21
Control of asthma, yes/no, <i>n</i>	NA	17/9	1/41*
Exacerbations (last year)	NA	0.40 ± 0.70	3.43 ± 2.83*
Last FEV ₁ , % predicted (<i>N</i> = 96)	97.4 ± 13.7	90.8 ± 17.8	73.3 ± 17.6*†
Best FEV ₁ , % predicted (<i>N</i> = 84)	102.7 ± 6.7	93.9 ± 12.7	78.9 ± 19.9*†
Blood eosinophils/μl (<i>N</i> = 83)	115.3 ± 69.1	265.9 ± 353.4	662.7 ± 1863.1†
Allergy, <i>n</i> (<i>N</i> = 86)	1	10†	20†
ICS, <i>n</i> (<i>N</i> = 93)	0	17†	41*†
LABA, <i>n</i> (<i>N</i> = 94)	1	13†	42*†
SABA, <i>n</i> (<i>N</i> = 94)	1	11†	36*†
OCS, <i>n</i> (<i>N</i> = 94)	1‡	0	21*†
Available samples, <i>n</i>			
Tissue IHC	15	8	8
Tissue RNA	7	13	26
HBEC culture	8	10	13

Definition of abbreviations: HBEC = human bronchial epithelial cells; ICS = inhaled corticosteroids; IHC = immunohistochemistry; LABA = long-acting β_2 agonist; NA = not applicable; OCS = oral corticosteroids; SABA = short-acting β_2 agonist.

Demographic data, lung function tests, and asthma-related features are stated for the patient groups. *N* is specified (in the left-hand-side column) when data are missing. Values represent mean ± SD, unless specified otherwise.

* $P < 0.05$ severe asthma versus mild asthma; Kruskal-Wallis test used for comparison among the three groups.

† $P < 0.05$ asthma versus control subjects; Kruskal-Wallis test used for comparison among the three groups.

‡One patient was treated by OCS for a connective-tissue disease not affecting the lungs. Primary BEC were derived from 31 patients (see Table 2).

were quantified by qRT-PCR and reported to those of RPS18 (ribosomal protein S18), RNA18S, and GAPDH (glyceraldehyde-3-phosphate dehydrogenase) housekeeping genes, using the iCycler IQ5 PCR (Bio-Rad). Data analysis was performed using Bio-Rad iQ5 software (see online supplement for primers sequences).

Immunohistochemistry for plgR and Epithelial Markers

Paraffin-embedded sections of bronchial biopsies were incubated with antihuman SC rabbit IgG (SC577) prepared in our laboratory, as previously reported (22). Slides were scanned using the SCN400 scanner and plgR epithelial staining was quantified by the TissueIA software (Leica Microsystems). Results were expressed as the percentage of stained area. Paraffin sections of BEC were stained for plgR/SC (SC577), p63, MUC5AC (mucin 5AC) (Leica Biosystems), and β -tubulin IV (Sigma). Quantification of the staining intensity was performed with ImageJ software on tissue section stained during a same run, using the same threshold.

Western Blot for plgR/SC

Proteins from BEC extracts were run onto sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred onto a polyvinylidene fluoride membrane probed with rabbit polyclonal antibody against human plgR/SC (SC577). Rabbit polyclonal antibody against GAPDH (G9545, Sigma) was used for standardization, and horseradish peroxidase-conjugated antirabbit IgG (SantaCruz) as secondary antibody. Blots were developed with an enhanced chemiluminescence kit (Biorad) and densitometric analysis was performed by using Alpha view Software (ProteinSimple).

ELISA for SC and TGF- α and - β

SC production by ALI-BEC was assessed using goat antihuman plgR/SC Ab (SC606 made in our laboratory) as capture Ab (1, 2). Biotin-conjugated Ab SC606 and horseradish peroxidase-conjugated streptavidin (BD Biosciences) were used for detection. TGF- α , TGF- β 1, and TGF- β 2 were assayed by using the DuoSet kits (DY239, DY240, and DY302, respectively) from R&D Systems.

Transcytosis Assay of D-IgA

The ability of BEC to transcytose d-IgA was assessed after incubation with d-IgA (added in the basolateral compartment) and by quantifying S-IgA in the apical wash after 48 hours. To detect S-IgA, the same goat anti-plgR/SC (SC606) was used as capture Ab and horseradish peroxidase-conjugated goat anti-IgA Ab (A0295, Sigma) as detection Ab.

RNA Sequencing

BEC treated with IL-13 or IL-4 were harvested in TRI Reagent and stocked at -80°C until extraction was performed with the RiboPure Kit from Ambion (Life Technologies) according to manufacturer's instructions. Quality and integrity of eluted RNAs were assessed by running aliquots on the NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies) and on the Agilent Bioanalyzer. All samples exhibited 260/280 ratios greater than 1.80 and RNA Integrity Number comprised between 9.5 and 10. Precise quantification was also performed on the Qubit 2.0 Fluorometer (Molecular probes, Life Technologies) and 50 ng of each sample were used for the subsequent RNA sequencing experiment using reagents and protocol provided with the TruSeq Targeted RNA Expression custom panel kit by Illumina. Libraries were equimolarly pooled and sequenced on the MiSeq sequencer with a MiSeq Reagent Kit v3 (Illumina). Among the 138 interrogated transcripts, targets exhibiting read counts above noise threshold were first filtered and counts of the 80 remaining were normalized to the mean of three stable housekeeping genes (EP300 [E1A-associated protein p300], HRTPI [human receptor transporter protein 1], and RPS13) determined with the GeNorm algorithm on Log2 transformed counts (stability value $M = 0.55$) (23). Differential expression analysis was then performed using the DESeq2 method (24) and fold changes induced by IL-4 and IL-13 were calculated.

Statistical Analysis

Differences between two groups were analyzed using nonparametric tests: Mann-Whitney *U* test (unpaired data), Wilcoxon rank test (paired data), or Kruskal-Wallis test for multiple comparisons (Table 1). Correlation coefficients were calculated using Spearman rank method. A probability value less than 0.05 was considered as statistically significant. Statistical

Table 2. Clinical Characteristics of Patients from Whom ALI-BEC Were Derived

	Control Subjects	Asthma	
		Mild	Severe
Subjects, <i>N</i>	8	10	13
Age, yr	40.7 ± 17.8	51.2 ± 12.0	53.8 ± 11.5
Sex, M/F, <i>n</i>	4/4	4/6	8/5
Control of asthma, yes/no, <i>n</i>	NA	10/0	1/13*
Exacerbations (last year)	NA	0.22 ± 0.44	5.70 ± 3.56*
Last FEV ₁ , % predicted (<i>n</i> = 25)	104.5 ± 8.8	89.9 ± 15.7	72.9 ± 22.2 [†]
Best FEV ₁ , % predicted (<i>n</i> = 25)	104.5 ± 8.8	92.5 ± 12.4	75.8 ± 18.8 [†]
Blood eosinophils/μl (<i>n</i> = 25)	160.0 ± 29.4	217.2 ± 206.5	530.9 ± 655.3
Allergy, <i>n</i> (<i>N</i> = 29)	0	5 [†]	4 [†]
ICS, <i>n</i> (<i>N</i> = 28)	0	5 [†]	13* [†]
LABA, <i>n</i> (<i>N</i> = 28)	0	5 [†]	13* [†]
SABA, <i>n</i> (<i>N</i> = 28)	0	5 [†]	10* [†]
OCS, <i>n</i> (<i>N</i> = 28)	0	0	6* [†]

Definition of abbreviations: ALI = air-liquid interface; BEC = bronchial epithelial cells; ICS = inhaled corticosteroids; LABA = long-acting β_2 agonist; NA = not applicable; OCS = oral corticosteroids; SABA = short-acting β_2 agonist.

Demographic data, lung function tests, and disease history and therapies. Primary BEC were derived from 31 patients with or without asthma (*n* = 8 for control subjects, *n* = 10 for patients with mild asthma, and *n* = 13 for patients with severe asthma). *N* is specified (in the left-hand-side column) when data are missing. Values represent mean ± SD, unless specified otherwise.

**P* < 0.05 versus patients with mild asthma; Kruskal-Wallis test.

[†]*P* < 0.05 versus control subjects; Kruskal-Wallis test.

analyses and figures were performed using GraphPad Prism version 5.00 for Windows (www.graphpad.com).

Ethical Considerations

All patients gave signed informed consent to the study protocol, which was approved by our local ethical committees (Ref. #2005/22sep/149 and 2013/21mar/136; and comité de protection des personnes Sud Méditerranée II IDRCB 2013R10).

Results

Patient Characteristics

Endobronchial biopsies from patients with asthma with mild-to-moderate or severe disease, as compared with control subjects, were assayed by immunohistochemistry and PCR analyses (Table 1). An allergic phenotype was observed in 38.5% of mild versus 47.6% in severe patients with asthma. In parallel, ALI-BEC were derived from 31 subjects (8 control subjects, 10 mild asthma, 13 severe asthma) (Table 2) to reconstitute *ex vivo* a mucociliary airway epithelium.

pIgR Expression in Bronchial Biopsies from Patients with Asthma

A reduced pIgR immunostaining was observed in the epithelium of patients with asthma as compared with control subjects,

both in mild-to-moderate (*P* = 0.0002, vs. control subjects) and severe groups (*P* = 0.0009, vs. control subjects), and irrespectively of the allergic status (Figures 1A–1C). Although it was not related to the global severity level of asthma, pIgR expression was (modestly) correlated with airway function in terms of FEV₁ (see Figure E1 in the online supplement) (*r* = 0.37; *P* = 0.03). No significant change could be observed at the pIgR mRNA level (Figure 1D).

pIgR Expression in Bronchial Epithelial Cultures from Patients with Asthma

We observed no constitutive change in pIgR expression, SC release, or d-IgA transcytosis by ALI-BEC from patients with asthma compared with those from control subjects (Figure 2). Because these data suggest that the *in vivo* microenvironment could be involved in epithelial pIgR downregulation, the relationship between pIgR expression and airway mucosal inflammation was ascertained in bronchial tissue by assessing tissue eosinophils (major basic protein/proteoglycan-2, encoding the eosinophil granule 2⁺) and Th2 (GATA-3⁺) cells, but no significant correlation was observed with pIgR expression (see Figure E1).

It was then assessed *ex vivo* whether Th2/eosinophilic inflammation could provide key microenvironmental factors inducing pIgR downregulation. First, no significant effect of activated eosinophils was observed on SC release or pIgR expression after 24 hours (see Figure E2A) or 48 hours (not shown) of coculture. Considering impaired resolution mechanisms in asthma, with defect in LXA4 and overexpression of 15 lipoxigenase 1/2 (18, 25–28), and data showing increased S-IgA in 12/15 LO^{−/−} mice (29), we wondered whether LXA4 could directly regulate pIgR expression, but no significant effect was observed (see Figure E2B).

IL-4/IL-13–Driven Downregulation of Bronchial Epithelial pIgR

In contrast to eosinophils or LXA4, exogenous IL-13 significantly inhibited SC release and pIgR expression (Figure 3). This effect was observed in cultures performed in ALI for 2–4 weeks and was also observed at the mRNA level at 4 weeks (when pIgR expression is maximal, presumably in relation to optimal epithelial differentiation) (Figure 3B). IL-13-driven pIgR inhibition was observed in ALI-BEC irrespectively of the patient group (see Figure E3). Accordingly, the epithelium treated with IL-13 for 2 weeks had a lower capacity to transcytose d-IgA (Figure 3E). A strong pIgR inhibition was also observed when treating ALI-BEC with IL-4 for 2, 3, and 4 weeks, suggesting that the effect was mediated through the IL-4R α shared by IL-4 and IL-13 (Figures 4A and 4B). In contrast, no significant effect was observed in short-term cultures stimulated for 48 hours with IL-13 or IL-4 (Figure 4C).

Considering these kinetics, it was next evaluated whether this IL-4/IL-13 effect involved changes in bronchial epithelial differentiation. First, Th2-related signatures were confirmed in ALI-BEC exposed to IL-13 in terms of induction of eotaxin-3 mRNA (Figure E4), and release of periostin (30). IL-13 treatment did not favor goblet or ciliated cell differentiation, but a trend for a global reduction in mucociliary differentiation was noted along with increased p63⁺ basal cells (Figures 5A and 5B). Accordingly, a trend (not reaching statistical significance, *P* = 0.07) for reduced expression of USF2, a key transcription factor for constitutive pIgR expression, was seen in the biopsies from patients with severe asthma (Figure

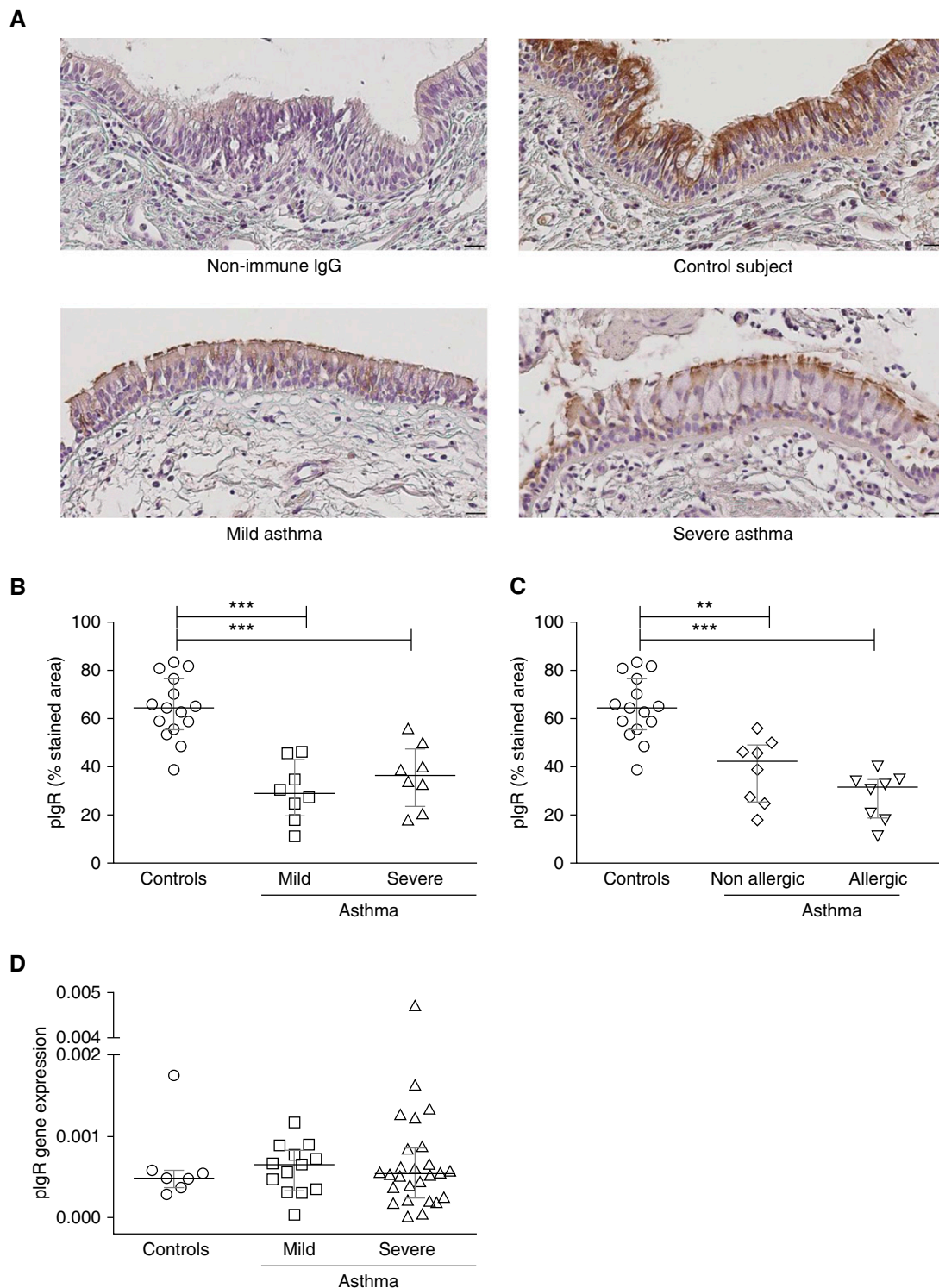


Figure 1. Polymeric immunoglobulin receptor (pIgR) immunostaining of bronchial biopsies from control subjects and patients with mild-to-moderate and severe asthma. (A) Representative pIgR staining in bronchial tissue from patients with or without asthma (scale = 20 μ m). (B) Percentage of epithelial staining of pIgR in bronchial tissue of patients with ($n = 8$ mild asthma, $n = 8$ severe asthma) or without asthma ($n = 15$ control subjects). (C) Percentage of epithelial staining of pIgR in bronchial tissue of patients with asthma with or without allergic phenotype ($n = 15$ control subjects, $n = 8$ allergic asthma, $n = 8$ nonallergic asthma). (D) Expression of pIgR mRNA corrected for three housekeeping genes (RPS18, GAPDH, and 18S rRNA) in bronchial tissue ($n = 8$ control subjects, $n = 13$ mild asthma, $n = 26$ severe asthma). Circles, control subjects; squares, mild-to-moderate asthma; triangles, severe asthma; diamond, nonallergic asthma; and inverted triangle, allergic asthma. Lines depict medians and interquartile ranges. $**P < 0.01$, $***P < 0.001$ (Mann-Whitney U test).

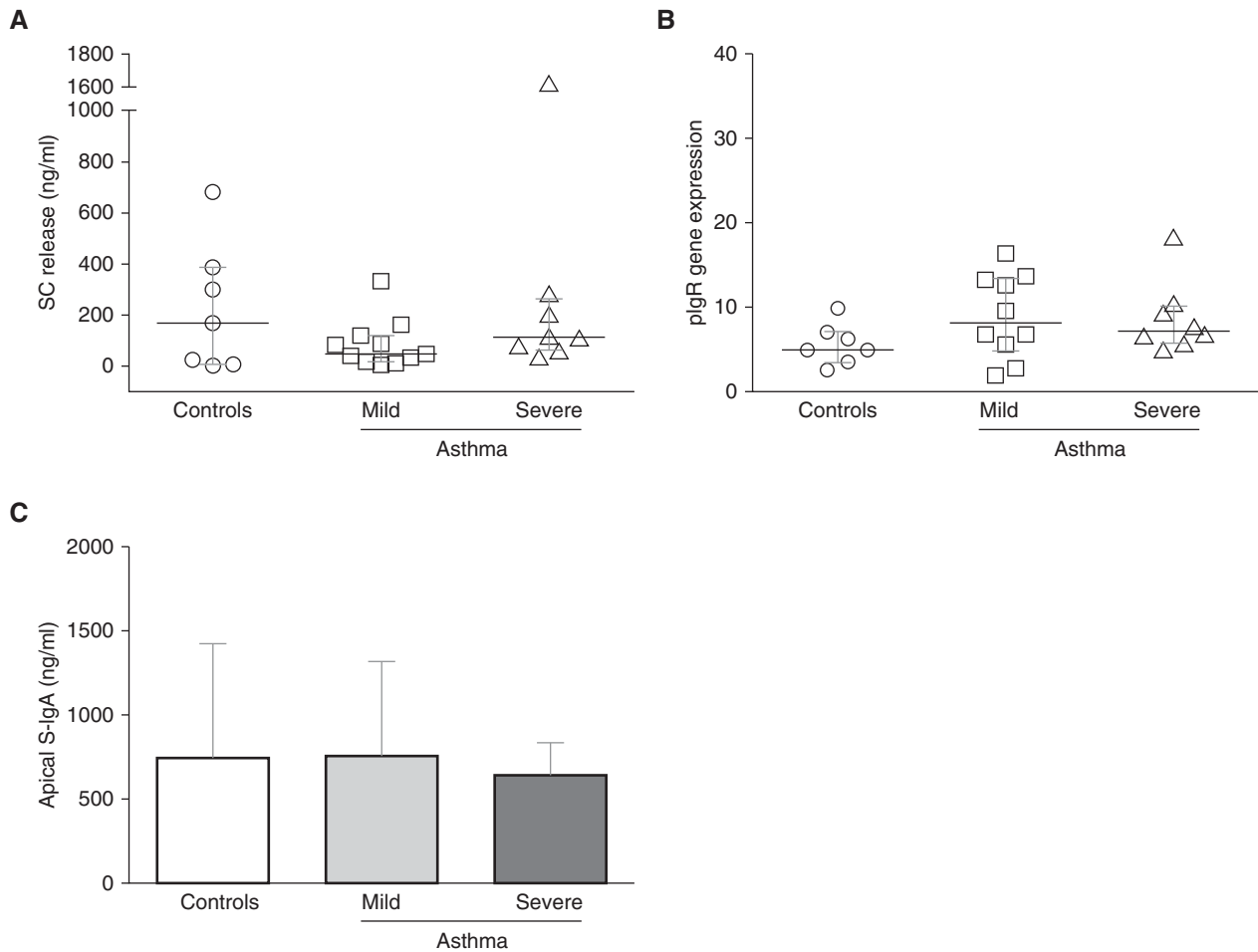


Figure 2. Polymeric immunoglobulin receptor (pIgR) expression by the cultured airway epithelium from patients with asthma and control subjects. Secretory component release (A), pIgR mRNA expression corrected to RPS18 (B), and dimeric IgA transcytosis (apical release of secretory IgA) by bronchial epithelial cells (C) at 3 weeks of air-liquid interface culture. Circles, control subjects ($n = 7$); squares, mild asthma ($n = 10$); and triangles, severe asthma ($n = 8$). Lines depict medians and interquartile ranges. SC = secretory component; S-IgA = secretory IgA.

E5). In addition, the effects of IL-4/IL-13 were studied more extensively by using RNA-sequencing targeted to several epithelial-related genes (Figures 5C and 5D; Table E1). The analysis of the top deregulated genes on IL-4 and IL-13 activation suggests altered potential of proliferation and differentiation, suggested by upregulation of p63 and keratin 6a (expressed by basal and intermediate cells) along with KRT16 (keratin 16), and downregulated BIRC5 (survivin, also called baculoviral inhibitor of apoptosis repeat-containing 5) and KAT2B/PCAF (catenin 2B/histone acetyltransferase). This was accompanied by downregulated expression of SOX (sex determining region Y-box)-2 as transcription factor regulating bronchial epithelial differentiation and decreased VANGL2 (Vang-like protein 2) as marker of polarity, and upregulated SOX9, TGF- α ,

and TEAD1/TEF-1 (transcriptional enhancer factor 1). Intriguingly, occludin upregulation was observed, whereas *wingless* (Wnt)-related factors were either upregulated (PORCN [porcupine homolog-Drosophila], DKK1 [Dickkopf-related protein 1]) or downregulated (Wnt4, Axin2).

These findings corroborate a profound deregulation of bronchial epithelial differentiation on IL-13 or IL-4 activation, which may lead to pIgR downregulation.

Mechanism of IL-4/IL-13-Driven pIgR Downregulation

Based on RNA-seq data showing upregulation of TGF- α (~twofold increase) and on previous data in BEC showing induction of TGF- β expression by IL-4 (31) and pIgR downregulation by TGF- β (32), the role of TGF superfamilies was further explored. First, it was confirmed that TGF- α

was increased at the protein level in BEC cultures stimulated by IL-13 (Figure 6A). A trend for TGF- β 2 upregulation was also observed in those cultures (Figures 6A and E6), whereas TGF- β 1 was not detected. When ALI-BEC were stimulated by TGF- β 2 for 2–4 weeks, pIgR/SC production was almost completely abrogated (residual SC production, 5–10 ng/ml) (Figures 6B and 6C). A similar effect with TGF- β 1 and a partial inhibition with TGF- α (~50% at 4 wk) were seen (Figures 6B and 6C), and confirmed at the mRNA level by qRT-PCR (Figure E7A). Neutralization experiments were then performed to assess the contribution of TGF- β and TGF- α /EGFR pathways to IL-4/IL-13-driven pIgR downregulation. The blockade of TGF- β , by using a blocking Ab to TGF- β RII during the 4 weeks of culture, almost completely abolished the effect of IL-13

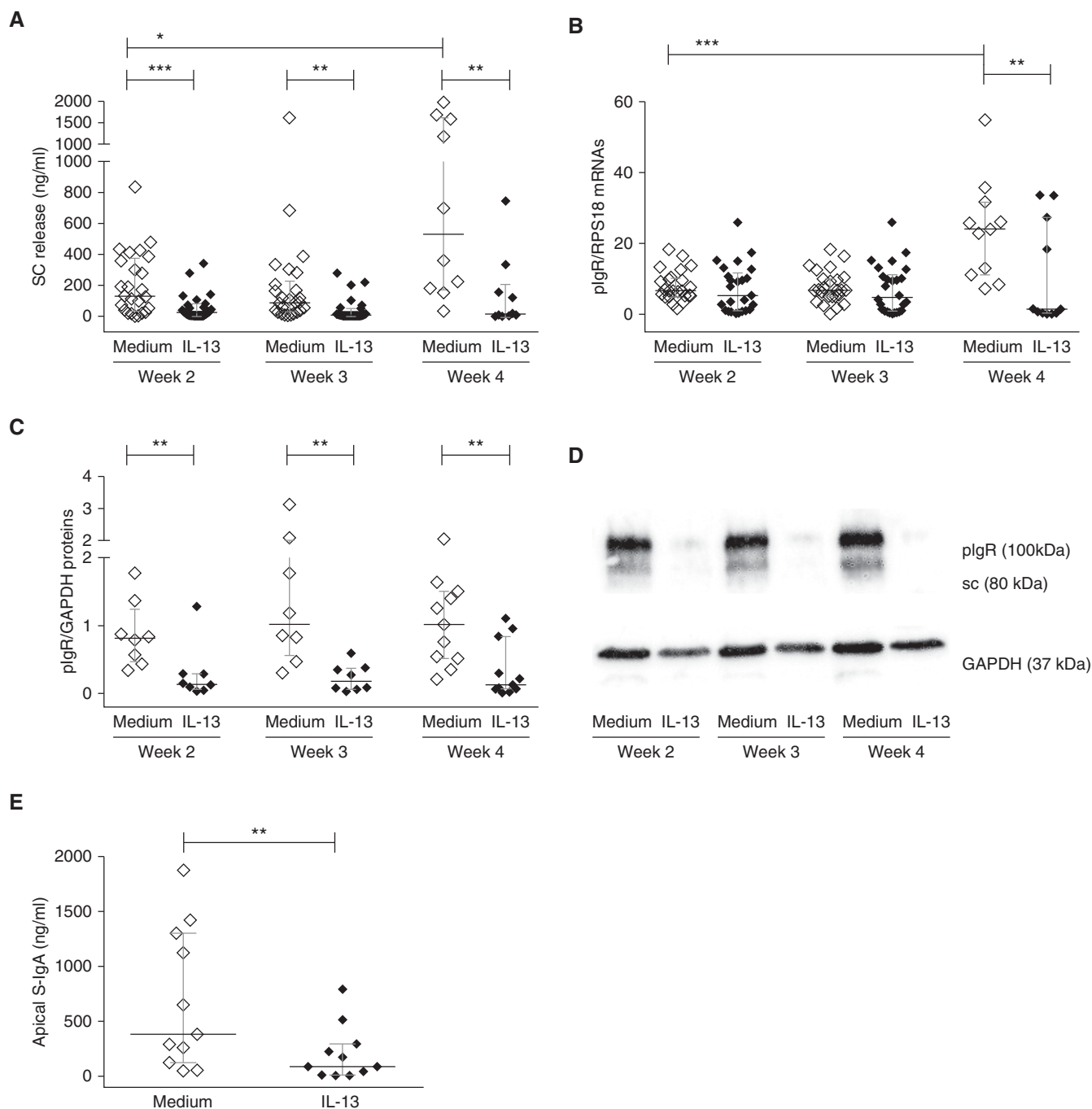


Figure 3. IL-13-driven polymeric immunoglobulin receptor (pIgR) downregulation in air-liquid interface (ALI) bronchial epithelial cells (BEC). (A) Secretory component release measured respectively at 2, 3, or 4 weeks of ALI. (B) pIgR mRNA corrected to RPS18 measured at 2, 3, or 4 weeks of ALI. (C) pIgR immunoblot quantification and (D) representative pIgR Western blot in BEC treated or not with IL-13, corrected to GAPDH, measured at 2, 3, or 4 weeks of ALI and (E) dimeric IgA transcytosis (and apical release of secretory IgA) by BEC treated or not with IL-13. Open symbols represent untreated BEC and solid symbols represent IL-13 (10 ng/ml)-treated BEC (added every other day) for 2, 3, or 4 weeks of ALI culture. Lines depict medians and interquartile ranges. Mann-Whitney *U* test for unpaired comparisons and Wilcoxon test for paired data; **P* < 0.05, ***P* < 0.01, ****P* < 0.001. Readers may view the uncut gels for D in the online supplement. SC = secretory component; S-IgA = secretory IgA.

and IL-4 on SC release (Figures 7A and 7B) and pIgR expression (Figure 7C), whereas the EGFR-tyrosine kinase inhibitor erlotinib achieved approximately

50% inhibition (46% for IL-13, 48% for IL-4) (Figure 7). In addition, the STAT6 inhibitor also abrogated the IL-13 and IL-4 effects on pIgR/SC production (Figure 7),

confirming that IL-13 and IL-4 mediate their effect through IL-4Rα/STAT6 signaling pathway. No significant effect was observed with DMSO vehicle or control

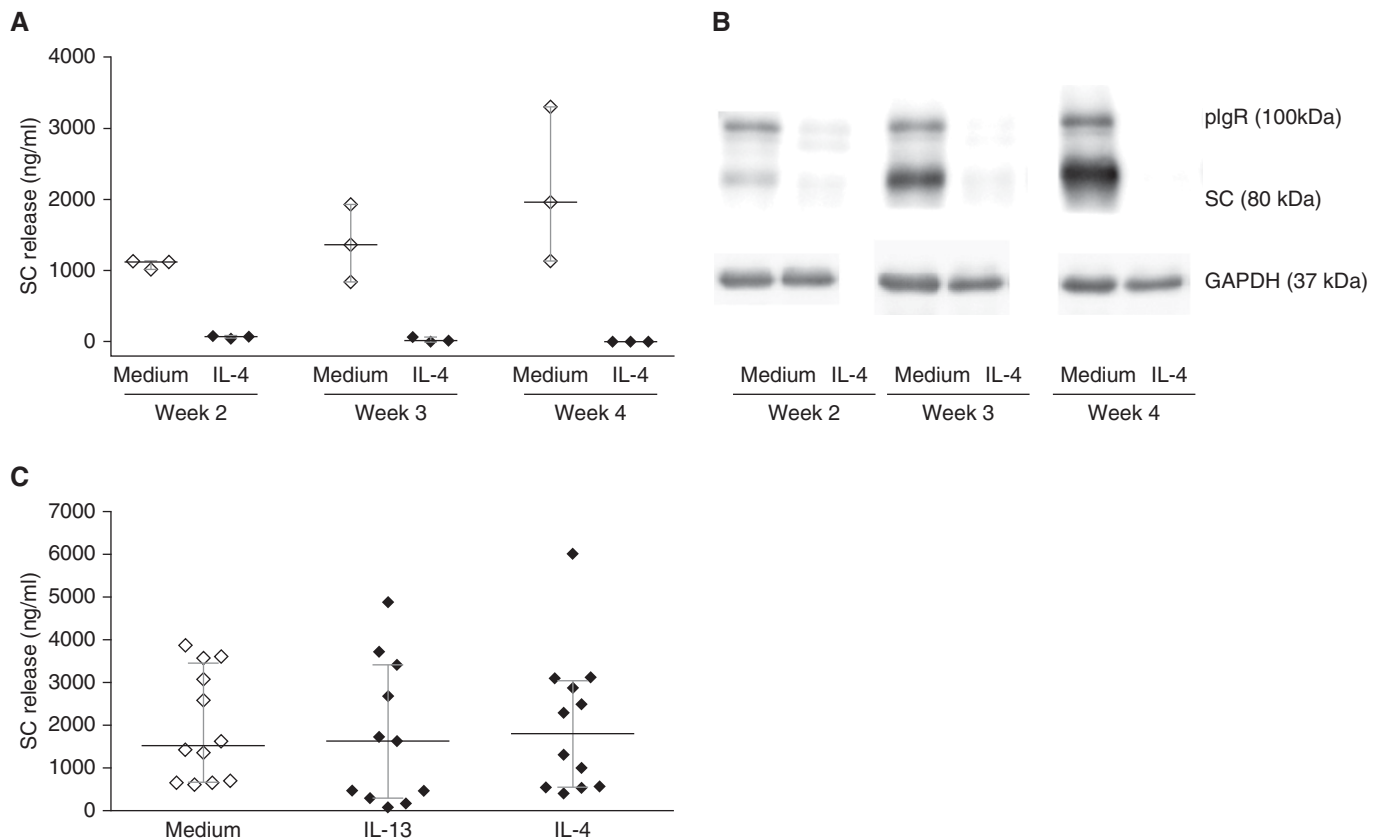


Figure 4. IL-13- and IL-4-driven polymeric immunoglobulin receptor (pIgR) regulation in air-liquid interface (ALI) bronchial epithelial cells (BEC) in long- versus short-term cultures. (A) Secretory component release measured at 2, 3, or 4 weeks of ALI, (B) representative pIgR Western blot in BEC treated or not with IL-4 at 2, 3, or 4 weeks of ALI, and (C) secretory component release measured after 48 hours of treatment with IL-13 or IL-4. Open symbols represent untreated BEC and solid symbols represent IL-13 (10 ng/ml)– or IL-4 (10 ng/ml)–treated BEC (added every other day) for 2, 3, or 4 weeks of ALI culture or only for 2 days. Lines depict medians and interquartile ranges. Readers may view the uncut gels for *B* in the online supplement. SC = secretory component.

IgG (not shown). Furthermore, it was observed that IL-13 is able to downregulate the expression levels of three transcription factors underlying the constitutive expression of pIgR (USF1, USF2, and AP2) (Figure E7B).

These data indicate that IL-13 and IL-4 inhibit pIgR expression by activating the IL-4R α /STAT6 signaling pathway and by affecting the differentiation state of the airway epithelium through autocrine TGF- β and, to a lesser extent, TGF- α /EGFR pathways.

Discussion

This study shows that bronchial epithelial pIgR expression is decreased in patients with asthma. In contrast with COPD (12), this epithelial defect is independent of the severity of the disease (or its allergic phenotype) and does not recapitulate *ex vivo*. It is found that activation through the IL-4R α (by IL-4 or IL-13) inhibits pIgR

expression and IgA transcytosis, operating through autocrine TGF- β and inhibition of mucociliary differentiation, with an additional contribution of upregulated TGF- α /EGFR pathway.

The first finding of this study is a downregulation of pIgR content in the asthmatic epithelium, as previously observed in COPD (13). However, in contrast with COPD (32), this epithelial defect is observed irrespective of disease severity, and of its allergic phenotype, even though pIgR expression correlated positively with airway function (in terms of FEV₁). This may suggest that pIgR downregulation is an early abnormality of the airway epithelium in asthma, which is characterized by an impaired barrier function with disruption of epithelial tight junctions that allows increased passage of inhaled substances into the airway wall (33, 34). It also includes loss of epithelial integrity and shedding of columnar cells

(35–37), hypertrophy of submucosal glands (38) and enhanced deposition of matrix proteins (39). BEC from patients with asthma express increased levels of surface receptors associated with tissue repair, such as adhesion molecule CD44 (40). These features, and pIgR downregulation, are observed in mild and severe asthma, suggesting they represent early epithelial abnormalities. In contrast, thickening of the epithelial layer with expression of markers of activation and repair (2, 20, 41), goblet cell hyperplasia, mucus hypersecretion (42–44), increased MUC5AC/5B (45, 46), and squamous cell metaplasia (37) are more consistently reported in severe disease as compared with mild asthma.

The *in vitro* model of bronchial epithelium reconstituted from patients' bronchial tissue on ALI has been shown as a validated tool to explore intrinsic changes of the airway epithelium in the diseased lung. Recently, it was shown that the

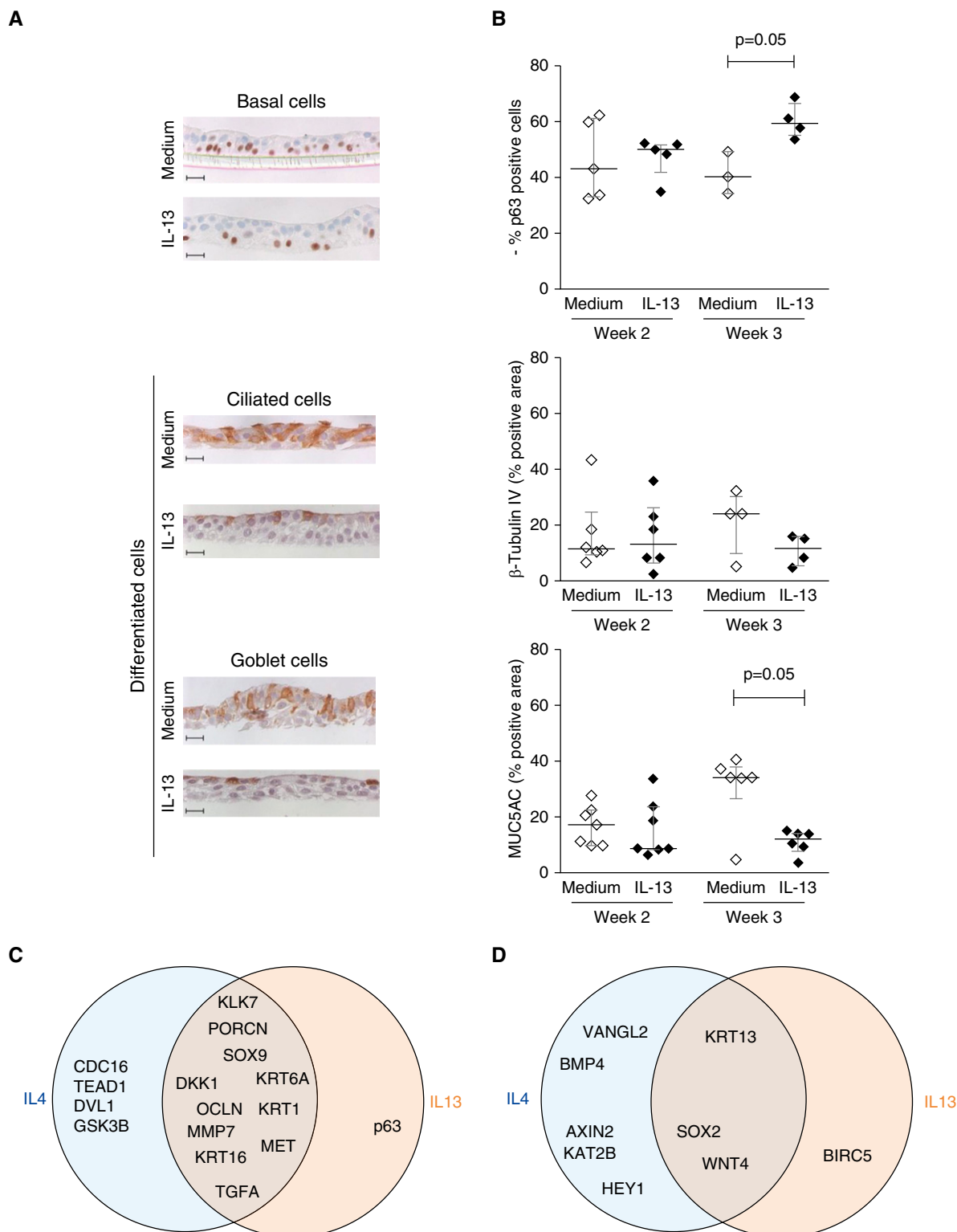


Figure 5. Bronchial epithelial specification in IL-13-treated cultures. (A) Representative bronchial epithelial phenotyping of air-liquid interface bronchial epithelial cells treated or not with IL-13: basal cells (p63⁺), and differentiated cells as goblet cells (Muc5AC⁺) or ciliated cells (β-tubulin IV⁺); and (B) quantification of the epithelial markers; scale bar = 20 μm. Open symbols represent untreated bronchial epithelial cells and solid symbols represent IL-13 (10 ng/ml)-treated bronchial epithelial cells at 2, 3, or 4 weeks of air-liquid interface. Lines depict medians and interquartile ranges. (C and D) Scheme of differentially expressed genes (RNA sequencing) on IL-13 or IL-4 treatment as compared with control nontreated cells. (C) Upregulated and (D) downregulated genes.

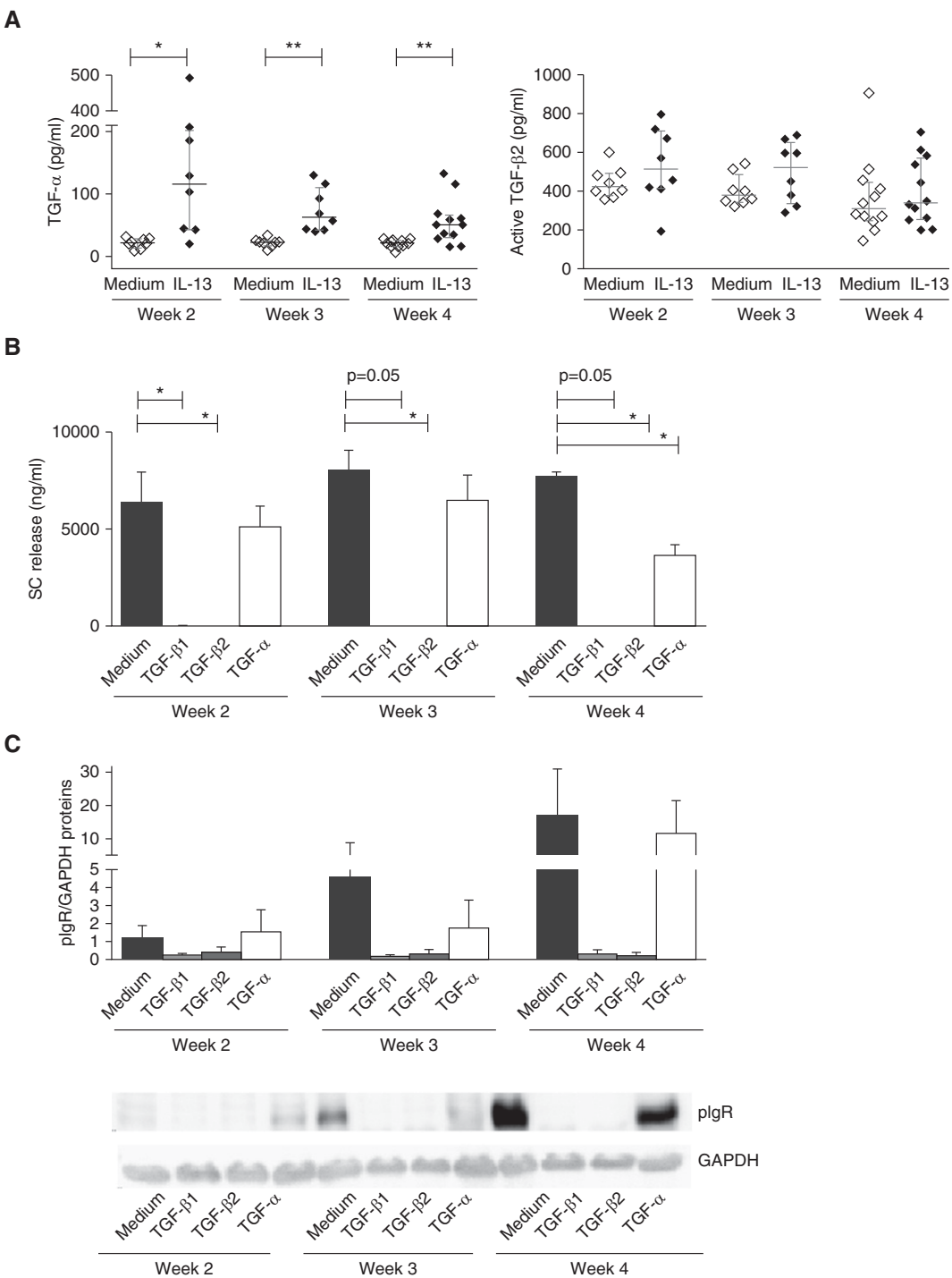


Figure 6. IL-13-driven downregulation of bronchial epithelial polymeric immunoglobulin receptor (pIgR) is mediated by transforming growth factors (TGFs). (A) TGF- α and TGF- β 2 release measured at 2, 3, or 4 weeks of air-liquid interface in bronchial epithelial cells treated or not with IL-13 (10 ng/ml). (B) Secretory component release and (C) pIgR Western blot assayed at 2, 3, or 4 weeks of air-liquid interface in bronchial epithelial cells treated or not with TGF- β 1, TGF- β 2, or TGF- α . Lines depict medians and interquartile ranges (data from four independent cultures). Mann-Whitney U test: * $P < 0.05$, ** $P < 0.01$. SC = secretory component.

ALI-reconstituted bronchial epithelium from patients with severe asthma produces increased amounts of mucins and IL-8/CXCL8 and decreased LXA4 (20). Other bronchial epithelial abnormalities evidenced in asthma using this model, include dyssynchronous inflammation and mitosis, decreased resolution processes, defects in junctional proteins, and remodeling factors (20, 47–49). In contrast with COPD, where pIgR downregulation was recapitulated in ALI-BEC, in relation

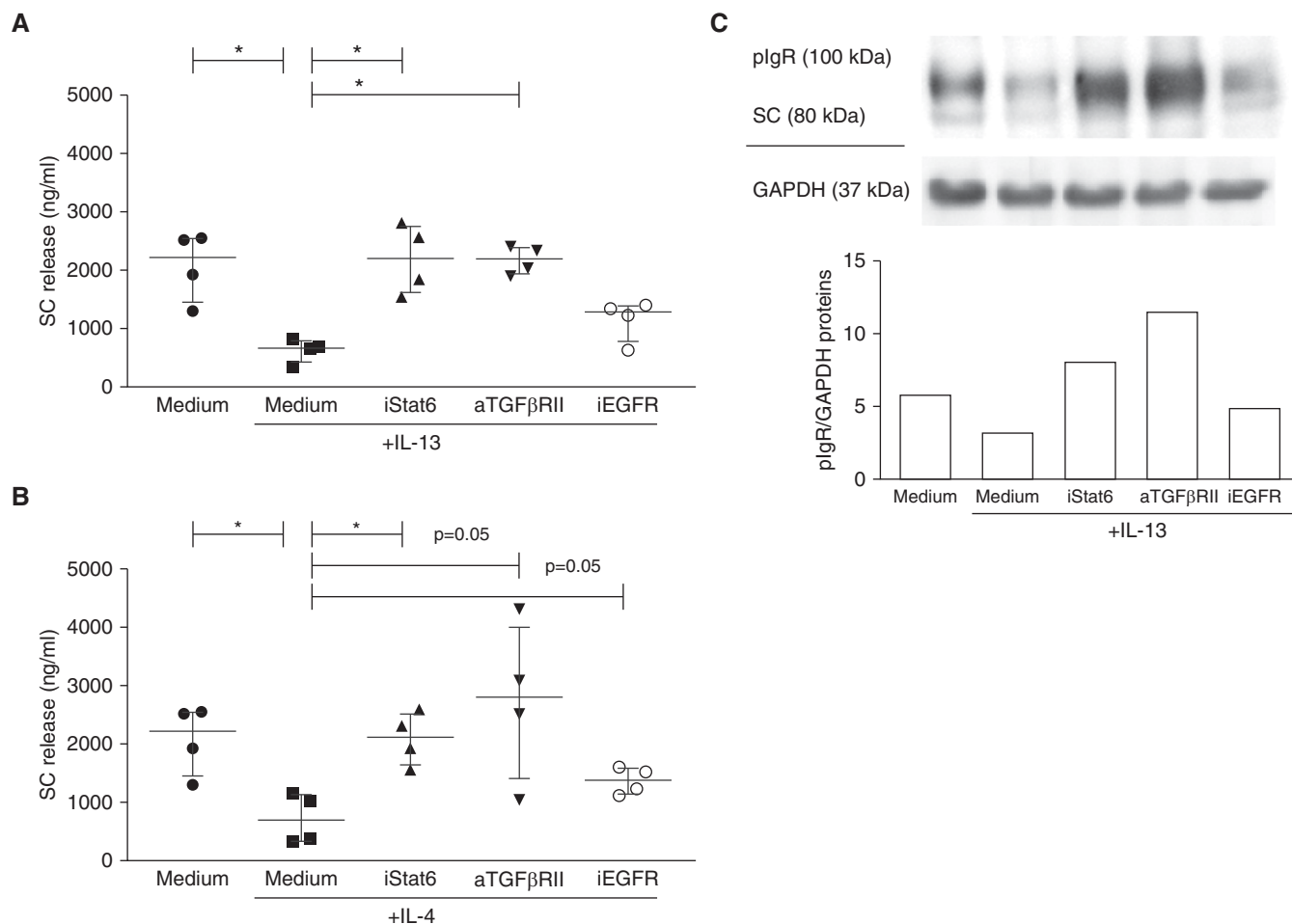


Figure 7. Blockade of the IL-4/IL-13 effect on polymeric immunoglobulin receptor (pIgR)/secretory component (SC) following neutralization of IL-4R α /STAT6, transforming growth factor (TGF)- β , and TGF- α /epidermal growth factor receptor (EGFR) pathway. SC release on blockade of STAT6, TGF- β RII, or EGFR in the presence of (A) IL-13 or (B) IL-4 measured at 4 weeks of air-liquid interface culture. (C) Western blot for pIgR in bronchial epithelial cells incubated for 4 weeks with IL-13 and STAT6 inhibitor, anti-TGF- β RII Ab, or the EGFR-tyrosine kinase inhibitor erlotinib. Lines depict medians and interquartile ranges (data from four independent cultures). Readers may view the uncut gels for C in the online supplement. Mann-Whitney U test $^*P < 0.05$. aTGF β RII = anti-TGF- β RII antibody; iEGFR = (tyrosine kinase) inhibitor of EGFR; iStat6 = inhibitor of signal transducer and activator of transcription 6.

to an intrinsic dysprogramming of the epithelium partly related to TGF- β 1 (12, 50), the pIgR defect observed in asthma did not persist in the cultured epithelium from such patients. This finding suggested that microenvironmental factors could condition, *in vivo*, this epithelial feature.

Despite previous data published in 12/15LO $^{-/-}$ mice, which display dramatic increases in S-IgA (29), no direct effect was observed on LXA4 exposure of the epithelium. In addition, links between IgA and eosinophils prompted us to explore a role for these cells on epithelial pIgR. Thus, it was reported that eosinophils may degranulate on activation with S-IgA (51), whereas conversely, eosinophils promote generation and maintenance of IgA-expressing plasma cells (52, 53). Moreover, pIgR downregulation

in chronic upper airway disease was related to eosinophilic inflammation (54). However, no direct effect of activated eosinophils was seen *in vitro* on epithelial pIgR expression, suggesting that other factors could link pIgR downregulation with type 2 mucosal inflammation.

IL-13 represents an important mediator in asthma (55, 56), originally described as inducing, like IL-4, IgE production by activated human B cells (57, 58). IL-13 acts through the IL-4R α , inducing salient features of asthma in experimental models, such as airway hyperresponsiveness, goblet cell hyperplasia, and mucus overproduction (59, 60), and disruption of junctional complexes (61, 62). In our study, IL-13 was found to inhibit pIgR/SC production

following long-term (2–4 wk), but not short-term, exposure. This inhibition was also observed with IL-4 and abrogated (for both IL-4 and IL-13) by using a STAT6 inhibitor, indicating that the IL-4R α , which is shared by IL-4 and IL-13, mediates this effect through a classical IL-4R α /STAT6 signaling pathway. It was previously reported that IL-13 (and IL-4) increases goblet cell density when used at low concentration (1 ng/ml, inducing 5- to 10-fold increase), whereas a concentration of 10 ng/ml (as used in our study) induced a reduced goblet cell density in one study (63) and an increase in the proportion of secretory cells in another study (64). Whereas a stimulatory effect was observed with IL-4 on pIgR production in short-term cultures of Calu-3 cells (65), our data in

primary BEC indicate that activation through IL-4/IL-13-IL4R α induces pIgR downregulation, at least in part through a reduction in mucociliary differentiation, mediated by alterations in several factors controlling bronchial epithelial differentiation (Figures 5C and 5D), and which conditions pIgR expression (32). Although we could not address which cells are specifically involved in this negative regulation of epithelial pIgR, it could be speculated that Th2 and innate lymphocytes are probably involved because they represent the main cell sources of IL-4 and IL-13 (66), with a potential contribution of other immune (e.g., mast cells) or structural cells, such as smooth muscle cells. Interestingly recent trials showed that anti-IL-13 and anti-IL-13/anti-IL-4R therapies improve several asthma outcomes (67, 68).

We could also demonstrate that autocrine TGF- β and, to a lesser extent TGF- α /EGFR pathway, are involved in pIgR downregulation on IL-4/IL-13 stimulation. Our data show that IL-4R activation triggers pIgR downregulation in the bronchial epithelium as part of a TGF- β -driven reprogramming toward

epithelial dedifferentiation, suggesting that TGF- β is a key negative regulator of pIgR expression in COPD (31) and asthma. Upregulation of TGF- α , a main ligand of EGFR, which has been implicated in several epithelial changes occurring in chronic disease, such as for repair or production of mucins and IL-8/CXCL8 (69), also contributes to a lower extent to pIgR downregulation on IL-4 or IL-13 activation.

Reduced pIgR-mediated transport of IgA across the epithelium, which has been reported in COPD and now in asthma, may abort IgA trafficking and limit the recognition of inhaled pathogens. S-IgA has a protective function against various forms of bacterial and viral aggression through the general mechanism of immune exclusion (70–72), and through intracellular neutralization and secretion of viruses (73). Moreover, IgA antibodies may probably also act as scavengers for allergens, this antibody response being upregulated after allergen-specific immunotherapy (74). Thus, a defect in S-IgA system could limit the capacity of the airway mucosa to neutralize inhaled antigens.

Altogether, this study identifies a defect in bronchial epithelial pIgR expression in human asthma, potentially altering the S-IgA-mediated frontline in the airway mucosa. It also provides evidence of a negative link between type 2 immune activation and S-IgA lung defense, which operates through autocrine TGF- β and EGFR signaling, and suggests that targeting the IL-4/IL-13 axis in asthma could benefit to IgA-mediated immune protection of the airways. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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Broncho-epithelial IgA secretion is impaired in asthma: role of IL-4/IL-13

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Charles Pilette

ONLINE DATA SUPPLEMENT

Supplemental material

Primary human bronchial epithelial cells (BEC) co-culture with eosinophils. BEC were cultured in polarized and ALI conditions, to reconstitute a pseudostratified mucociliary epithelium. At three weeks of ALI, BEC were co-cultured for two days with Eo1-1 human cell line (0.5 million/condition) and in the presence or not of IL-3 or IL-5 at 5 to 25ng/mL (miltenybiotec, Germany) to activate eosinophils.

Treatment of primary human bronchial epithelial cells with lipoxin A4. BEC were cultured in polarized and ALI conditions, to reconstitute a pseudostratified mucociliary epithelium, and in the presence or not of 10^{-7} M LXA4 (sigma Aldrich, USA) since the beginning of the ALI phase. The medium was replaced every two days and analyses performed at 2, 3 or 4 weeks of culture.

Primers sequences for real time quantitative PCR. pIgR Forward: CTC TCT GGA GGA CCA CCG T; pIgR Reverse: CAG CCG TGA CAT TCC CTG; Eotaxin-3 Forward: AAT ACA GCC ACA AGC CCC TT; Eotaxin-3 Reverse: GCC TCT TTT GGT AGT GAA TAT CAC A; PRG2 Forward: TGG-ATT-GGA-GGC-AGG-ATC-A; PRG2 Reverse: CAG-TGG-CCT-CCT-CGG-GTA; RNA18S Forward: CGG CTA CCA CAT CCA AGG AA; RNA18S Reverse: ATA CGC TAT TGG AGC TGG AAT ACC; glyceraldehyde-3-phosphate deshydrogenase (GAPDH) Forward: TTC TTT TGC GTC GCC AGC CGA; GAPDH Reverse: GTG ACC AGG CGC CCA ATA CGA; ribosomal protein S18 (RPS18) Forward: TGT GGG CCG AAG ATA TGC T; RPS18 Reverse: TGA TCA CAC GTT CCA CCT CAT); AP2 Forward: TCCGGAGGGCGAAGTCTAAA; AP2 Reverse CAAAGTCCCTGGCTAGGTGG; USF1 Forward: AGC-TTC-GGC-AGA-GTA-ACC-AC, USF1 Reverse: AGC-CCC-TGA-ATC-CCC-ATA-GT; USF2 Forward: CGG-ACA-CAC-CCT-TAC-TCT-CC, USF2 Reverse: GAT-CCC-TCC-TTT-ACT-CGC-TCC.

Table E1. Mean fold change of RNA sequences upon IL-13 or IL-4 treatment as compared to control non-treated cells.

Gene	Treatment (IL-13)	p value	Gene	Treatment (IL-4)	p value
KLK7	6.11	0	KRT13	0.025	0
KRT13	0.09	0	MMP7	15.301	0
PORCN	2.58	0.0003	KLK7	4.844	0
MMP7	3.882	0.0017	KRT16	2.648	0.0003
OCLN	2.283	0.0018	HEY1	0.16	0.0007
HPRT1	0.552	0.0036	SOX9	8.733	0.0008
KRT16	2.427	0.0046	AXIN2	0.101	0.0008
TP63	1.945	0.0082	KAT2B	0.384	0.001
SOX9	5.426	0.0109	KRT6A	2.021	0.0011
TGFA	2.16	0.0116	SOX2	0.484	0.0023
BIRC5	0.204	0.0138	BMP4	0.121	0.0025
KRT1	1.896	0.0185	OCLN	1.964	0.0026
DKK1	2.081	0.0201	WNT4	0.403	0.0041
SOX2	0.54	0.0278	MET	1.73	0.0059
MET	1.682	0.0279	VANG2	0.203	0.0069
WNT4	0.466	0.0372	KRT1	1.862	0.0069
KRT6A	1.674	0.0439	CDC16	1.969	0.0087
AXIN2	0.262	0.0519	TEAD1	2.551	0.0108
HEY1	0.328	0.055	TGFA	1.905	0.0133
BMP4	0.285	0.071	PORCN	1.706	0.0171
TEAD1	2.075	0.0811	DVL1	2.546	0.031
NOTCH1	2.314	0.0852	DKK1	1.783	0.0318
LRP5	0.453	0.1032	GSK3B	1.459	0.0384
FZD3	1.705	0.1053	FOSL1	3.482	0.0678
RUNX1	0.709	0.1152	NOTCH1	2.167	0.0768
WNT7B	0.464	0.1216	FZD7	2.487	0.083
TLE1	1.763	0.1296	RUNX2	1.852	0.09
DAAM1	1.71	0.1341	CHUK	1.68	0.0937
APC	1.488	0.1786	HPRT1	0.754	0.0987
CCNE1	0.394	0.1798	WNT7B	0.488	0.1072
CBL	2.162	0.1816	SPDEF	1.957	0.1209
RPS13	1.196	0.1983	PSEN2	1.969	0.1288
FZD7	2.051	0.2067	POFUT1	0.47	0.1395
PPARG	2.321	0.2221	EP300	1.367	0.1564
GSK3B	1.3	0.2276	TLE1	1.54	0.1831
PRICKLE1	2.288	0.233	HES2	2.404	0.2002
HES2	2.262	0.236	TP63	1.311	0.2022
PSEN2	1.751	0.2588	FZD2	0.412	0.204
FZD2	0.457	0.2591	SUFU	0.53	0.206
TFAP2A	1.703	0.2612	BTRC	1.384	0.2077
SPDEF	1.708	0.2682	TWIST1	2.415	0.2111

TWIST1	2.072	0.295	MAPK8	1.53	0.238
TCF7L1	0.607	0.3087	AKT1	1.378	0.2459
STIL	0.502	0.3182	NLK	0.678	0.275
SUFU	0.622	0.3865	PPARG	2.109	0.2767
BTRC	1.297	0.3904	TFAP2A	1.507	0.3321
KAT2B	0.753	0.4014	ADAM17	1.435	0.3385
ADAM17	1.413	0.4197	LRP5	0.66	0.3404
MMP9	0.75	0.4239	RFNG	0.588	0.3596
CTBP1	1.224	0.4642	PRICKLE1	1.826	0.387
BCL9	0.645	0.4838	FZD3	0.794	0.4142
HDAC1	0.697	0.4863	JAG2	1.488	0.4163
FOSL1	1.579	0.5067	AXIN1	1.572	0.463
PSEN1	0.848	0.5295	PSEN1	1.167	0.4872
SMO	0.672	0.5643	NOTCH2NL	1.089	0.5067
RUNX2	0.789	0.5672	ERBB2	0.626	0.5097
AKT1	1.197	0.5768	DAAM1	1.218	0.5239
DVL2	1.403	0.6019	SMO	0.652	0.5342
RFNG	1.37	0.6082	CTBP1	1.141	0.573
RUVBL1	1.147	0.6412	TCF7L1	0.783	0.5792
JAG2	0.779	0.6416	PDPK1	1.153	0.5796
NOTCH2NL	1.069	0.6683	MMP9	0.842	0.5806
CHUK	1.166	0.6685	RUVBL1	0.883	0.6199
WNT5A	0.747	0.6754	APC	0.891	0.6472
NFATC1	1.301	0.7048	HDAC1	0.808	0.6517
PDPK1	0.895	0.7137	TCF7	0.744	0.6706
CDC16	1.094	0.7659	MAP2K7	0.795	0.6855
POFUT1	0.864	0.7925	STIL	0.765	0.6971
EP300	1.071	0.7935	CCNE1	0.808	0.7636
NLK	1.109	0.7995	RUNX1	0.952	0.7876
MAP2K7	1.149	0.8176	CSNK2A1	1.051	0.8388
AXIN1	0.866	0.8233	NFATC1	0.878	0.8524
TCF7	0.871	0.8418	WNT5A	1.117	0.8755
HEY2	0.882	0.8561	BCL9	1.085	0.8909
ERBB2	0.89	0.8659	DVL2	1.077	0.9052
CTNNBIP1	1.029	0.9225	BIRC5	1.073	0.9093
MAPK8	1.022	0.957	RPS13	1.011	0.9218
VANGL2	1.029	0.9632	CBL	0.972	0.9584
DVL1	0.982	0.9695	HEY2	1.031	0.9654
CSNK2A1	1.008	0.9787	CTNNBIP1	0.998	0.9944

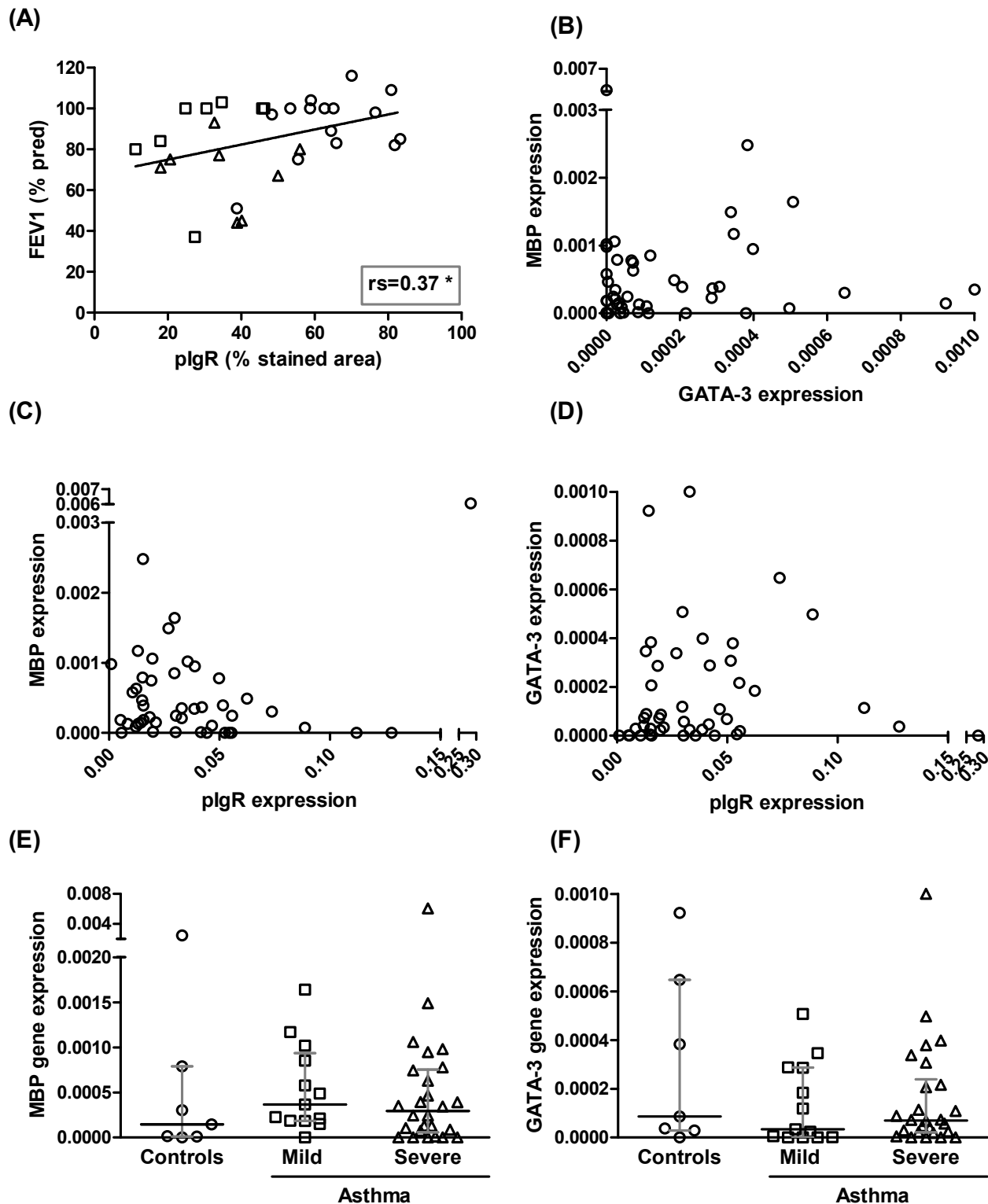


Figure E1: Correlation between plgR staining and airway function (FEV1 predicted); Eosinophil-related MBP marker and Th2-related GATA-3 transcription factor expressions and correlation with plgR expression.

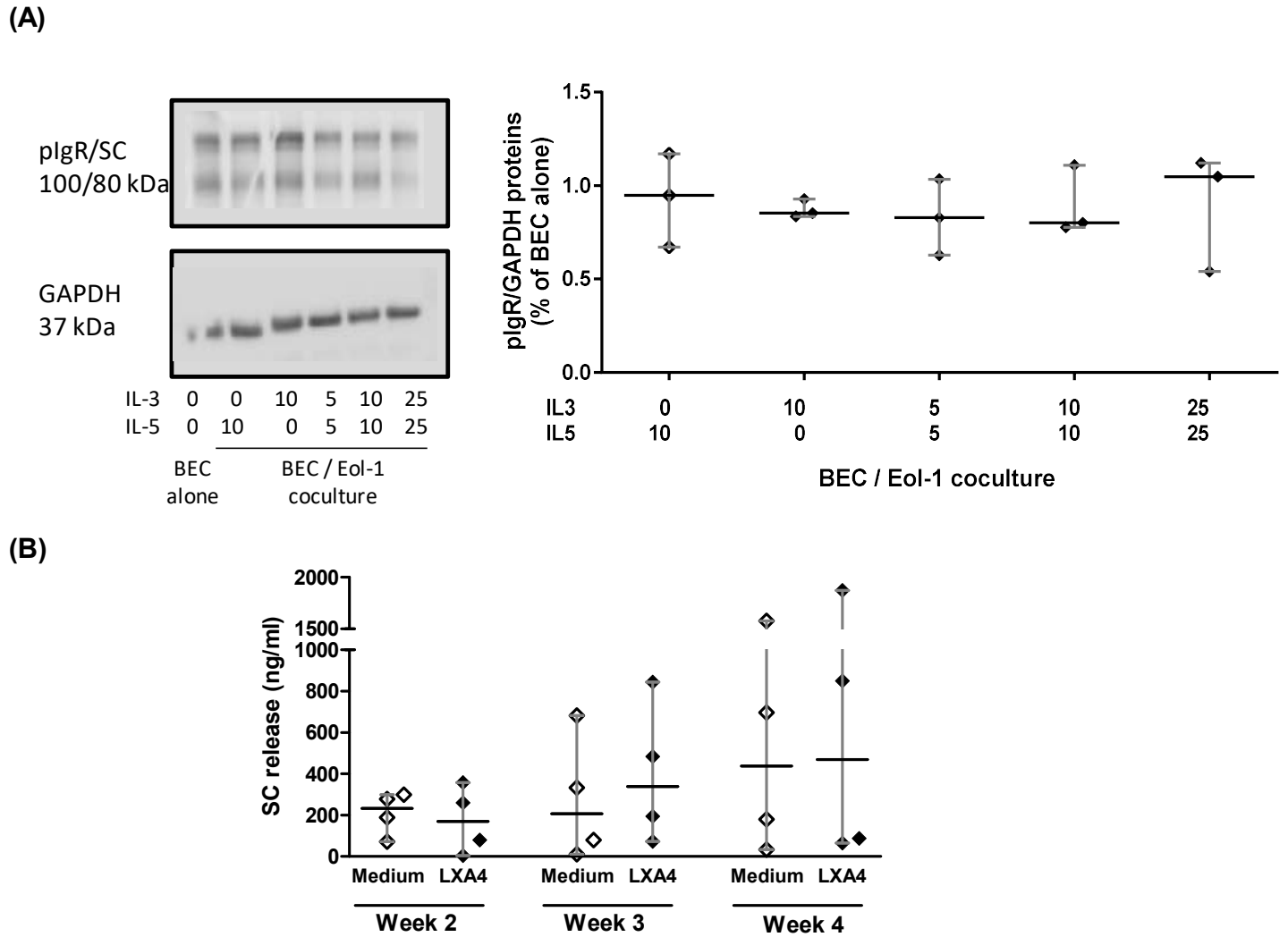


Figure E2: pIgR regulation in ALI-BEC by eosinophils (A) pIgR protein measured by western blot; or (B) SC release regulation by LXA4 (10^{-7} M).

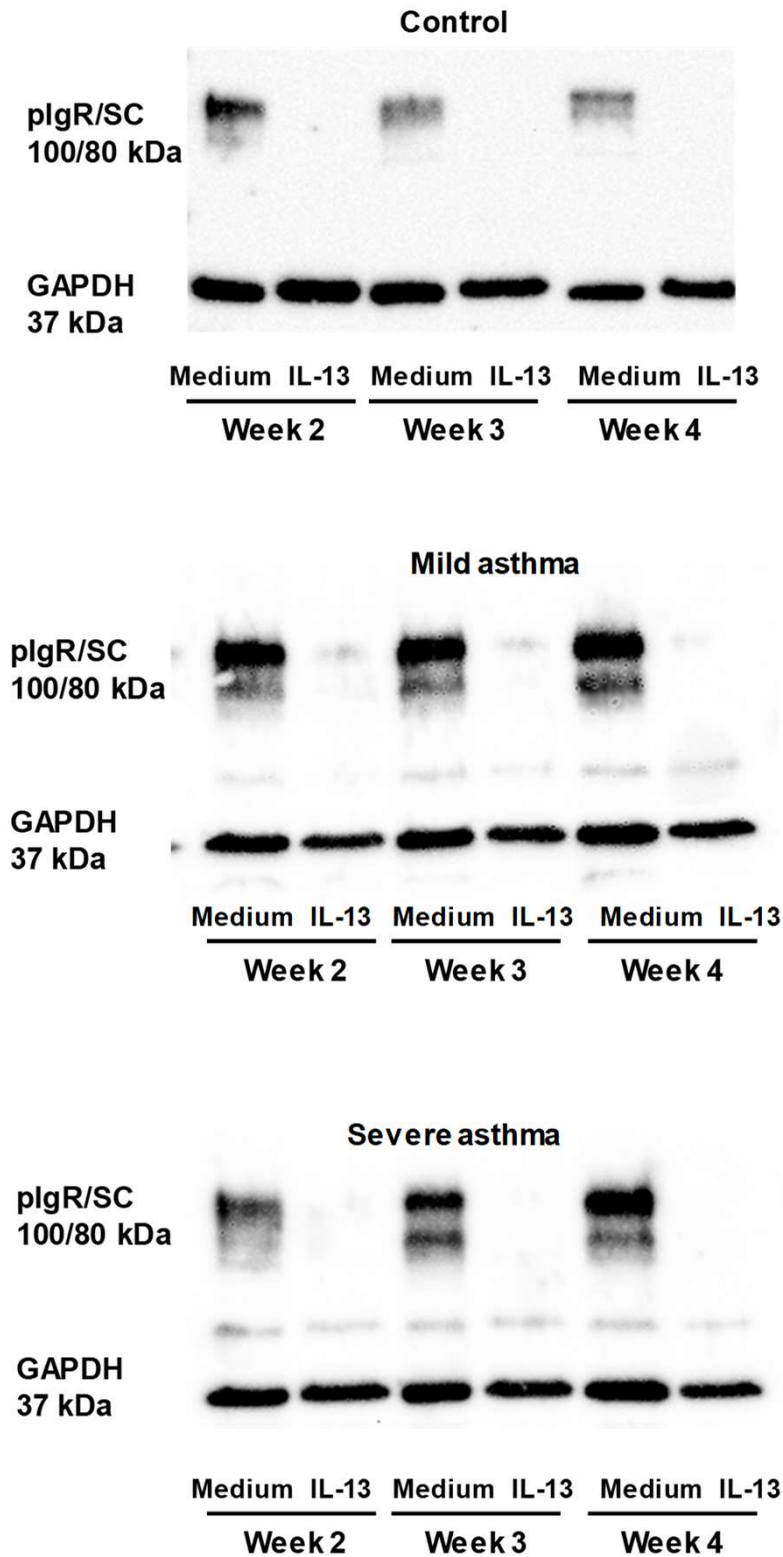


Figure E3: IL-13-driven pIgR downregulation in ALI-BEC (irrespective of the patient' group). Representative pIgR Western Blot in BEC treated or not with IL-13 for 2, 3 or 4 weeks of ALI

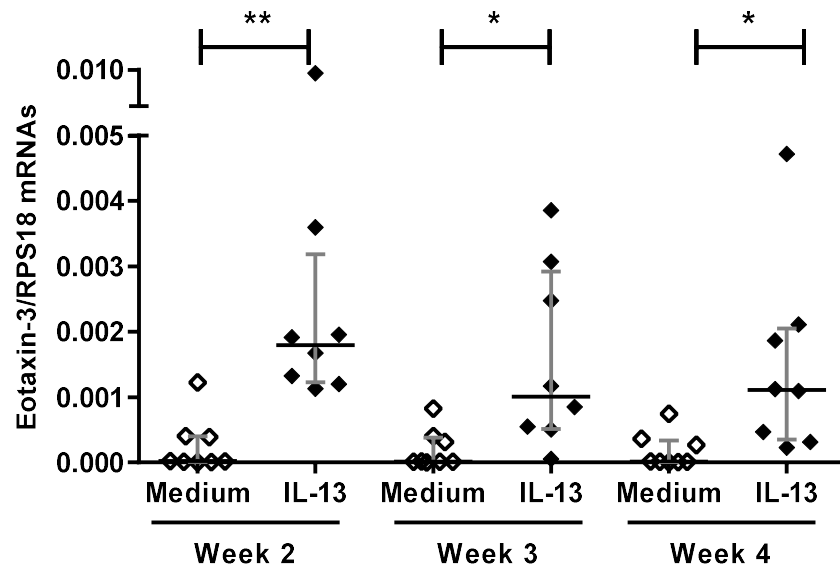
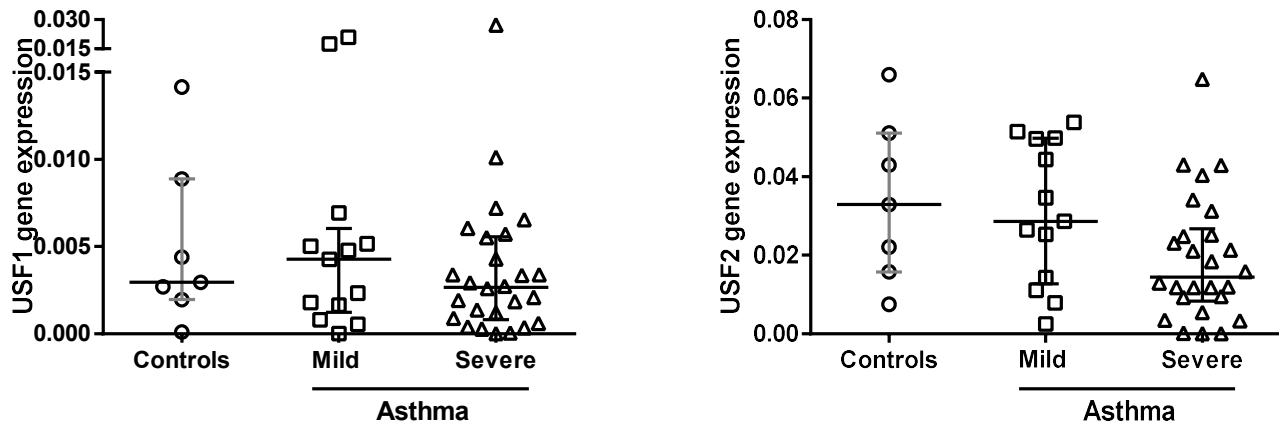


Figure E4: Eotaxin-3 expression by HBEC upon IL-13 stimulation (10 ng/ml, every other day) versus control (medium alone), as assessed at week 2, 3 and 4 in ALI conditions. Data are from HBEC of 8 different donors; lines depict median (interquartile range).

(A)



(B)

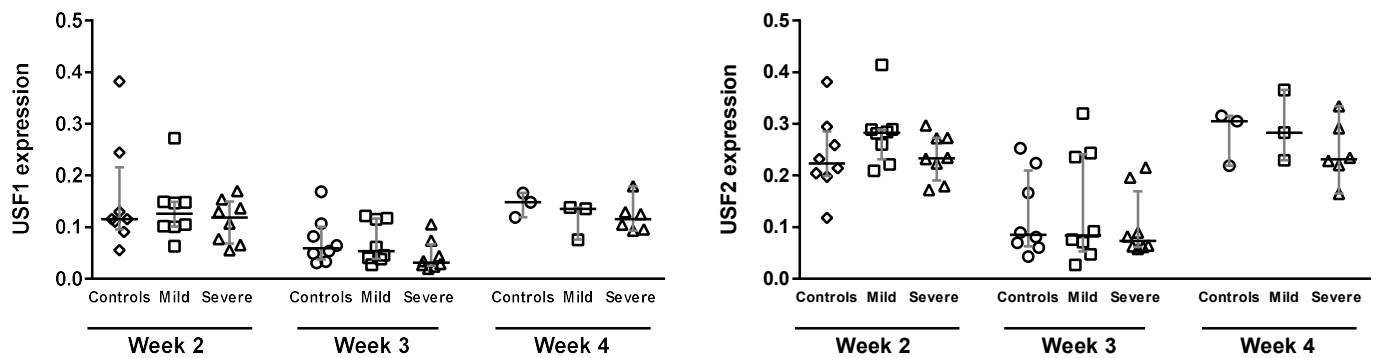


Figure E5: Expression of USF1 and USF2 mRNA corrected for the housekeeping gene RPS18 in (A) bronchial biopsies (n=7 controls, 13 mild asthma, 26 severe asthma) and (B) cultured primary BEC for 2, 3 or 4 weeks of ALI (n=3/8 controls, 3/8 mild asthma, 6/8 severe asthma) . White circles (Controls), squares (mild-to-moderate asthma patients) and triangles (severe asthma). Lines depict medians and interquartile ranges.

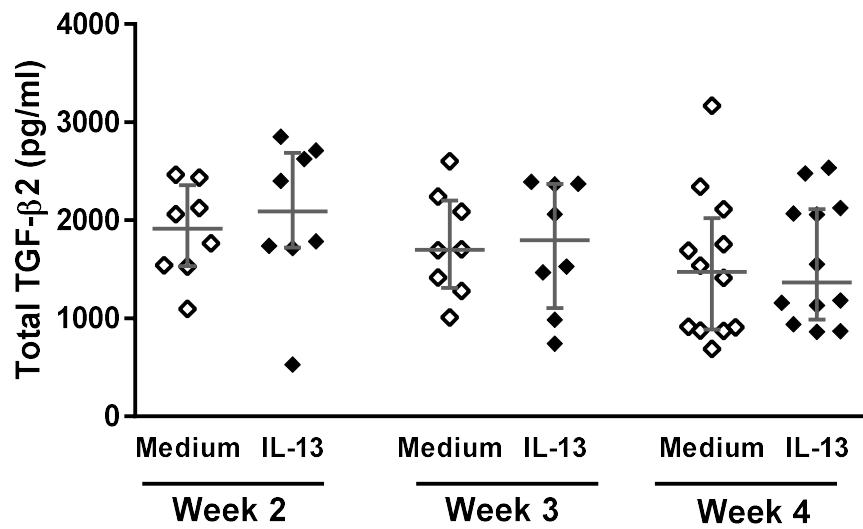
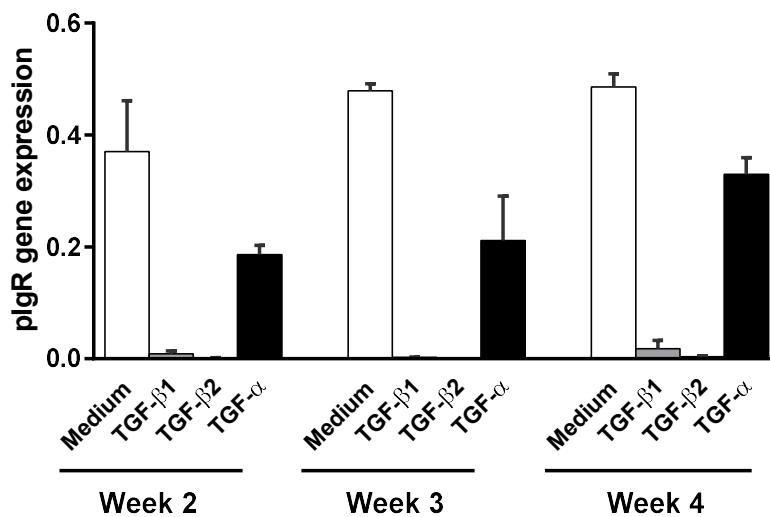


Figure E6: TGF-β2 release measured at 2, 3 or 4 weeks of ALI in BEC treated or not with IL-13; total TGF-β2. White symbols represent untreated BEC and black symbols represent IL-13 (10ng/ml) treated BEC (added every other day) for 2, 3 or 4 weeks of ALI culture or only for two days. Lines depict medians and interquartile ranges.

(A)



(B)

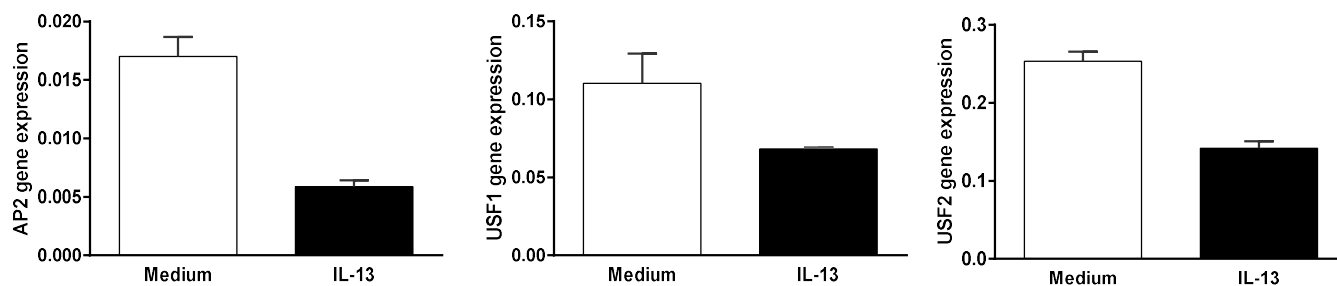


Figure E7: Regulation of (A) plgR expression by TGF- β 1, TGF- β 2 and TGF- α after 2, 3 or 4 weeks of ALI culture ; and (B) transcription factors expression AP2, USF1 and 2 by IL-13 after 4 weeks of ALI culture . Bars depict medians and interquartile ranges.

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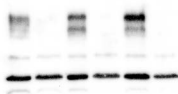


Figure 3



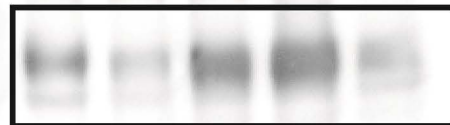
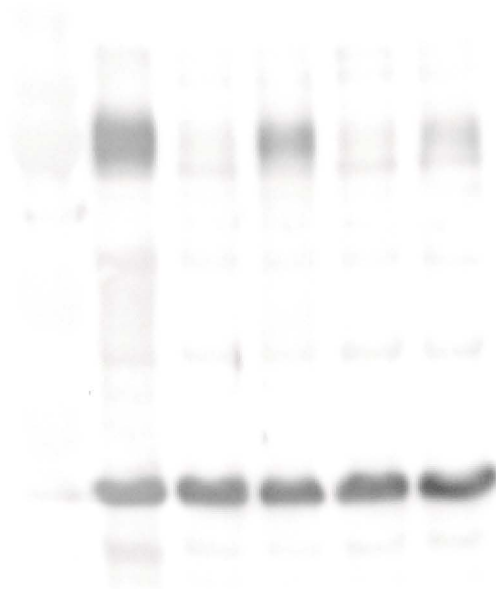
Figure 4 GAPDH



Figure 4 pIgR



Figure 7



pIgR
SC



GAPDH