

Prostaglandin E₂ stimulates adaptive IL-22 production and promotes allergic contact dermatitis

Calum T. Robb, PhD,^a Henry J. McSorley, PhD,^a Jinju Lee, MSc,^{b,c} Tomohiro Aoki, MD, PhD,^{b,c} Cunjing Yu, PhD,^a Siobhan Crittenden, MSc,^a Anne Astier, PhD,^a Jennifer M. Felton, PhD,^a Nicholas Parkinson, MA, MS, VetMB,^a Adane Ayele, MD,^d Richard M. Breyer, PhD,^{e,f} Stephen M. Anderton, PhD,^a Shuh Narumiya, MD, PhD,^{b,c} Adriano G. Rossi, PhD, DSc,^a Sarah E. Howie, PhD,^a Emma Guttman-Yassky, MD, PhD,^{g,h} Richard B. Weller, MD, FRCP,^a and Chengcan Yao, PhD^a
 Edinburgh, United Kingdom, Kyoto, Japan, Addis Ababa, Ethiopia, Nashville, Tenn, and New York, NY

Background: Atopic dermatitis (AD) and allergic contact dermatitis (ACD) are both forms of eczema and are common inflammatory skin diseases with a central role of T cell-derived IL-22 in their pathogenesis. Although prostaglandin (PG) E₂ is known to promote inflammation, little is known about its role in processes related to AD and ACD development, including IL-22 upregulation.

Objectives: We sought to investigate whether PGE₂ has a role in IL-22 induction and development of ACD, which has increased prevalence in patients with AD.

Methods: T-cell cultures and *in vivo* sensitization of mice with haptens were used to assess the role of PGE₂ in IL-22 production. The involvement of PGE₂ receptors and their downstream signals was also examined. The effects of PGE₂ were evaluated by using the oxazolone-induced ACD mouse model. The relationship of PGE₂ and IL-22 signaling pathways in skin inflammation were also investigated by using genomic profiling in human lesional AD skin.

Results: PGE₂ induces IL-22 from T cells through its receptors, E prostanoid receptor (EP) 2 and EP4, and involves cyclic AMP signaling. Selective deletion of EP4 in T cells prevents hapten-

induced IL-22 production *in vivo*, and limits atopic-like skin inflammation in the oxazolone-induced ACD model. Moreover, both PGE₂ and IL-22 pathway genes were coordinately upregulated in human AD lesional skin but were at less than significant detection levels after corticosteroid or UVB treatments.

Conclusions: Our results define a crucial role for PGE₂ in promoting ACD by facilitating IL-22 production from T cells. (J Allergy Clin Immunol 2017;■■■:■■■-■■■.)

Key words: Allergic contact dermatitis, atopic dermatitis, prostaglandin E₂, CD4⁺ T cells, T_H22 cells, T_H17 cells, IL-22

Atopic dermatitis (AD) and allergic contact dermatitis (ACD) are both forms of eczema and are common chronic inflammatory skin diseases. The prevalence of AD is up to 3% to 7% in adults and up to 25% among children,^{1,2} and the prevalence of ACD is up to 7% among the general population.³ ACD and AD potentially share common cellular mechanisms, and there is an increased rate of ACD in patients with AD.³ Histologic features of affected

From ^athe Medical Research Council (MRC) Centre for Inflammation Research, Queen's Medical Research Institute, University of Edinburgh; ^bthe Center for Innovation in Immunoregulatory Technology and Therapeutics (AK Project) and ^cCore Research for Evolutional Science and Technology, Medical Innovation Center, Kyoto University Graduate School of Medicine; ^dthe Department of Dermatovenereology, School of Medicine, College of Health Sciences, Addis Ababa University, Addis Ababa; ^ethe Department of Veterans Affairs, Tennessee Valley Health Authority, Nashville; ^fthe Department of Medicine, Vanderbilt University Medical Center, Nashville; ^gthe Department of Dermatology and the Laboratory for Inflammatory Skin Diseases, Icahn School of Medicine at Mount Sinai, New York; and ^hthe Laboratory for Investigative Dermatology, Rockefeller University, New York.

Supported in part by the University of Edinburgh start-up funding and Wellcome Trust Institutional Strategic Support Fund (to C. Yao and H.J.M.), Asthma UK (to H.J.M.), Psoriasis Association UK (to R.B.W.), Medical Research Council (MRC) UK (A.G.R. C.T.R., and J.M.F. were supported by MR/K013386/1 and to S.M.A., R.B.W. and H.J.M.), National Institutes of Health grants DK37097 and R56HL127218 and a VA Merit 1BX000616 (to R.M.B.), and Core Research for Evolutional Science and Technology (CREST) of Japan Science and Technology Agency (to S.N.). C. Yu and C.S. receive scholarship supports from the Chinese Scholarship Council and the University of Edinburgh, respectively.

Disclosure of potential conflict of interest: C. T. Robb and A. G. Rossi have received grants from the Medical Research Council UK. H. J. McSorley is employed by the University of Edinburgh and has received or applied for grants from Asthma UK, the Medical Research Council, and the Wellcome Trust. T. Aoki is supported by the Coordination Fund from the Ministry of Education, Culture, Sports, Science and Technology of Japan (MEXT) and Astellas Pharma. N. Parkinson's salary is supported by a grant from the Wellcome Trust. R. M. Breyer has received grants from the

National Institutes of Health, has received payment for lectures from Dalian Medical University and 2016 Symposium on Polyunsaturated Fatty Acid and Metabolism, and has received royalties from Amgen. S. M. Anderton is employed by and is director of Aquila BioMedical. S. Narumiya has consultant arrangements with Astellas Pharma and Toray; is employed by Ono Pharmaceutical, Dainippon Sumitomo Pharma, Mitsubishi-Tanabe Pharma, and Kyorin Pharmaceutical; and has received grants from Astellas Pharma. E. Guttman-Yassky is on advisory boards for Sanofi Aventis, Regeneron, Stiefel/GlaxoSmithKline, MedImmune, Celgene, Anacor, AnaptysBio, Celcus, Dermira, Galderma, Glenmark, Novartis, Pfizer, Vitae, and Leo Pharma; has received consultancy fees from Regeneron, Sanofi, MedImmune, Celgene, Stiefel/GlaxoSmithKline, Celcus, BMS, Amgen, Draize, AbbVie, Anacor, AnaptysBio, Dermira, Galderma, Glenmark, LEO Pharma, Novartis, Pfizer, Vitae, Mitsubishi Tanabe, and Eli Lilly; has received research support from Janssen, Regeneron, Celgene, BMS, Novartis, Merck, LEO Pharma, and Dermira; and has received grants from Regeneron, Celgene, BMS, Janssen, Dermira, LEO Pharma, Merck, Novartis, and Pfizer. R. B. Weller has received payment for lectures from Johnson & Johnson. C. Yao has received a grant from the Wellcome Trust (WT-UoE ISSF2). The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication November 30, 2016; revised April 10, 2017; accepted for publication April 11, 2017.

Corresponding author: Chengcan Yao, PhD, Medical Research Council (MRC) Centre for Inflammation Research, Queen's Medical Research Institute, University of Edinburgh, 47 Little France Crescent, Edinburgh EH16 4TJ, United Kingdom. E-mail: Chengcan.Yao@ed.ac.uk.

0091-6749/\$36.00

© 2017 American Academy of Allergy, Asthma & Immunology

<http://dx.doi.org/10.1016/j.jaci.2017.04.045>

Abbreviations used

ACD:	Allergic contact dermatitis
AD:	Atopic dermatitis
AHR:	Aryl hydrocarbon receptor
cAMP:	Cyclic AMP
db-cAMP:	Dibutyl cAMP
DC:	Dendritic cell
EP:	E prostanoïd receptor
EtOH:	Ethyl alcohol
<i>Gapdh</i> :	Glyceraldehyde-3-phosphate dehydrogenase
ILC3:	Group 3 innate lymphoid cell
LN:	Lymph node
PG:	Prostaglandin
PKA:	Protein kinase A

eczematous skin include epidermal hyperplasia and spongiosis and infiltration of immune cells (eg, T cells, dendritic cells [DCs], and eosinophils) in the dermis.^{1,3,4} Barrier dysfunction is also recognized as important for AD development.⁵⁻⁷ In response to antigen and hapten sensitization, keratinocytes are activated to produce proinflammatory cytokines. Concurrently, skin-resident innate immune cells, such as DCs, capture antigens and migrate to secondary lymphocytes, where they present antigens to T lymphocytes for their activation and differentiation into effector T cells, resulting in ACD initiation when the antigen or hapten is repeatedly applied to the skin.³ Both T_H1 and T_H2 cells, which are characterized by production of IFN- γ or IL-4/IL-13, respectively, have been thought to contribute to ACD pathogenesis, depending on the antigens.⁸ For example, although the majority of haptens (eg, 1-fluoro-2,4-dinitrobenzene) induce a T_H1-dominant response, others, like fluorescein isothiocyanate, induce T_H2-dominant responses.⁹ Although single-hapten elicitation induces delayed-type hypersensitivity, as a mouse model of human contact dermatitis,¹⁰ repeated hapten challenge results in a chronic late-phase and immediate-type hypersensitivity response, which is clinically relevant to the acute phase of AD in human subjects.¹¹⁻¹³ However, recent studies have shown that IL-17 and IL-22, which are produced by T_H17 and other activated T cells, including T_H22 cells, are also critical in mediating the initiation and progression of ACD and AD.¹⁴⁻²⁰ In patients with ACD to nickel, there were upregulated levels of IL-22 in both serum and inflamed skin.^{19,20} Furthermore, IL-22 levels in serum from patients with AD are greater than those in healthy subjects,^{21,22} and IL-22⁺ T cells infiltrate into lesional AD skin,²³ where IL-22 induces epidermal hyperplasia and inhibits epidermal differentiation.^{24,25} Importantly, a neutralizing anti-IL-22 antibody (NCT01941537) or targeting components of the IL-23/T_H17 pathway are currently being tested in clinical trials.^{4,26} To refine future therapies, there is a need to better understand the mechanisms that drive IL-22 production in response to cutaneous antigen stimulation.

Prostanoids, including prostaglandin (PG) D₂, PGE₂, PGF_{2 α} , PGI₂, and thromboxane A₂, are bioactive lipid mediators that are generated from arachidonic acid by COXs and then respective PG synthases. PGs have various roles in patients with inflammatory skin diseases through regulating functions of immune cells, including T_H1/T_H17 T cells, regulatory T cells, mast cells, and DCs.²⁷ PGE₂ is synthesized by microsomal PGE synthases (mPGES1 and mPGES2) and has essential roles in modulating various inflammatory responses by binding to E prostanoïd

receptors 1 to 4 (EP1-4) on cell surfaces. Many cutaneous cells, including keratinocytes, mast cells, eosinophils, fibroblasts, DCs, and lymphocytes, express PGs and PG receptors.²⁸ Increased PGE₂ expression in biologically active amounts has been reported in both lesional and nonlesional skin from patients with AD.²⁹ Blockade of PG production by a COX2 inhibitor was reported to enhance eosinophil infiltration and increase IL-4 expression in lesions of an ovalbumin-sensitized mouse model.³⁰ In contrast, PGE₂ was also suggested to induce AD by favoring a T_H2 immune milieu and directly enhancing B-cell production of IgE.^{31,32} Furthermore, we and others have recently reported that PGE₂, through EP2 and EP4 receptors, augmented IL-17 and IL-22 production, and blockade of PGE₂ signaling during T-cell differentiation limited acute contact hypersensitivity.³³⁻³⁵ These findings suggest that PGE₂ can have both suppressive and provocative roles in the development of ACD and AD.²⁸ However, it is unclear how PGE₂ regulates IL-22 production and chronic atopic skin inflammation.

Here we report that PGE₂ promotes IL-22 production from T_H17 and T_H22 T cells through its receptors EP2 and EP4. This effect is mediated by cyclic AMP (cAMP)–protein kinase A (PKA) signaling and induction of aryl hydrocarbon receptor (AHR), a transcription factor critical for both adaptive and innate IL-22 expression.³⁶ T cell–specific EP4 deficiency diminishes hapten-induced IL-22–expressing T cells in skin-draining lymph nodes (LNs). Accordingly, inhibition of PGE₂ production limits skin inflammation in an animal model for ACD induced by repeated oxazolone challenges in mice. Furthermore, genes related to PGE₂ signaling are overexpressed in human atopic lesional skin, and their expression correlates positively with expression of IL-22 pathway genes. This relationship between expression of PGE₂ and IL-22–related genes is no longer evident after successful corticosteroid or UVB treatments. These findings suggest that PGE₂ facilitates ACD through promoting adaptive IL-22 signaling.

METHODS**Mice**

Wild-type C57BL/6 mice were purchased from Harlan UK (Bicester, United Kingdom). Lck-Cre mice were crossed to lox-flanked *Ptger4* (EP4-floxed) mice³⁷ to generate mice with selective EP4 deficiency in T cells (EP4cKO), as previously reported.^{34,35} All mice were bred and maintained under specific pathogen-free conditions in accredited animal facilities in the University of Edinburgh and Kyoto University. Mice were aged greater than 7 weeks at the beginning of use and sex matched. All experiments were conducted in accordance with the UK Scientific Procedures Act of 1986 and had local institutional ethical approval.

Reagents

Antibodies to mouse CD3 (clone 145-2C11), CD28 (clone 37.51), CD45 (clone 30-F11), CD4 (clone L3T4), CD8 (clone 53-6.7), IL-17A (clone eBio17B7), and IL-22 (clone IL22J0P) were from eBioscience (San Diego, Calif) or BioLegend (San Diego, Calif). Mouse CD4 microbeads were from Miltenyi Biotec (Bergisch Gladbach, Germany). Recombinant human TGF- β 1 and recombinant mouse IL-6 and IL-23 were purchased from BioLegend. PGE₂, 17-phenyl trinor PGE₂ (EP1/3 agonist), butaprost (EP2 agonist), CAY10598 or L-902,688 (EP4 agonist), PF-04418948 (EP2 antagonist), and L-161,982 (EP4 antagonist) were from Cayman Chemicals (Ann Arbor, Mich). Dibutyl cAMP (db-cAMP), PKA inhibitor 14-22, CH-223191, oxazolone, indomethacin, phorbol 12-myristate 13-acetate, and ionomycin were from Sigma (St Louis, Mo) or Calbiochem (Nottingham, United Kingdom).

Oxazolone-induced ACD model

The oxazolone-induced mouse ACD model was induced, as previously reported.¹¹ Briefly, mice were sensitized with 3% oxazolone in ethyl alcohol (EtOH) on shaved abdominal skin and after 5 days were repeatedly challenged with 0.6% oxazolone in EtOH on one ear once every 2 days for a total of 5 challenges. The opposing ear was challenged with vehicle (pure EtOH) to serve as a control. Mice were culled at 6 hours after the last challenge, and the ears and ear-draining LNs were collected for further analysis. Ear samples were fixed with 10% neutral-buffered formalin solution (Sigma) and embedded in paraffin wax, and 5- μ m sections were used for staining with hematoxylin and eosin. In some experiments EP4cKO or control mice were sensitized with 0.5% dinitrofluorobenzene, and 5 days later, skin-draining LNs were collected for further analysis.

T-cell isolation and culture

Mouse CD4⁺ T cells were isolated from spleens by using autoMACS (Miltenyi Biotec). Cells were cultured in complete RPMI 1640 medium containing 10% FBS and stimulated with plate-bound anti-CD3 (5 μ g/mL) and anti-CD28 (5 μ g/mL) antibodies plus various cytokines and other compounds, as indicated in the figures. For certain experiments, T_H17 cells were differentiated from CD4⁺ T cells by TGF- β 1 and IL-6 for 3 to 4 days. IL-22 levels in supernatants were measured by using mouse IL-22 ELISA Ready-SET-Go! kits (eBioscience).

Surface and intracellular staining

For surface staining, cells were stained on ice for 30 minutes with anti-CD45, anti-CD3e, anti-CD4, and anti-CD8 antibodies. For intracellular staining of IL-22, cells were stimulated with phorbol 12-myristate 13-acetate and ionomycin for 4 to 5 hours in the presence of GolgiPlug (BD Biosciences, San Jose, Calif). Cells were then harvested and fixed with BD Cytofix/Cytoperm Fixation Buffer (BD Biosciences) for 30 minutes and then stained with anti-human/mouse IL-22 (clone IL22JOP, eBioscience) or anti-human IL-22 in BD Perm/Wash Buffer for 1 hour. Flow cytometry was performed on a BD LSRFortessa (BD Biosciences) and analyzed by using FlowJo software (TreeStar, Ashland, Ore).

Real-time PCR

RNA purification from T cells was performed with the RNeasy Mini Kit (Qiagen, Hilden, Germany). cDNA was obtained by using reverse transcription with High-capacity cDNA Reverse Transcription Kits (Applied Biosystems, Foster City, Calif). Samples were analyzed by using real-time PCR with SYBR Premix Ex Taq II (Tli RNase H Plus) kit (Takara Bio, Mountain View, Calif) or GoTaq qPCR Master Mix (Promega, Madison, Wis) on the Applied Biosystems 7900HT Fast machine. The following primers were used: glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*) forward, 5'-TGAACGGGAAGCTCACTGG-3'; *Gapdh* reverse, 5'-TCCACCACCTGTTGCTGTA-3'; *Il22* forward, 5'-CATGCAGGAGGTGGTACCTT-3'; *Il22* reverse, 5'-CAGACGAAGCATTTCTCAG-3'; *Ahr* forward, 5'-TGACAAGGAGTGGACGA-3'; and *Ahr* reverse, 5'-AGG AAGCTGGTCTGGGGTAT-3'. Expression was normalized to mouse *Gapdh* and presented as relative expression to control group by using the $2^{-\Delta\Delta C_t}$ method.

Gene expression of human skin biopsy specimens from microarray data sets

Microarray gene expression data of human skin biopsy specimens were retrieved from Gene Expression Omnibus data sets (GSE16161, GSE32924, GSE36842, GSE32473, and GSE27887).³⁸⁻⁴² Patients' information and skin sample have been described previously.^{38-40,42} In brief, skin biopsy specimens were collected from patients with moderate-to-severe AD (SCORAD score, 20-97) and healthy volunteers under institutional review board-approved protocols (written consent obtained). Patients (age, 16-81 years; mean age,

40 years [from 3 cohorts]) with an acute exacerbation of chronic AD and without any therapy for more than 4 weeks were included. Biopsy specimens were obtained from acute lesional skin that was actively involved and erythematous lesions with AD and were frozen in liquid nitrogen for RNA extraction.^{38-40,42} Gene expression levels were transformed to z-score values to standardize data across a wide range of experiments and to allow for the comparison of microarray data independent of the original hybridization intensities.⁴³ *P* values were calculated by using the nonparametric Wilcoxon-Mann-Whitney test, paired nonparametric tests with *post hoc* Dunn multiple comparisons, or paired 2-way ANOVA test with the *post hoc* Bonferroni multiple comparisons test. Correlations between expression levels of 2 genes were calculated by using the nonparametric Spearman correlation test.

Statistical analyses

All data were expressed as means $\pm$ SEMs or scatter dot plots in which each dot represents 1 mouse, 1 patient with AD, or 1 healthy subject. Statistical significance between 2 groups was examined by using the Student *t* test or Mann-Whitney test, whereas 1-way and 2-way ANOVA with the *post hoc* Bonferroni multiple comparisons test were used to evaluate multiple groups, unless otherwise indicated in figure legends. Statistical analyses were performed with Prism 6 software (GraphPad Software, La Jolla, Calif), and a *P* value of less than .05 was considered statistically significant.

RESULTS

PGE₂ promotes IL-22 production *in vitro* through its receptors EP2 and EP4

Recently, we reported that PGE₂ promotes IL-22 production from group 3 innate lymphoid cells (ILC3s).³⁵ Thus we evaluated whether PGE₂ also promoted IL-22 production from T cells. To address this question, we isolated CD4⁺ T cells from mouse spleens activated with anti-CD3 and anti-CD28 antibodies and cocultured with various cytokines. Addition of exogenous PGE₂ enhanced *Il22* gene expression with IL-6 or IL-23 alone, IL-6 plus IL-23 (T_H22-priming condition), or IL-6 plus IL-23 plus TGF- β (T_H17-priming condition; Fig 1, A). IL-22 protein production in supernatants of primary cell cultures was also increased by PGE₂ in the presence of IL-23 or IL-23 plus IL-6 (Fig 1, B). It is important to note that these data are in agreement with previous findings⁴⁴ that TGF- β inhibits IL-22 production, even in the presence of PGE₂ (Fig 1, A and B). Moreover, when the same numbers of PGE₂-stimulated or control T cells were washed and then restimulated with T-cell receptors (ie, anti-CD3 and anti-CD28), PGE₂-stimulated T cells produced more IL-22 than control T cells, even in the presence of TGF- β (Fig 1, C), suggesting that PGE₂-treated cells have higher capability to produce IL-22 at the single-cell level.

To define which PGE₂ receptor mediates IL-22 production, we cultured T cells under T_H22-priming conditions and selectively activated EP2 and EP4. Both EP2 and EP4 agonists individually mimicked augmentation of IL-22 production (Fig 1, D). To further confirm the roles of PGE₂-EP2/EP4 signaling in inducing IL-22 production of T cells, we also cultured differentiated T_H17 T cells and found that PGE₂ still promotes IL-23-driven IL-22 gene and protein expression (Fig 1, E and F). Moreover, enhancement of IL-22 production by EP2 or EP4 agonists was prevented by cotreatment of antagonists against EP2 or EP4, respectively (Fig 1, F); increased IL-22 production by PGE₂ was also diminished by coadministration of EP2 and EP4 antagonists (Fig 1, F). In addition, PGE₂ also increased IL-22 production in T_H17 cells in the absence of other cytokines (Fig 1, E and F), suggesting a potentially direct action of PGE₂ to promote *Il22*

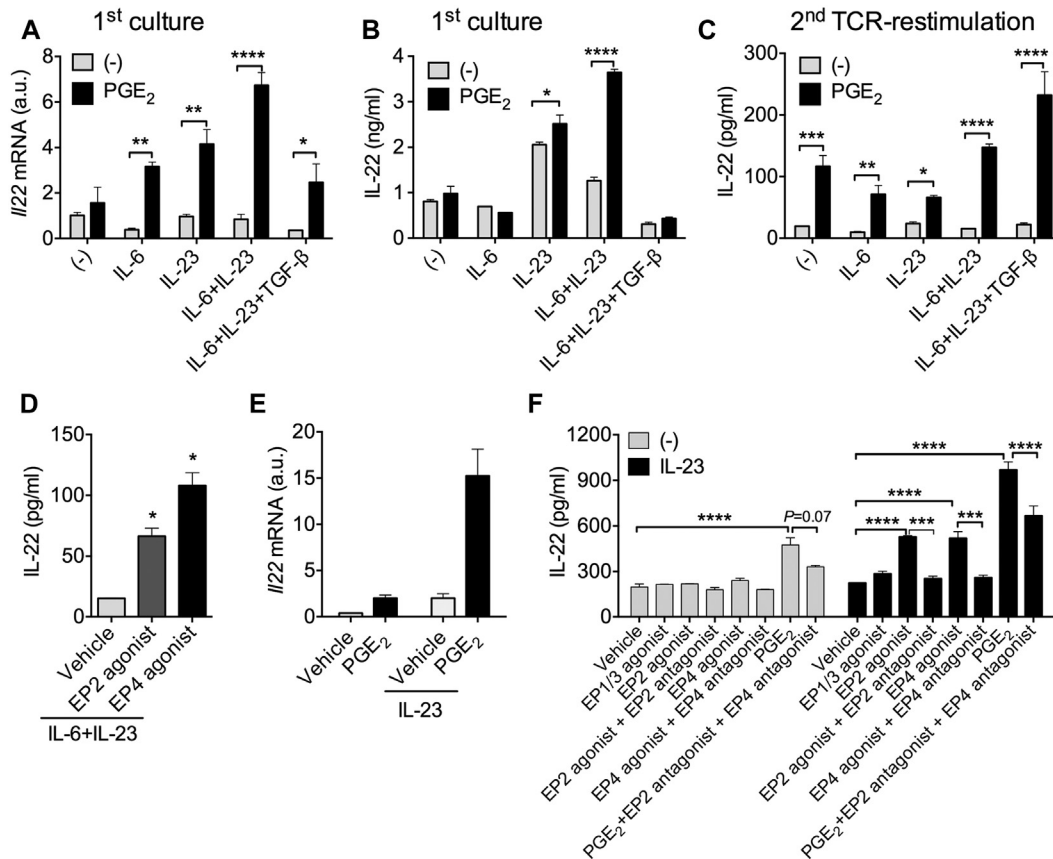


FIG 1. PGE₂ promotes T-cell production of IL-22 through its receptors EP2 and EP4. **A**, //22 mRNA expression in naive CD4⁺ T cells stimulated with anti-CD3 and anti-CD28 (anti-CD3/CD28) antibodies with indicated cytokines in the absence or presence of PGE₂ (100 nmol/L) for 3 days. **B**, IL-22 levels in supernatants of cultures in Fig 1, A. **C**, IL-22 levels in supernatants of naive CD4⁺ T cells were cultured as in Fig 1, A, and then the same numbers of T cells were restimulated with anti-CD3/CD28 antibodies for another 3 days. **TCR**, T-cell receptor. **D**, IL-22 levels in supernatants of naive CD4⁺ T cells were stimulated with anti-CD3/CD28 antibodies with IL-6 plus IL-23 with or without an EP2 agonist (butaprost, 1 μmol/L) or EP4 agonist (L-902,688, 1 μmol/L) for 3 days, and then the same numbers of T cells were restimulated with anti-CD3/CD28 antibodies for another 3 days. **E**, IL-22 levels in supernatants from T_H17 cells restimulated with anti-CD3/CD28 antibodies without or with IL-23 and various small-molecule compounds activating or inhibiting PGE₂ receptors for 3 days. **F**, //22 mRNA of restimulated T_H17 cells with PGE₂, IL-23, or both for 3 days. The EP1/3 agonist 17-phenyl trinor PGE₂ was used at 1 μmol/L, whereas the EP2 antagonist PF-04418948 and EP4 antagonist L-161,982 were used at 10 μmol/L. All experiments were performed in duplicates or triplicates and repeated 2 to 3 times independently. **P* < .05, ***P* < .01, ****P* < .001, and *****P* < .0001.

gene expression. These data indicate the involvement of EP2 and EP4 in PGE₂ facilitation of IL-22 production from T cells *in vitro*.

cAMP promotes IL-22 production from T cells through the transcription factor AHR

Because both EP2 and EP4 receptors activate the cAMP-PKA pathway in T cells,³³ we next examined whether cAMP mediates PGE₂ facilitation of adaptive IL-22 production. Like PGE₂, db-cAMP promoted IL-22 production in a concentration-dependent manner in either the presence or absence of IL-23 (Fig 2, A), confirming a direct action of cAMP on IL-22 induction. The increased IL-22 production by cAMP was prevented by PKA inhibitor 14-22 in a concentration-dependent manner (Fig 2, B). cAMP also upregulated //22 gene expression in IL-23-stimulated T cells, which

was prevented by cycloheximide, an inhibitor for eukaryote protein synthesis (Fig 2, C and D), suggesting that the PGE₂-cAMP pathway promoted adaptive IL-22 production through synthesis of a new protein or new proteins.

The transcription factor AHR has been reported to regulate IL-22 production in both T cells and ILC3s.³⁶ Thus we investigated whether AHR contributes to the PGE₂-cAMP signaling-dependent increase in adaptive IL-22 production. We first checked that both PGE₂ and cAMP upregulated *Ahr* gene expression in T cells independently of cytokine stimuli (Fig 2, E and F). Importantly, the small-molecule AHR inhibitor CH-223191 effectively suppressed cAMP-dependent IL22 gene expression and protein production from T cells (Fig 2, G and H), although it had no effect on *Ahr* expression itself (Fig 2, F). These results suggest that PGE₂-cAMP signaling promotes IL-22 production from T cells through induction of AHR.

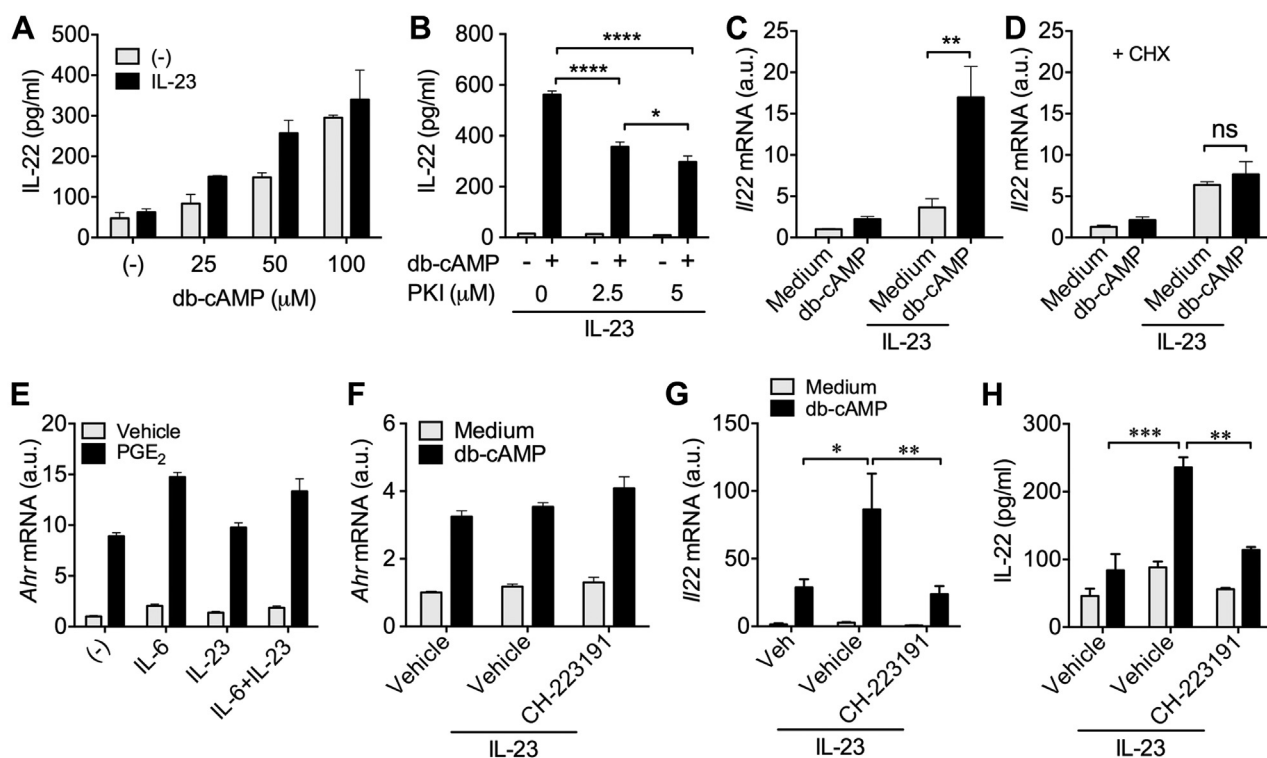


FIG 2. cAMP promotes IL-22 production from T cells through induction of AHR. **A**, IL-22 levels in supernatants from T_H17 cells restimulated with anti-CD3/CD28 antibodies without or with IL-23 and indicated concentrations of db-cAMP for 3 days. **B**, IL-22 levels in supernatants from T_H17 cells restimulated with anti-CD3/CD28 antibodies with IL-23, db-cAMP (100 μmol/L), and indicated concentrations of PKA inhibitor 14-22 (PKI) for 3 days. **C** and **D**, IL-22 mRNA expression levels in T_H17 cells restimulated with anti-CD3/CD28 antibodies with IL-23 and db-cAMP in the absence (Fig 2, C) or presence (Fig 2, D) of cycloheximide (CHX; 1 μmol/L) for 3 days. **E**, Ahr mRNA expression in naive CD4⁺ T cells cultured with anti-CD3/CD28 antibodies and indicated cytokines in the absence or presence of PGE₂ for 3 days. **F-H**, Ahr (Fig 2, F) and IL-22 (Fig 2, G) mRNA expression and IL-22 levels (Fig 2, H) in supernatants of T_H17 cells cultured with anti-CD3/CD28 antibodies, IL-23, db-cAMP, and CH-223191 (10 μmol/L) for 3 days. All experiments were performed in duplicates or triplicates and repeated 2 to 3 times independently. **P* < .05, ***P* < .01, ****P* < .001, and *****P* < .0001. ns, Nonsignificant.

PGE₂-EP4 signaling promotes adaptive IL-22 production *in vivo*

To investigate whether PGE₂ promotes adaptive IL-22 production *in vivo*, we topically sensitized the abdominal skin of control mice and mice with specific EP4 deletion in T cells (EP4cKO mice³⁴) with dinitrofluorobenzene and measured IL-22-producing T-cell numbers in skin-draining LNs 5 days after sensitization using flow cytometry. Both percentages and absolute numbers of IL-22-producing T-cells were reduced markedly in EP4cKO compared with control mice (Fig 3, A and B). Furthermore, EP4 deficiency reduced *Il22* gene expression in skin-draining LN CD4⁺ T cells (Fig 3, C). These data indicate that PGE₂-EP4 signaling promotes adaptive IL-22 production *in vivo*.

Endogenous PGE₂-EP4 signaling in T cells promotes ACD

We next investigated whether PGE₂ has a pathogenic role in development of allergic skin inflammation. To address this question, we used an animal model mimicking human ACD. We sensitized wild-type C57BL/6 mice with 3% oxazolone on abdominal skin, and after 5 days, we repeatedly challenged mice with 0.6%

oxazolone every 2 days for a total of 5 challenges. Indomethacin was administered to mice in drinking water to inhibit endogenous PGE₂ production. Once DCs in the skin capture the antigen oxazolone, they migrate to skin-draining LNs and present antigen to T cells for their activation. Because the migration of antigen-capturing DCs to skin-draining LNs peaks between 18 and 24 hours requires PGE₂-EP4 signaling,⁴⁵ we only treated mice with indomethacin 24 hours after the oxazolone sensitization (Fig 4, A). Consistent with previous reports,¹¹ repeated challenge with oxazolone induced allergic skin inflammation (Fig 4, B and C). Ears of oxazolone-challenged, indomethacin-treated mice showed decreased swelling compared with those of vehicle-treated mice (Fig 4, B). Indomethacin had no effect on the thickness of vehicle (EtOH)-challenged ears. Compared with EtOH-challenged ears, the histology of oxazolone-challenged ear skin showed features of eczema, namely parakeratosis, acanthosis, and focal spongiosis, with a dense and diffuse dermal infiltrate containing eosinophils (Fig 4, C). However, in skin from indomethacin-treated mice, epidermal changes are less pronounced, and dermal infiltrates are markedly reduced (Fig 4, C). Furthermore, although repeated oxazolone challenge recruited CD4⁺ and CD8⁺ T cells to ear-draining LNs (Fig 4, D),

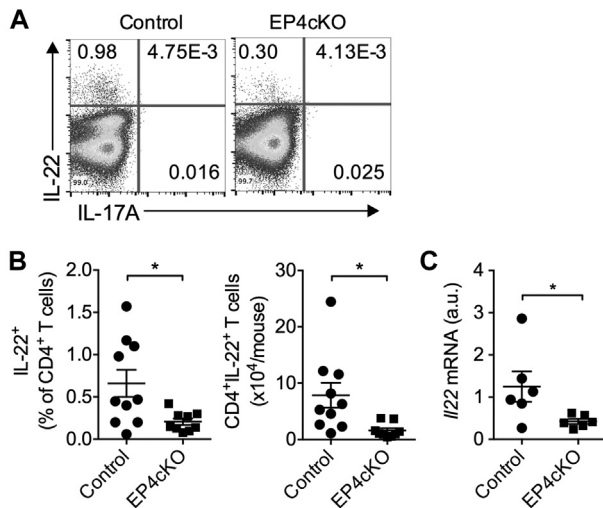


FIG 3. PGE₂-EP4 signaling in T cells facilitates IL-22 production *in vivo*. **A**, Representative flow cytometric dot plot of IL-22 and IL-17 expression in skin-draining LN CD4⁺ T cells from T cell-specific EP4-deficient (EP4cKO, n = 9) or control (n = 10) mice 5 days after sensitization with 0.5% dinitrofluorobenzene. **B**, Percentages and numbers of IL-22⁺ CD4⁺ T cells. Each dot represents 1 mouse. **C**, IL22 mRNA expression levels in CD4⁺ T cells isolated from skin-draining LNs of EP4cKO (n = 6) or control (n = 6) mice. Each dot represents 1 mouse. *P < .05.

indomethacin treatment reduced both this recruitment and the T-cell capacity to produce IL-22 (Fig 4, E). To further investigate whether this proinflammatory effect of PGE₂ on oxazolone-induced ACD development was mediated by EP4 and T cells, we performed this model on EP4cKO and control (EP4^{fl/fl}) mice. Oxazolone induced significantly less ear skin inflammation in EP4cKO compared with control mice (Fig 4, F), suggesting that PGE₂-EP4 signaling in T cells promotes ACD development.

PGE₂-dependent IL-22 production and signaling in human atopic lesional skin

Because ACD and AD potentially share common cellular mechanisms⁴ and the repeated hapten challenge-induced animal model is relevant to the acute phase of AD in human subjects,¹¹ we next investigated whether PGE₂-dependent adaptive IL-22 production and signaling can be found in human atopic skin. We analyzed mRNA expression of genes related to PGE₂ metabolism (Fig 5, A) and IL-22 signaling pathways in skin biopsy specimens from human patients with lesional AD and healthy subjects. As reported previously,³⁹ mRNA expression levels for T_H17/T_H22-related genes (*IL17A*, *IL22*, *IL23R*, and *AHR*), IL-22-induced products (eg, *S100A7*, *S100A8*, and *S100A9*), and skin-homing chemokines (*CCR4*) were overexpressed in lesional AD compared with normal skin (Fig 5, B).^{38-40,43} Strikingly, mRNA expression levels for PGE₂ synthases (*PTGS2*, *PTGES*, and *PTGES2*) were also upregulated in human atopic lesional skin, and in contrast, expression levels for the PGE₂ degrading enzyme *HPGD* (hydroxyprostaglandin dehydrogenase 15-[NAD]) was downregulated in lesional AD skin (Fig 5, B). These gene expression data are consistent with previous reports showing increased PGE₂ levels in lesional AD skin.²¹ There was a trend that expression of the *PTGER4* gene (encoding PGE₂ receptor EP4) was upregulated in lesional AD skin but expression of

PTGER2 (encoding PGE₂ receptor EP2) was significantly downregulated in lesional AD skin (Fig 5, B). Expression of T_H2 cytokines (eg, IL-4, IL-5, and IL-13) was not significantly upregulated in lesional AD skin; however, T_H2 chemokines, such as *CCL26*, *CCL18*, *CCL22*, *CCL17*, *CCL11*, and *CCL5*, were upregulated in lesional AD skin compared with normal skin (Fig 5, B). Interestingly, expression of IL-22 pathway genes showed strongly positive correlation with those of *PTGES* in biopsy samples from lesional AD but not normal skin (Fig 5, C). There were no correlations between *PTGES* expression and T_H2 cytokine levels (data not shown), but weak correlations between *PTGES* gene expression and T_H2 chemokine levels in lesional AD skin were observed (Fig 5, D). These results suggest that PGE₂ signaling is activated and positively correlates with the IL-22 signaling pathway and, probably, the T_H2 pathway in human atopic skin.

Finally, we investigated whether current therapies for AD modulate PGE₂ signaling in human AD. We analyzed changes in expression levels of IL-22 and PGE₂ signaling genes in lesional AD skin before and after treatments, such as betamethasone (a corticosteroid), by reanalyzing a public microarray data set.⁴¹ Compared with baseline levels, treatments with betamethasone reduced *IL22* gene expression in atopic lesional skin (Fig 4, A). Betamethasone also significantly reduced expression of IL-22-related genes, such as *AHR*, *S100A8*, and *S100A9* (Fig 6, A).⁴¹ Expression of PGE₂ synthases (eg, *PTGES* and *PTGES2*) and the EP4 receptor (*PTGER4*) was significantly downregulated by betamethasone treatment, whereas EP2 receptor (*PTGER2*) expression was not changed (Fig 6, A). This observation is consistent with findings that betamethasone treatment reduces PGE₂ production.^{46,47} Interestingly, expression of *PTGES*, *PTGES2*, and *PTGER4*, but not *PTGER2*, positively correlated with *IL22* expression in atopic biopsy specimens at baseline, whereas expression of *HPGD*, which mediates PGE₂ degradation, negatively correlated with *IL22* expression (Fig 6, B). Furthermore, we have also reanalyzed gene expression of skin biopsy specimens before and after treatment with UVB from our previous data set.⁴² Expression of the PGE₂ synthases *PTGS1* and *PTGES* in lesional AD skin was downregulated after treatment with UVB (Fig 6, C).⁴² Interestingly, although *IL22* gene expression was not changed by UVB treatment, most likely because of the small number of samples, changes in expression of IL-22-activated genes (eg, *S100A8* and *S100A9*) positively correlated with changes in expression of PGE₂ synthases (ie, *PTGS1*, *PTGES*, and *PTGES2*) in atopic lesional but not nonlesional biopsy specimens (Fig 6, D, and data not shown). Together, these data indicate that PGE₂, most likely through the EP4 receptor, plays a role in IL-22 regulation in human skin.

DISCUSSION

The cytokine IL-22, which is notably produced by T_H22 and T_H17 T cells, is emerging as a key player in AD,^{3,16,23} leading to a new therapy targeting IL-22 signaling in AD clinical trials (NCT01941537). Genome-wide association studies have identified that gene polymorphisms of the PGE₂ receptor EP4 were associated with T cell-mediated human autoimmune and allergic inflammatory diseases.^{48,49} However, the role of PGE₂ in promoting IL-22 production and development of AD remains to be determined. To address this, ideally, a model that showed the features of AD would be used. However, because no single animal model can fully capture all aspects of the human AD profile⁵⁰ and there is

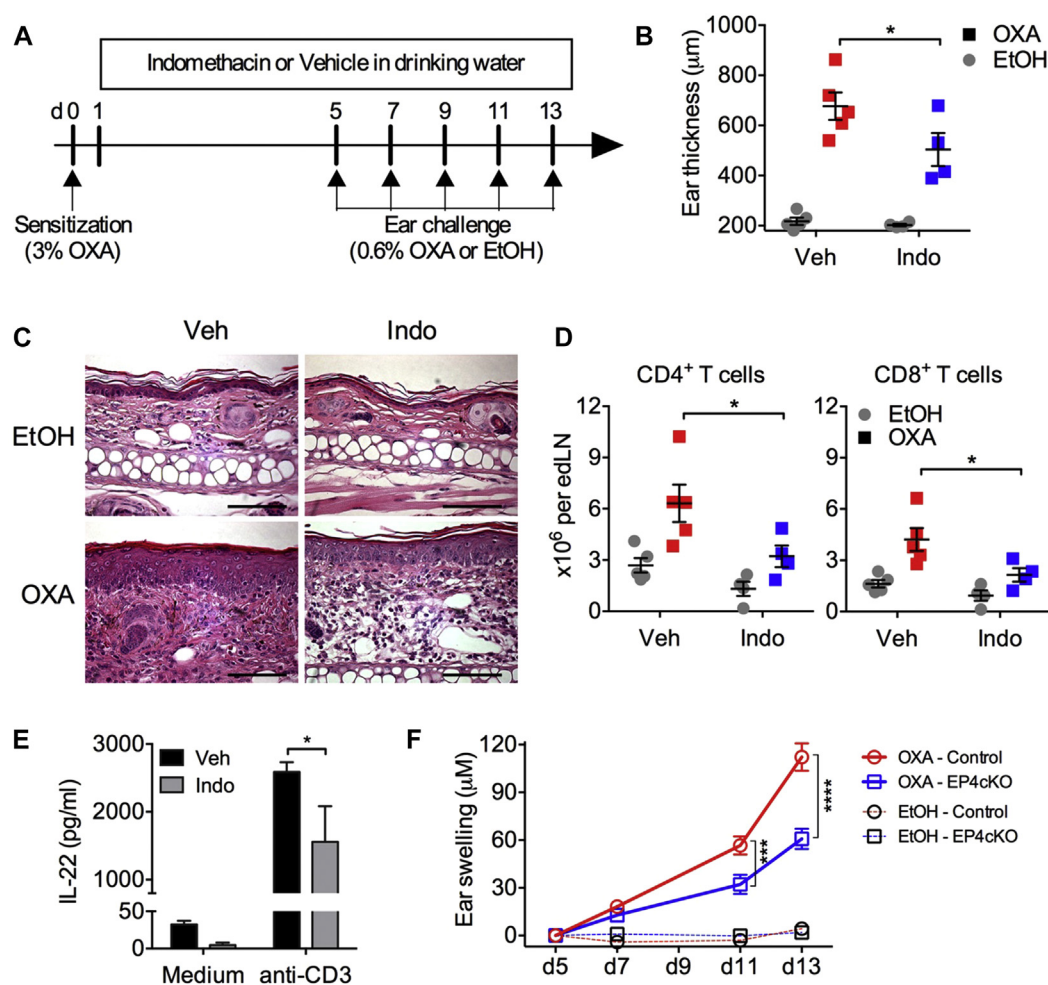


FIG 4. PGE₂ exacerbates atopic skin inflammation in the repeated oxazolone (OXA) challenge model. **A–E**, WT C57BL/6 mice were sensitized with 3% oxazolone on abdominal skin on day 0 and then challenged with 0.6% oxazolone or EtOH on ears 5 days later. Challenge with oxazolone was repeated once every 2 days for a total of 5 challenges. Ears and ear-draining LNs were collected 6 hours after the last oxazolone challenge (n = 4–5). Fig 4, A, Schematic representation of the experimental protocol. Fig 4, B, Ear thickness. Fig 4, C, Ear histology. Scale bar = 50 μmol/L. Fig 4, D, CD4⁺ and CD8⁺ T cells in ear-draining LNs. Fig 4, E, IL-22 production by ear-draining LN cells cultured with soluble anti-CD3 or medium only *in vitro* for 3 days. Indo, Indomethacin. **F**, Swelling of ears from control and EP4cKO mice (n = 9–13) sensitized and repeatedly challenged with OXA as in Fig 4, A, but without indomethacin treatment. *P < .05, ***P < .001, and ****P < .0001.

acceptance that there are shared common cellular mechanisms between AD and ACD,³ we used a repeated hapten challenge ACD model to study the role of PGE₂ signaling in IL-22⁺ T cells and development of chronic skin inflammation. We also analyzed gene expression in human AD lesional skin before and after common treatments for AD to examine the PGE₂–IL-22 axis in the relevant human skin disease in both the chronic inflammatory phase and after therapeutic intervention.

We show that PGE₂ markedly stimulates IL-22 production from T cells, which complements our previous findings that PGE₂ promotes innate IL-22 production from ILC3s.³⁵ It is worth noting that PGE₂ promotes IL-22 production from freshly activated CD4⁺ T cells under various conditions, including T_H17- and T_H22-priming conditions. The effect of PGE₂ was mimicked by activation of EP2 and EP4 receptors and was prevented by antagonists against these receptors, confirming the involvement of

these 2 receptors in adaptive IL-22 production *in vitro*. Further studies showed that the cAMP-PKA signaling pathway, which is activated by engagement of EP2 and EP4, mediates PGE₂ enhancement of IL-22 production through induction of the newly expressed AHR. Thus our results uncover a new and targetable molecular mechanism for regulation of adaptive IL-22 by the PGE₂–cAMP–AHR axis.

The role of PGE₂ in promoting adaptive IL-22 production was also confirmed *in vivo*. T cell-specific EP4 deficiency led to a large reduction of IL-22⁺ T cells in skin-draining LNs in response to hapten sensitization and attenuated repeated oxazolone challenge-induced allergic skin inflammation in the mouse model of ACD, indicating a critical role of endogenous EP4 signaling in T cells for IL-22 expression and function *in vivo*. This is similar to innate IL-22 expression, which also requires PGE₂–EP4 signaling,³⁵ suggesting a shared mechanism for regulating both

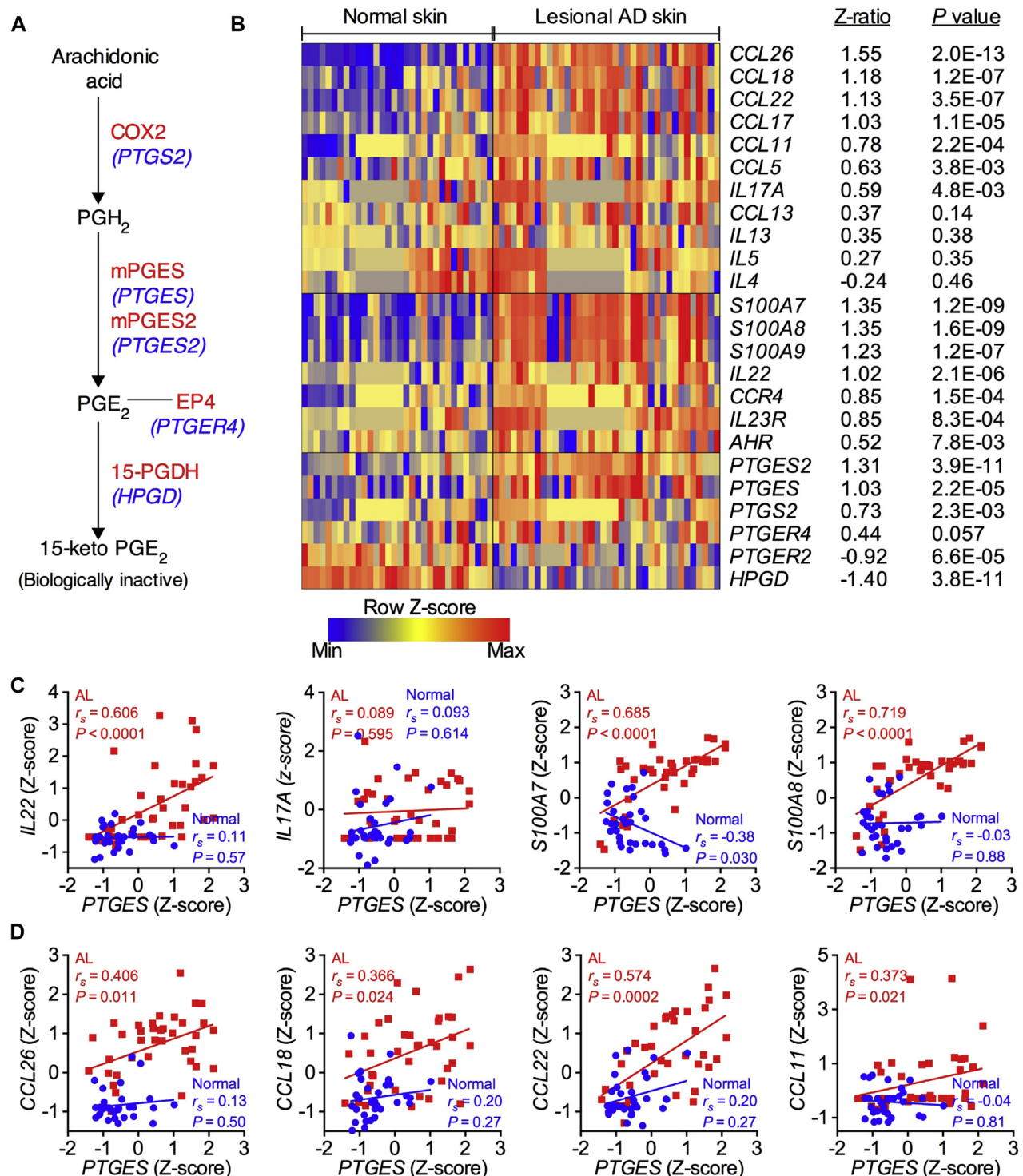


FIG 5. Overexpression of PGE₂ signaling genes in human lesional AD skin, which positively correlates with expression of IL-22-signaling genes. **A**, Schematic depicting synthesis and metabolism of PGE₂ mediated by COX2 (encoding by *PTGS2*), PGE synthases (encoding by *PTGES* or *PTGES2*), and 15-PGDH (encoding by *HPGD*), respectively. The PGE₂ receptor EP4 (encoding by *PTGER4*) is also shown. **B**, Heat map of expression profiles of T_H2 cytokine and chemokine genes and genes related to IL-22 and PGE₂ pathways in human lesional AD skin (n = 38) and normal skin (n = 32). A color scale bar indicates the z-score transformed values of microarray gene expression data.³⁸⁻⁴⁰ Ratios of z scores represent changes in gene expression levels between the healthy and AD lesional groups.⁴³ P values were calculated by using nonparametric Wilcoxon-Mann-Whitney tests. Probes with the largest z-score ratios were chosen when several probes represented single genes. **C** and **D**, Correlations of *PTGES* gene expression versus expression of IL-22 pathway genes (Fig 5, C) or T_H2 chemokines (Fig 5, D) in atopic lesional and normal skin. P values were calculated by using the nonparametric Spearman correlation test.

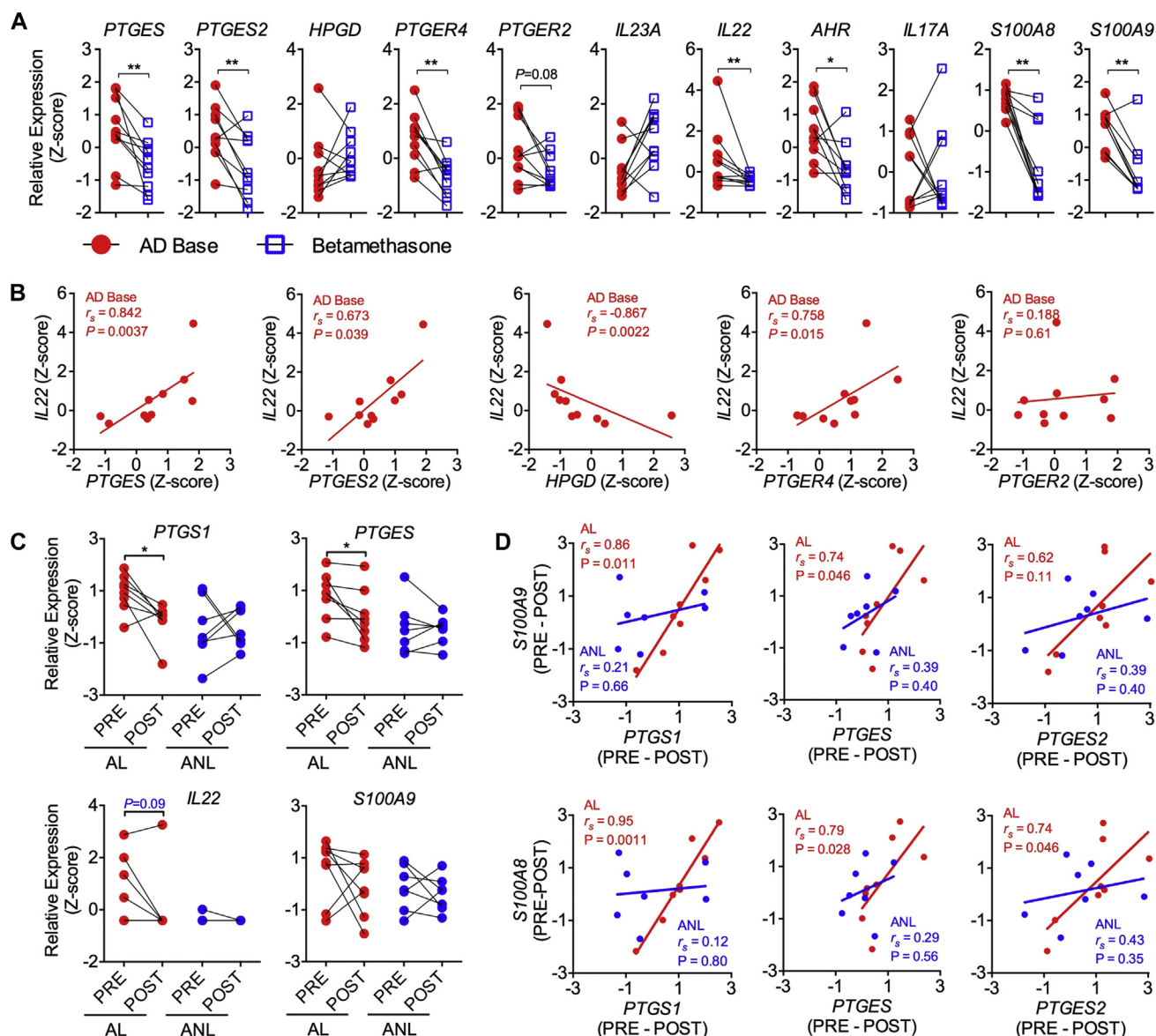


FIG 6. Steroid and UVB therapies downregulate PGE₂ and IL-22 signaling pathway genes in human atopic lesional skin. **A**, Expression profiles of genes related to PGE₂ and IL-22 pathways in human lesional AD skin ($n = 10$) at baseline (AD Base) or after treatment with betamethasone for 3 weeks. The z-score transformed values of microarray gene expression data⁴¹ were used. P values were calculated by using paired nonparametric tests with the *post hoc* Dunn multiple comparisons test. **B**, Correlations of *PTGES*, *PTGES2*, *HPGD*, and *PTGER4* gene expression versus that of the *IL22* gene in human atopic lesional skin at baseline. P values were calculated by using the nonparametric Spearman correlation test. **C**, Expression of genes related to PGE₂ and IL-22 signaling in human atopic lesional (AL, $n = 8$) and nonlesional (ANL, $n = 7$) skin before (PRE) or 12 weeks after (POST) treatment with UVB. The z-score transformed values of microarray gene expression data⁴² were used. P values were calculated by using the paired 2-way ANOVA test with the *post hoc* Bonferroni multiple comparisons. **D**, Correlations of changes in expression of IL-22-induced genes (*S100A8*, and *S100A9*) and PGE₂ synthase genes (*PTGS1*, *PTGES*, and *PTGES2*) before (PRE) and after (POST) UVB therapy in atopic lesional or nonlesional skins. P values were calculated by using the nonparametric Spearman correlation test.

the adaptive and innate IL-22 responses. Inhibition of endogenous PGE₂ production by a COX inhibitor successfully prevented accumulation of T cells in ear-draining LNs, reduced T-cell production of IL-22, and attenuated allergic skin inflammation induced by repeated oxazolone challenges. Given that PGE₂ also promotes IL-17 production from both mouse and human

T_H17 T-cells^{33,51} and that IL-17 might participate in creating the ACD phenotype,¹⁴⁻²⁰ IL-17 might also be involved in the PGE₂-facilitated ACD pathogenesis.

Facilitation of IL-22 production by PGE₂ might contribute to several human inflammatory diseases, such as AD and psoriasis.⁵² Indeed, parallel upregulations of IL-22 pathway genes and PGE₂

synthases were observed in human atopic lesional skin, whereas levels of the PGE₂ degrading enzyme 15-PGDH (encoded by *HPGD*) were downregulated in AD skin. This is consistent with previous observations showing increased PGE₂ levels in lesional AD skin.²⁹ Moreover, our data indicate that IL-22 signaling positively correlated with PGE₂ signaling in atopic lesional skin, and these correlations were absent in both normal and lesional AD skin after successful steroid or UVB treatments. These findings potentially suggest a PGE₂-dependent IL-22 production and signaling in human atopic skin.

In addition to IL-22⁺ T cells, T_H2 cells (especially the cutaneous lymphocyte antigen-positive population with skin-homing capacity) are expanded in patients with severe AD.⁵³ T_H2 cells suppress major terminal differentiation proteins (ie, filaggrin and loricrin) and predominate in the acute phase of AD through production of cytokines (eg, *IL4* and *IL13*) and chemokines (eg, *CCL17*, *CCL18*, and *CCL26*).^{40,54} Thus the T_H2 response is critical for AD pathogenesis. Inhibition of COX2 has been reported to enhance T_H2 cytokine production and the T_H2 response to ovalbumin-induced epicutaneous sensitization,³⁰ suggesting a potential inhibitory role of PGs in T_H2 cell-driven allergic inflammation. In human lesional AD skin, however, there was no correlation between the PGE₂ pathway and T_H2 cytokines, and in contrast, there were weak positive correlations between the PGE₂ pathway and T_H2 chemokines, suggesting a possible promoting role of PGE₂ in T_H2 response in patients with AD. This might be because PGE₂ promotes IL-17 and IL-22 production^{33-35,49} and subsequently IL-17 exacerbates T_H2-type inflammation during the initiation of AD.^{11,55}

We acknowledge this report has several limitations. For example, our analysis of gene expression profiles in human skin biopsy specimens were retrieved from public Gene Expression Omnibus data sets, and confirmation of these findings was not performed by using methods with higher sensitivity, such as real-time PCR with fresh biopsy specimens. Moreover, cytokine expression in protein levels in different T-cell subsets were not measured by using flow cytometry in skin of patients and animals. Furthermore, although in mice the oxazolone repeated challenge-induced chronic allergic skin inflammation represents a robust model, additional animal models are required in attempt to fully mimic most pathogenic and physiologic processes during the development of human AD. In addition, because of a limitation of resources, the effects of PGE₂ signaling in T cells, especially the involvement of both EP2 and EP4 in T cells, on initiation and progression of ACD could not be interrogated, such as using mice with T cell-specific deficiency of both EP2 and EP4 receptors. Therefore future prospective studies are required to further understand not only the role of PGE₂ during perpetuation of ACD and AD skin inflammation but also during their onset.

In conclusion, our data ascertain that PGE₂ acts as a potent promoter of both adaptive and innate IL-22 production. We have previously found that PGE₂ signaling promotes DC production of IL-23, a cytokine essential for IL-22 expression, as well as for proliferation and maturation of T_H17 T cells.³³ Taken together, our results highlight the PGE₂ signaling pathway as an important stimulus of T-cell responses and ACD and, possibly, AD development. Thus targeting PGE₂ synthesis or its receptors might represent a possible therapeutic strategy for the treatment of ACD and AD and other inflammatory skin diseases, such as psoriasis, with an active role for IL-22.

We thank P. Ghazal (University of Edinburgh) for critically reading and editing the manuscript and S. Johnston, W. Ramsay, and F. Rossi for assistance with flow cytometry.

Clinical implications: ACD is a common disabling disease characterized by increased IL-22 levels. The identification of a tightly regulated PGE₂-driven pathway controlling IL-22 dysfunction offers a novel target for therapeutic intervention.

REFERENCES

- Bieber T. Atopic dermatitis. *N Engl J Med* 2008;358:1483-94.
- Malajian D, Guttman-Yassky E. New pathogenic and therapeutic paradigms in atopic dermatitis. *Cytokine* 2015;73:311-8.
- Gittler JK, Krueger JG, Guttman-Yassky E. Atopic dermatitis results in intrinsic barrier and immune abnormalities: implications for contact dermatitis. *J Allergy Clin Immunol* 2013;131:300-13.
- Noda S, Krueger JG, Guttman-Yassky E. The translational revolution and use of biologics in patients with inflammatory skin diseases. *J Allergy Clin Immunol* 2015;135:324-36.
- Cork MJ, Danby SG, Vasilopoulos Y, Hadgraft J, Lane ME, Moustafa M. Epidermal barrier dysfunction in atopic dermatitis. *J Invest Dermatol* 2009;129:1892-908.
- Egawa G, Kabashima K. Multifactorial skin barrier deficiency and atopic dermatitis: essential topics to prevent the atopic march. *J Allergy Clin Immunol* 2016;138:350-8.
- Kabashima K. New concept of the pathogenesis of atopic dermatitis: interplay among the barrier, allergy, and pruritus as a trinity. *J Dermatol Sci* 2013;70:3-11.
- Romagnani S. Immunologic influences on allergy and the TH1/TH2 balance. *J Allergy Clin Immunol* 2004;113:395-400.
- Zhao Y, Balato A, Fischelevich R, Chapoval A, Mann DL, Gaspari AA. Th17/Tc17 infiltration and associated cytokine gene expression in elicitation phase of allergic contact dermatitis. *Br J Dermatol* 2009;161:1301-6.
- Honda T, Egawa G, Grabbe S, Kabashima K. Update of immune events in the murine contact hypersensitivity model: toward the understanding of allergic contact dermatitis. *J Invest Dermatol* 2013;133:303-15.
- Nakajima S, Kitoh A, Egawa G, Natsuaki Y, Nakamizo S, Moniaga CS, et al. IL-17A as an inducer for Th2 immune responses in murine atopic dermatitis models. *J Invest Dermatol* 2014;134:2122-30.
- Man MQ, Hatano Y, Lee SH, Man M, Chang S, Feingold KR, et al. Characterization of a hapten-induced, murine model with multiple features of atopic dermatitis: structural, immunologic, and biochemical changes following single versus multiple oxazolone challenges. *J Invest Dermatol* 2008;128:79-86.
- Jin H, He R, Oyoshi M, Geha RS. Animal models of atopic dermatitis. *J Invest Dermatol* 2009;129:31-40.
- Guttman-Yassky E, Lowes MA, Fuentes-Duculan J, Zaba LC, Cardinale I, Nogales KE, et al. Low expression of the IL-23/Th17 pathway in atopic dermatitis compared to psoriasis. *J Immunol* 2008;181:7420-7.
- Eyerich K, Pennino D, Scarponi C, Foerster S, Nasorri F, Behrendt H, et al. IL-17 in atopic eczema: linking allergen-specific adaptive and microbial-triggered innate immune response. *J Allergy Clin Immunol* 2009;123:59-66.
- Souwer Y, Szegedi K, Kapsenberg ML, de Jong EC. IL-17 and IL-22 in atopic allergic disease. *Curr Opin Immunol* 2010;22:821-6.
- Czarnowicki T, Krueger JG, Guttman-Yassky E. Skin barrier and immune dysregulation in atopic dermatitis: an evolving story with important clinical implications. *J Allergy Clin Immunol Pract* 2014;2:371-9.
- Simon D, Aeberhard C, Erdemoglu Y, Simon HU. Th17 cells and tissue remodeling in atopic and contact dermatitis. *Allergy* 2014;69:125-31.
- Peiser M. Role of Th17 cells in skin inflammation of allergic contact dermatitis. *Clin Develop Immunol* 2013;2013:261037.
- Dyring-Andersen B, Skov L, Løvendør MB, Bzorek M, Søndergaard K, Lauritsen JP, et al. CD4(+) T cells producing interleukin (IL)-17, IL-22 and interferon-γ are major effector T cells in nickel allergy. *Contact Dermatitis* 2013;68:339-47.
- Hayashida S, Uchi H, Takeuchi S, Esaki H, Moroi Y, Furue M, et al. Significant correlation of serum IL-22 levels with CCL17 levels in atopic dermatitis. *J Dermatol Sci* 2011;61:78-9.
- Ungar B, Garcet S, Gonzalez J, Dhingra N, da Rosa JC, Shemer A, et al. An integrated model of atopic dermatitis biomarkers highlights the systemic nature of the disease. *J Invest Dermatol* 2017;137:603-13.
- Nogales KE, Zaba LC, Shemer A, Fuentes-Duculan J, Cardinale I, Kikuchi T, et al. IL-22-producing "T22" T cells account for upregulated IL-22 in atopic dermatitis despite reduced IL-17-producing TH17 T cells. *J Allergy Clin Immunol* 2009;123:1244-52.

24. Nograles KE, Suárez-Fariñas M, Shemer A, Fuentes-Duculan J, Chiricozzi A, Cardinale I, et al. Atopic dermatitis keratinocytes exhibit normal T(H)17 cytokine responses. *J Allergy Clin Immunol* 2010;125:744-6.
25. Boniface K, Bernard FX, Garcia M, Gurney AL, Lecron JC, Morel F, et al. IL-22 inhibits epidermal differentiation and induces proinflammatory gene expression and migration of human keratinocytes. *J Immunol* 2005;174:3695-702.
26. Guttman-Yassky E, Ungar B, Noda S, Suprun M, Shroff A, Dutt R, et al. Extensive alopecia areata is reversed by IL-12/IL-23p40 cytokine antagonism. *J Allergy Clin Immunol* 2016;137:301-4.
27. Hirata T, Narumiya S. Prostanoids as regulators of innate and adaptive immunity. *Adv Immunol* 2012;116:143-74.
28. Honda T, Kabashima K. Prostanoids in allergy. *Allergol Int* 2015;64:11-6.
29. Fogh K, Herlin T, Kragballe K. Eicosanoids in skin of patients with atopic dermatitis: prostaglandin E₂ and leukotriene B₄ are present in biologically active concentrations. *J Allergy Clin Immunol* 1989;83:450-5.
30. Laouini D, Elkhail A, Yalcindag A, Kawamoto S, Oettgen H, Geha RS, et al. COX-2 inhibition enhances the Th2 immune response to epicutaneous sensitization. *J Allergy Clin Immunol* 2005;116:390-6.
31. Chan S, Henderson WR Jr, Li SH, Hanifin JM. Prostaglandin E₂ control of T cell cytokine production is functionally related to the reduced lymphocyte proliferation in atopic dermatitis. *J Allergy Clin Immunol* 1996;97:85-94.
32. Fedyk ER, Phipps RP. Prostaglandin E₂ receptors of the EP2 and EP4 subtypes regulate activation and differentiation of mouse B lymphocytes to IgE-secreting cells. *Proc Natl Acad Sci U S A* 1996;93:10978-83.
33. Yao C, Sakata D, Esaki Y, Li Y, Matsuoka T, Kuroiwa K, et al. Prostaglandin E₂-EP4 signaling promotes immune inflammation through Th1 cell differentiation and Th17 cell expansion. *Nat Med* 2009;15:633-40.
34. Yao C, Hirata T, Soontrapa K, Ma X, Takemori H, Narumiya S. Prostaglandin E₂ promotes Th1 differentiation via synergistic amplification of IL-12 signalling by cAMP and PI3-kinase. *Nat Commun* 2013;4:1685.
35. Duffin R, O'Connor RA, Crittenden S, Forster T, Yu C, Zheng X, et al. Prostaglandin E₂ constrains systemic inflammation through an innate lymphoid cell-IL-22 axis. *Science* 2016;351:1333-8.
36. Cella M, Colonna M. Aryl hydrocarbon receptor: Linking environment to immunity. *Semin Immunol* 2015;27:310-4.
37. Schneider A, Guan Y, Zhang Y, Magnuson MA, Pettepher C, Loftin CD, et al. Generation of a conditional allele of the mouse prostaglandin EP4 receptor. *Genesis* 2004;40:7-14.
38. Guttman-Yassky E, Suárez-Fariñas M, Chiricozzi A, Nograles KE, Shemer A, Fuentes-Duculan J, et al. Broad defects in epidermal cornification in atopic dermatitis identified through genomic analysis. *J Allergy Clin Immunol* 2009;124:1235-44.
39. Suárez-Fariñas M, Tintle SJ, Shemer A, Chiricozzi A, Nograles K, Cardinale I, et al. Nonlesional atopic dermatitis skin is characterized by broad terminal differentiation defects and variable immune abnormalities. *J Allergy Clin Immunol* 2011;127:954-64.
40. Gittler JK, Shemer A, Suárez-Fariñas M, Fuentes-Duculan J, Gulewicz KJ, Wang CQ, et al. Progressive activation of T(H)2/T(H)22 cytokines and selective epidermal proteins characterizes acute and chronic atopic dermatitis. *J Allergy Clin Immunol* 2012;130:1344-54.
41. Jensen JM, Scherer A, Wanke C, Bräutigam M, Bongiovanni S, Letzkus M, et al. Gene expression is differently affected by pimecrolimus and betamethasone in lesional skin of atopic dermatitis. *Allergy* 2012;67:413-23.
42. Tintle S, Shemer A, Suárez-Fariñas M, Fujita H, Gilleaudeau P, Sullivan-Whalen M, et al. Reversal of atopic dermatitis with narrow-band UVB phototherapy and biomarkers for therapeutic response. *J Allergy Clin Immunol* 2011;128:583-93.
43. Cheadle C, Vawter MP, Freed WJ, Becker KG. Analysis of microarray data using z score transformation. *J Mol Diagn* 2003;5:73-81.
44. Rutz S, Noubade R, Eidenschenk C, Ota N, Zeng W, Zheng Y, et al. Transcription factor c-Maf mediates the TGF- β -dependent suppression of IL-22 production in Th17 cells. *Nat Immunol* 2011;12:1238-45.
45. Kabashima K, Sakata D, Nagamachi M, Miyachi Y, Inaba K, Narumiya S. Prostaglandin E₂-EP4 signaling initiates skin immune responses by promoting migration and maturation of Langerhans cells. *Nat Med* 2003;9:744-9.
46. Inada T, Kushida A, Sakamoto S, Taguchi H, Shingu K. Intrathecal betamethasone pain relief in cancer patients with vertebral metastasis: a pilot study. *Acta Anaesthesiol Scand* 2007;51:490-4.
47. Zulfakar MH, Abdelouahab N, Heard CM. Enhanced topical delivery and ex vivo anti-inflammatory activity from a betamethasone dipropionate formulation containing fish oil. *Inflamm Res* 2010;59:23-30.
48. Kurz T, Hoffjan S, Hayes MG, Schneider D, Nicolae R, Heinzmann A, et al. Fine mapping and positional candidate studies on chromosome 5p13 identify multiple asthma susceptibility loci. *J Allergy Clin Immunol* 2006;118:396-402.
49. Hinds DA, McMahon G, Kiefer AK, Do CB, Eriksson N, Evans DM, et al. A genome-wide association meta-analysis of self-reported allergy identifies shared and allergy-specific susceptibility loci. *Nat Genet* 2013;45:907-11.
50. Ewald DA, Noda S, Oliva M, Litman T, Nakajima S, Li X, et al. Major differences between human atopic dermatitis and murine models, as determined by using global transcriptomic profiling. *J Allergy Clin Immunol* 2017;139:562-71.
51. Boniface K, Bak-Jensen KS, Li Y, Blumenschein WM, McGeachy MJ, McClanahan TK, et al. Prostaglandin E₂ regulates Th17 cell differentiation and function through cyclic AMP and EP2/EP4 receptor signaling. *J Exp Med* 2009;206:535-48.
52. Noda S, Suárez-Fariñas M, Ungar B, Kim SJ, de Guzman Strong C, Xu H, et al. The Asian atopic dermatitis phenotype combines features of atopic dermatitis and psoriasis with increased TH17 polarization. *J Allergy Clin Immunol* 2015;136:1254-64.
53. Czarnowicki T, Gonzalez J, Shemer A, Malajian D, Xu H, Zheng X, et al. Severe atopic dermatitis is characterized by selective expansion of circulating TH2/TC2 and TH22/TC22, but not TH17/TC17, cells within the skin-homing T-cell population. *J Allergy Clin Immunol* 2015;136:104-15.
54. Sehra S, Yao Y, Howell MD, Nguyen ET, Kansas GS, Leung DY, et al. IL-4 regulates skin homeostasis and the predisposition toward allergic skin inflammation. *J Immunol* 2010;184:3186-90.
55. Dhingra N, Guttman-Yassky E. A possible role for IL-17A in establishing Th2 inflammation in murine models of atopic dermatitis. *J Invest Dermatol* 2014;134:2071-4.