

CCL2 mitigates cyclic AMP-suppressed Th2 immune response in human dendritic cells

To the Editor,

Allergic rhinitis (AR) is a Th2-type chronic inflammatory disease of the nasal mucosa, characterized by symptoms including rhinorrhea, obstruction, and sneezing. Induction of Th2 cell differentiation requires antigen-presenting cells, particularly dendritic cells (DCs). However, the mechanisms underlying induction of T helper effector cells in AR remain unclear.¹⁻³ A recent report showed that chemokine ligand 2 (CCL2) expression was increased in the bronchoalveolar lavage fluid of ovalbumin-sensitized/challenged

mice,⁴ suggesting that CCL2 plays a critical role in the Th2 response in upper airway allergic disease. Interestingly, in our previous study, when we deleted *Gnas*, encoding *Gαs* in mouse CD11c+ cells (*Gnas*^{ΔCD11c} mice), the resulting decrease in cyclic adenosine monophosphate (cAMP) concentration in CD11c+ cells provoked systemic Th2 polarization and a prominent spontaneous allergic asthma phenotype. Additionally, bone marrow (BM)-derived DCs (BMDCs) from *Gnas*^{ΔCD11c} mice induced a Th2 response when co-cultured with CD4 T cells, partially because of increased CCL2

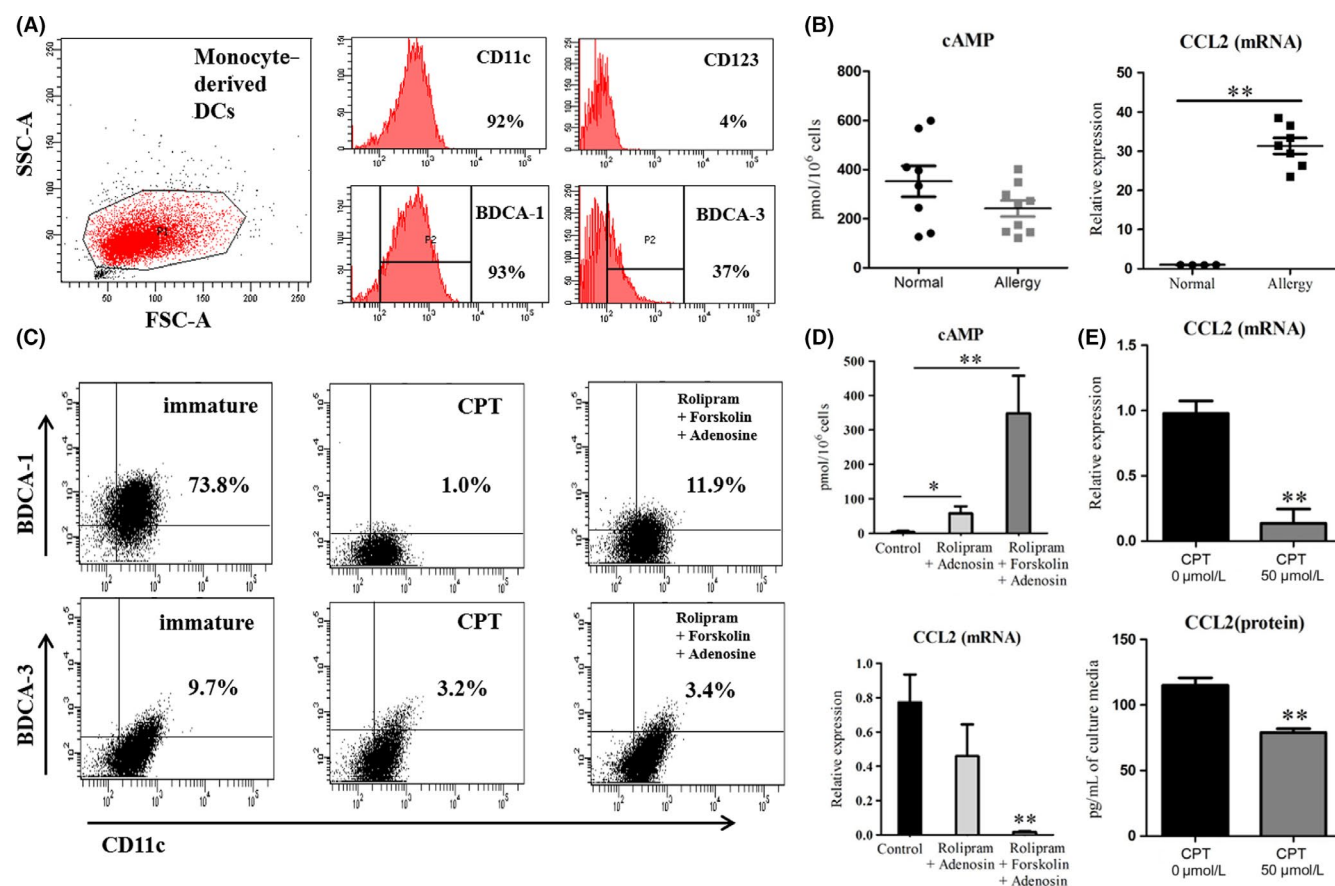


FIGURE 1 Evaluation of cAMP concentration and CCL2 expression in human MDDCs from healthy controls and patients with AR. A, Identification of human MDDCs by FACS analysis. B, The cAMP concentration and CCL2 expression in MDDCs from patients with AR and healthy subjects after stimulation of cAMP by chemical treatment. C, FACS analysis for DC marker expression in CPT-treated or endogenous cAMP-elevated MDDCs. D, Examination of the effects of different endogenous cAMP concentrations on the transcriptional expression of CCL2. E, The effects of CPT on protein and mRNA levels of CCL2. *, *P* value < 0.05; **, *P* value < 0.01

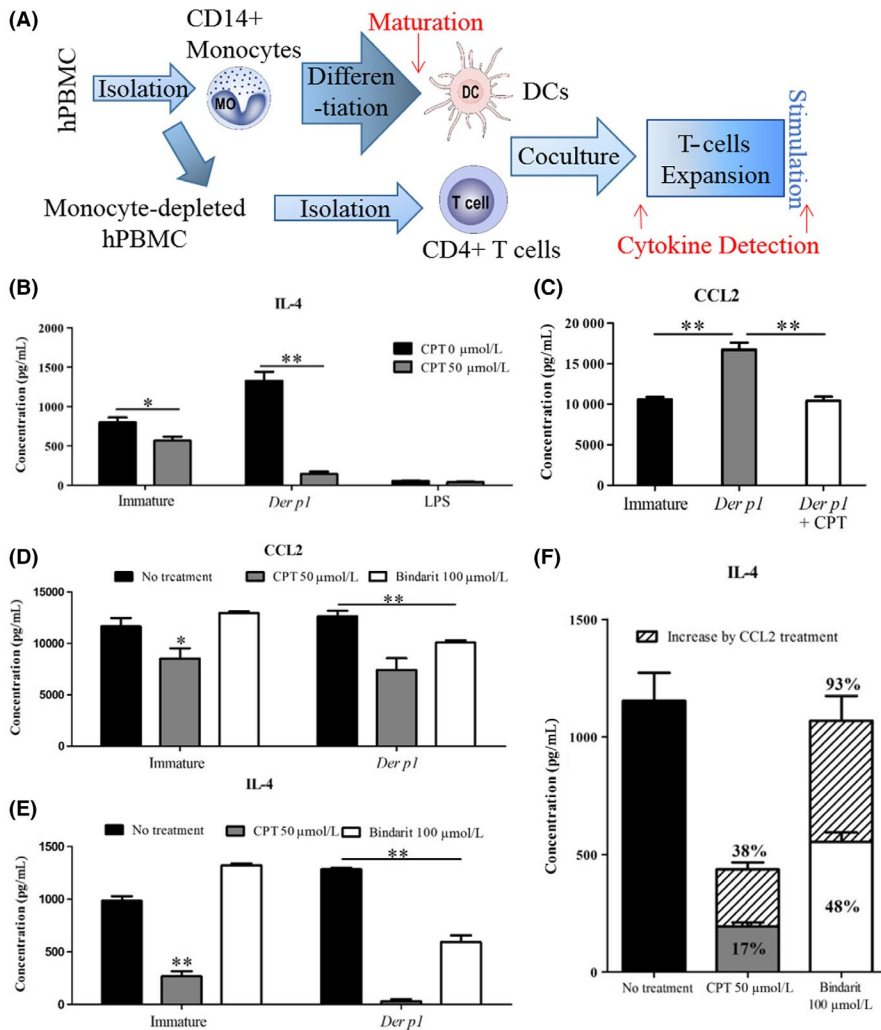


FIGURE 2 Examination of CPT and CCL2 effects on allergic responses in human MDDCs and T-cell coculture. A, Diagram of the MDDCs and T-cell coculture and cytokine detection. B, IL-4 production in coculture with Der p1-, LPS-, and/or CPT-matured MDDCs. C, CCL2 production in coculture with Der p1- and/or CPT-matured MDDCs. Production of (D) CCL2 and (E) IL-4 in coculture with immature or Der p1-matured MDDCs combined with treatment of CPT (50 μM) or bindarit (100 μM). (F) Rescue of the reduced IL-4 production in coculture with Der p1-matured MDDCs combined with treatment of CPT (50 μM) or bindarit (100 μM) by addition of CCL2 (100 ng/mL). *, P value < 0.05; **, P value < 0.01

production.⁵ Thus, we hypothesized that decreased cAMP concentration in DCs induces upper airway allergic responses in the nasal mucosa by enhancing CCL2 expression.

Before investigating our hypothesis in AR patients, we examined it in *Gnas*^{ΔCD11c} mice. In 6-month-old *Gnas*^{ΔCD11c} mice, nasal rubbing and sneezing symptom scores were significantly higher than in 2-month-old *Gnas*^{ΔCD11c} mice and *Cre*-*Gnas*^{fl/fl} control mice (Figure S1A), indicating a spontaneous upper airway allergic phenotype. Histological examination and cytokine analysis confirmed the nasal mucosa allergic response in *Gnas*^{ΔCD11c} mice (Figure S1B-D). These results suggest that the decreased concentration of cAMP in DCs may induce AR.

To compare the transcriptional expression profile of human DCs between individuals with AR and healthy controls, we isolated CD11c-BDCA3⁺ DCs from human peripheral blood mononuclear cells (hPBMCs) by flow cytometry (Figure S2A) and analyzed them by RNA sequencing (Figure S2B). The expression of BDCA-1, a Th2 inflammatory DC marker, was higher in AR DCs than in control DCs (Figure S2C). By comparing the RNA sequencing data to our previous microarray results from *Gnas*^{ΔCD11c} mice,⁵ we found that CCL2 and CCL7 mRNA levels were increased in both humans and mice (Figure S2D).

After obtaining these preliminary data, we examined our hypothesis in vitro. Monocyte-derived dendritic cells (MDDCs) were differentiated and matured from hPBMCs of patients with AR and healthy controls (Table S1). These cells were CD11c⁺ and BDCA-1⁺ myeloid DCs (Figure 1A), the same DC subtype of turbinate tissue that was increased in AR patients (Figure S3). When MDDCs were stimulated with forskolin, rolipram, and adenosine, the cAMP concentration in AR MDDCs tended to be lower than that of control MDDCs, while CCL2 mRNA was significantly higher in AR MDDCs than in control MDDCs (Figure 1B). However, CCL7 mRNA in AR MDDCs was not different from that in control MDDCs (Figure S4), suggesting that decreased cAMP levels in AR DCs induce the Th2 immune response through upregulation of CCL2. To obtain direct evidence of the interaction between cAMP concentration and CCL2 expression, we treated MDDCs with endogenous cAMP elevators (forskolin, adenosine, and rolipram) or the exogenous cAMP analogue 8-(4-chlorophenylthio)-adenosine-3',5'-cyclic monophosphate (CPT). Treating control MDDCs with endogenous cAMP elevators or CPT decreased BDCA-1 expression but not BDCA-3 expression. This suggested that enhanced cAMP levels may modulate Th2 inflammation (Figure 1C). Additionally, the transcription level of CCL2 was inversely correlated with that of endogenous

cAMP (Figure 1D), and both transcription and translation levels of CCL2 were reduced by the exogenous cAMP analogue (Figure 1E). These results suggest that cAMP concentration is critical for CCL2 expression in human DCs.

To investigate the function of these cAMP-regulated DCs, human MDDCs were matured with the allergen Der p 1 or liposaccharide (LPS) and then cocultured with CD4⁺ naïve T cells. The cytokines in coculture media were detected by ELISA (Figure 2A). Increased IL-4 levels were detected in coculture media with Der p 1-matured MDDCs; this effect was mitigated by CPT treatment. IL-4 was not detected in coculture with LPS-matured MDDCs (Figure 2B). In addition, the production patterns of CCL2 and IL-13 were similar to those of IL-4 (Figure 2C; Figure S5). However, in the coculture with Der p 1-matured and CPT-treated MDDCs, the production of IL-10 was decreased while that of IFN- γ was increased, suggesting that the regulation of allergic conditions by cAMP might be also related to the enhancement of Th1 inflammatory response (Figure S5). Treatment with bindarit, a CCL2 inhibitor, reduced the amount of CCL2 and IL-4 only in the coculture with Der p 1-matured MDDCs, but not to the degree observed in the coculture with CPT-treated MDDCs (Figure 2D and E). When recombinant CCL2 was added to coculture media, the reduced amount of IL-4 in bindarit-treated coculture media returned to control levels, while that in the coculture with CPT-treated MDDCs returned to 38% of control levels (Figure 2F). This indicates that the Th2 response regulated by cAMP in DCs is affected partially by CCL2 signaling.

Our findings suggest that decreased cAMP levels in DCs induce Th2 differentiation and consequently elicit chronic allergic responses in the upper airways of Th2-biased *Gnas* ^{Δ CD11c} mice. In addition, we demonstrated regulation of CCL2 expression by cAMP concentration in human MDDCs, and the allergy promoting effect of CCL2 on T-cell differentiation in human MDDCs cocultured with T cells. Regulation of CCL2 expression by intracellular cAMP concentration in several cell types has been reported previously.^{6,7} However, here, CCL2 treatment resulted in partial recovery of the reduced cAMP-mediated Th2 response and complete recovery of the reduced Th2 response mediated by the CCL2 inhibitor. This result is logical considering the multiple cAMP-related factors in DC involved in biased Th response.⁵ Generally, the mechanisms of cAMP affecting T-cell functions include several effector pathways such as protein kinase A and exchange protein activated by cAMP and diverse regulators such as phosphodiesterases and adenylyl cyclases.⁸ These mechanisms are also mediated by chemokines including CCL2 and enzymes such as hematopoietic prostaglandin D synthases.⁵ Thus, CCL2 probably exerted a partial role in enhancing the Th2 response in cAMP-controlled MDDCs.

In conclusion, our findings demonstrate that local allergic responses in the nasal mucosa can be induced by decreased cAMP concentration in the DCs of patients with AR and are partially regulated through CCL2 expression.

KEYWORDS

allergic rhinitis, cAMP, CCL2, dendritic cells, *Gnas* ^{Δ CD11c} mice

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

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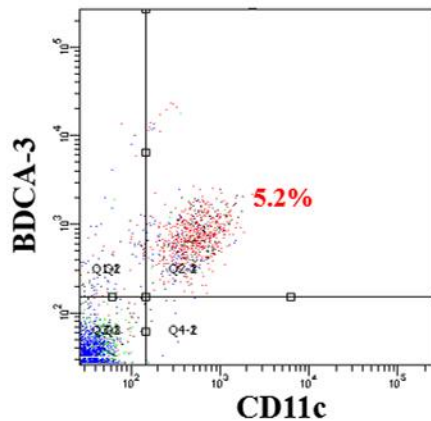
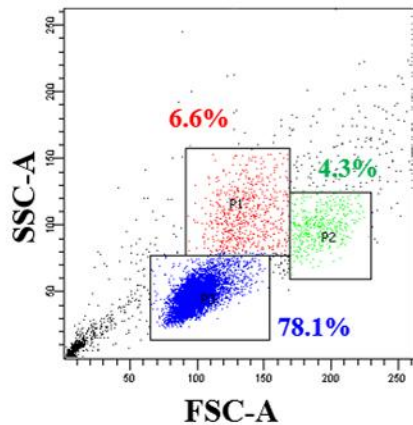
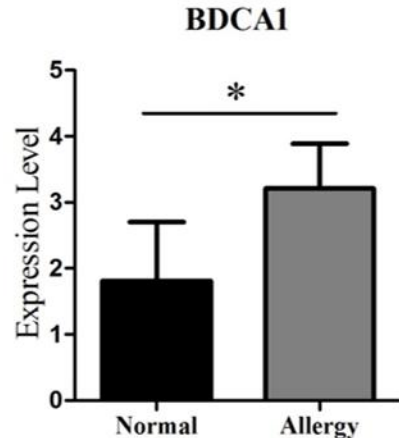
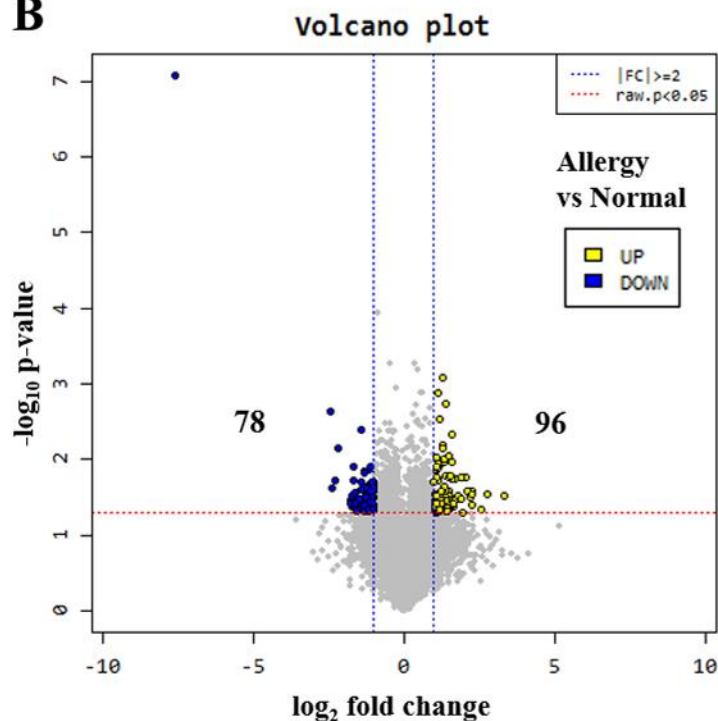
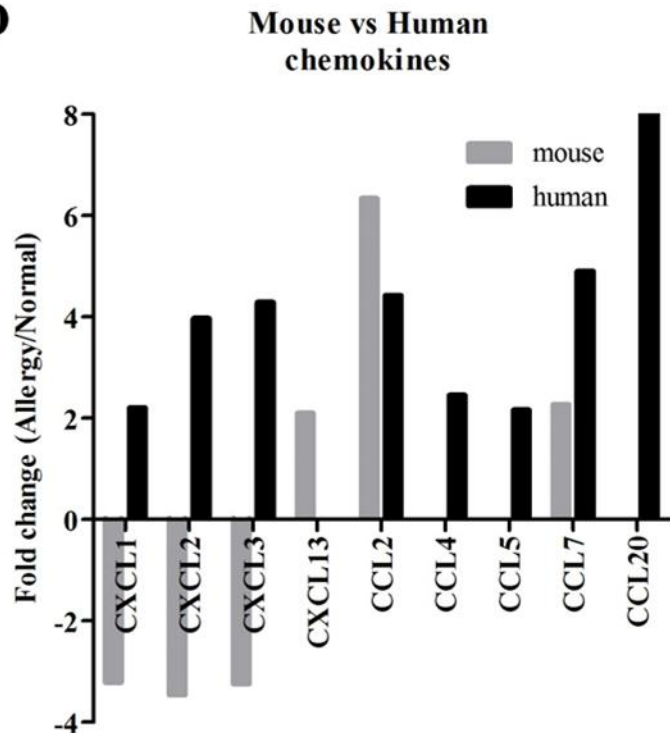
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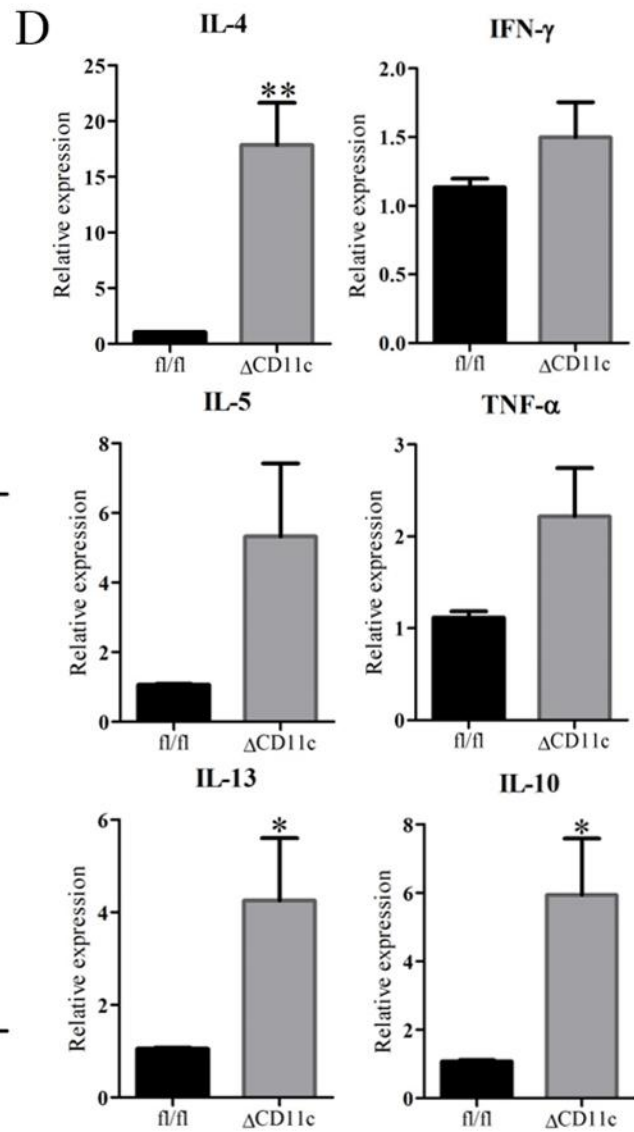
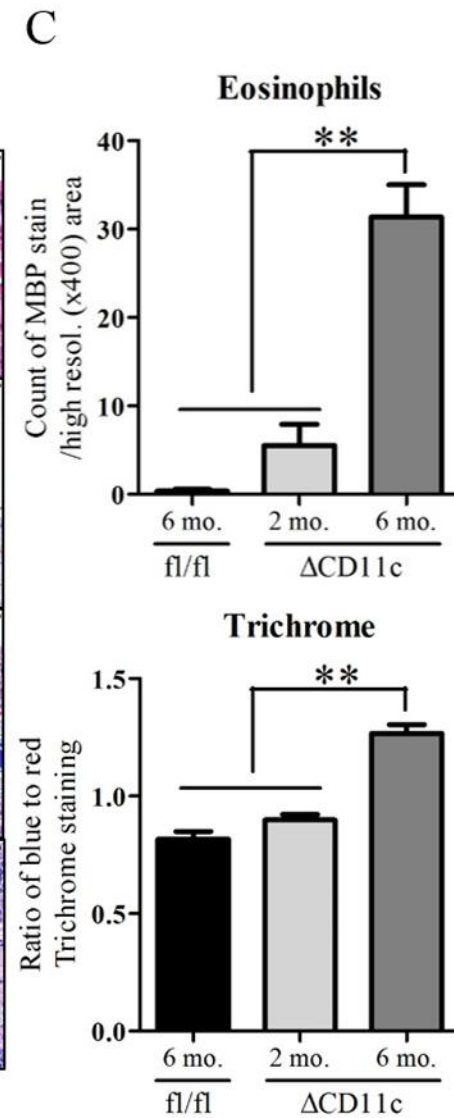
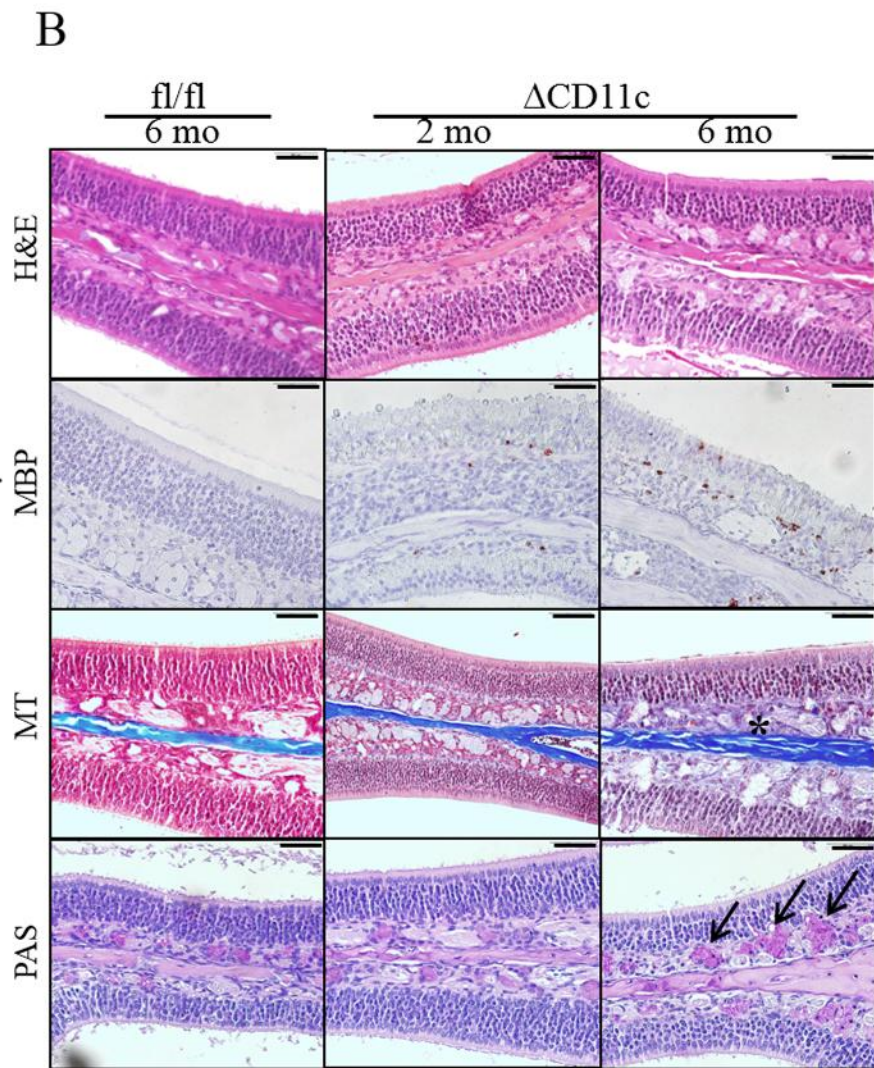
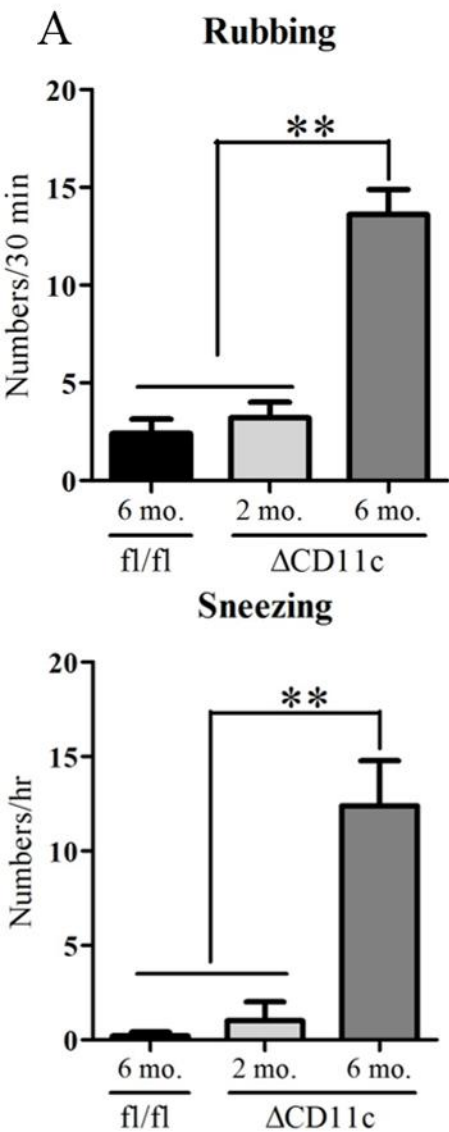
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SUPPORTING INFORMATION

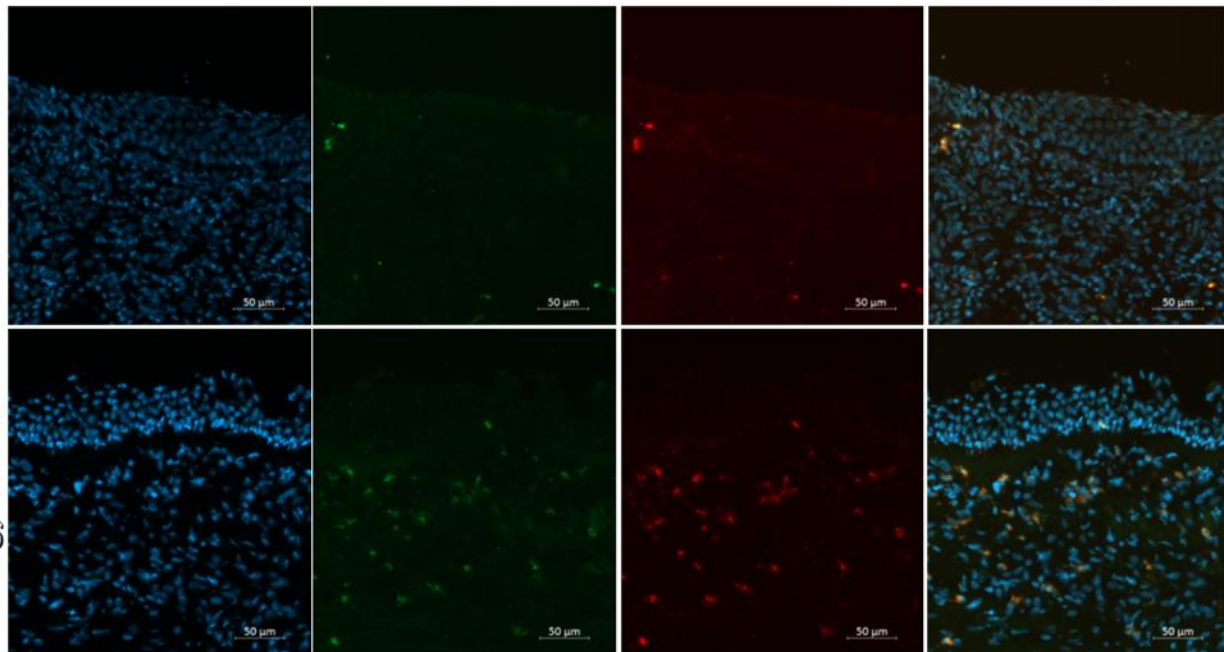
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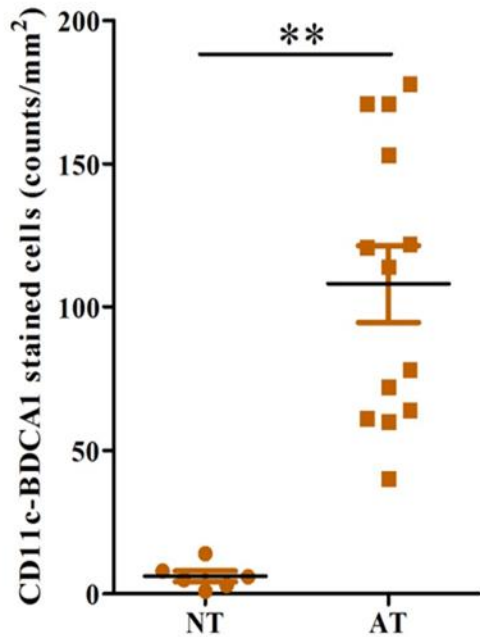


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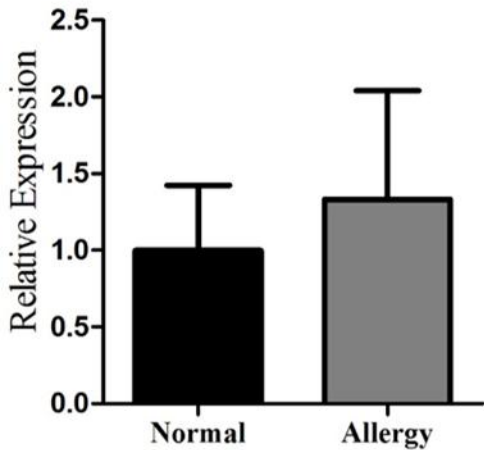
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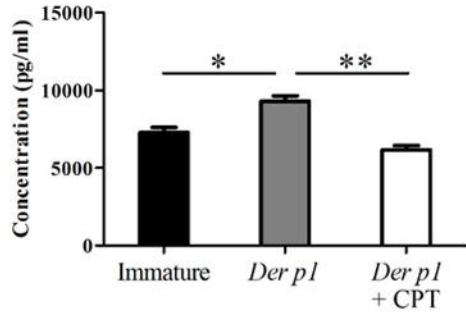
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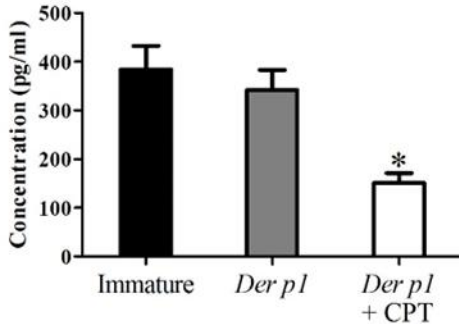
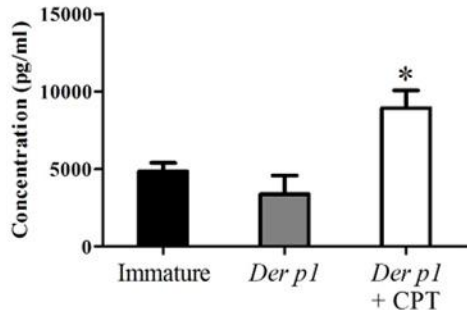
CCL7



IL-13



IL-10

IFN- γ 

Materials and methods

Animals Experiments

Gnas^{ΔCD11c} mice with a targeted deletion of *Gnas* in CD11c⁺ cells were gifted by Dr. Eyal Raz (Department of Medicine, University of California, San Diego, La Jolla, CA, USA).¹ The Cre⁻*Gnas*^{fl/fl} littermates were used as a control. All mice were kept in an SPF facility.

Symptom score

A blinded observer recorded the frequencies of nasal rubbing for 30 min and sneezing for 1 h of 2-month-old or 6-month-old *Gnas*^{ΔCD11c} mice and 6-month-old Cre⁻*Gnas*^{fl/fl} negative control mice. Sneezing was recorded over a longer period than nasal rubbing because it occurred intensively for a short time (< 5 min) and needed to be averaged by the extended time span.

Histology of the nasal mucosae of *Gnas*^{ΔCD11c} mice

Nasal mucosae of 2- and 6-month-old *Gnas*^{ΔCD11c} mice and 6-month-old Cre⁻*Gnas*^{fl/fl} negative control mice were decalcified, embedded in paraffin, and sectioned coronally (5 μm thick) approximately 5 mm from the nasal vestibule. Sections were stained with hematoxylin-eosin for morphological inspection, periodic acid Schiff (PAS) to stain the mucus-containing area, and Masson's trichrome (MT) to stain for subepithelial collagen. To detect eosinophils, we performed immunostaining using antibodies against the major basic protein (MBP) (Santa Cruz Biotechnology, USA). Slides were examined with an Olympus BX51 microscope, and pictures were captured using an Olympus DP72 camera with DP2-BSW software.

Cytokine profile of the nasal mucosae of *Gnas*^{ΔCD11c} mice

To measure immunologic cytokine expression, nasal mucosae of 6-month-old *Gnas*^{ACD11c} mice and 6-month-old Cre⁻*Gnas*^{fl/fl} negative control mice were harvested, and transcriptional levels of cytokines were analyzed by real-time PCR.

Patients characteristics

We enrolled 30 patients with AR and 30 healthy controls in our study. All patients with AR had typical symptoms of perennial nasal allergy, nasal eosinophilia, and a positive skin test or positive RAST to house dust mites, as well as positive reactions on provocation tests. Moreover, serum total IgE was determined by an enzyme-linked immunosorbent assay (ELISA) (CAP system). The detectable range of the assay for total IgE was 2–2000 IU/mL. Allergic patients with exacerbated upper respiratory tract infections during the 6 weeks preceding the study were not included. Other exclusion criteria included smoking, a medical history of asthma, recurrent infections of the respiratory tract, and use of topical or oral corticosteroids, leukotriene modifiers, or antihistamines during the 8 weeks that preceded the study. Clinical data of patients, including serum total IgE, are summarized in Table 1.

Cell preparation

Peripheral blood mononuclear cells (PBMCs)

The venous blood from patients with AR or healthy controls was collected directly into BD Vacutainer CPT (BD biosciences, Franklin Lakes, NJ, USA) containing 0.1 M sodium citrate, and human PBMCs (hPBMCs) were isolated following the manufacturer's instruction. Briefly, right after blood collection with the Vacutainer CPT, the tubes were centrifuged at $1500 \times g$ for 20 min at room temperature (RT) in a swinging-bucket rotor. Subsequently, the hPBMC layer was gently transferred to 15-mL conical tubes and washed with PBS by centrifugation at $300 \times g$ for 15 min. Precipitated hPBMCs were resuspended in

RPMI 1640 (WELGENE, Gyeongsan, Korea) containing 10% fetal bovine serum (FBS), penicillin/streptomycin, and 2 mM glutamine, and cultured for subsequent experiments or stored in liquid nitrogen for later. Prior to the blood collection, the protocol and informed consent form were approved by the Institutional Review Board for Human Studies at Korea University Hospital, and all participants provided written informed consent.

Monocyte-derived dendritic cells (MDDCs)

To prepare MDDCs, monocytes were isolated from hPBMCs with the EasySep™ Human CD14 positive selection kit II (STEMCELL technologies, Vancouver, Canada). The hPBMC suspension was mixed with 100 µL of EasySep™ Human CD14 positive selection cocktail. After incubation for 10 min at RT, the cell suspension was mixed with 100 µL of EasySep™ Dextran RapidSheres™ and incubated for 3 min at RT. After placing the cell suspension tube into the magnet (STEMCELL Technologies) for 3 min, the monocytes were collected and cultured in RPMI 1640 containing GM-CSF and IL-4 (50 ng/mL and 40 ng/mL, respectively; PeproTech, Rocky Hill, NJ, USA). At day 3, the cells were replenished with RPMI 1640 containing GM-CSF and IL-4 and cultured for 3 days into immature DCs. To mature the immature DCs, LPS (1 µg/mL, Sigma-Aldrich) or Der p1 (2 µg/mL, Indoor Biotechnologies, Charlottesville, VA, USA) was added into the media at day 6, and cells were cultured for 24 h. Mature DCs were analyzed by flow cytometry.

Naïve T cells

Human naïve T cells were isolated from hPBMCs with the EasySep™ Human Naïve CD4⁺ T cell isolation kit (STEMCELL technologies). The hPBMC suspension was mixed with 50 µL of EasySep™ biotinylated anti-CD45RO antibody and 50 µL of EasySep™ Human naïve CD4⁺ T cell isolation cocktail. After incubation for 5 min at RT, the cell

suspension was mixed with 50 μ L of EasySepTM Dextran RapidSheresTM and incubated for 5 min at RT. After placing the cell suspension tube into the magnet (STEMCELL technologies) for 5 min, the human naïve CD4⁺ T cells were collected and cultured in RPMI 1640.

Flow cytometry

The hPBMCs were washed with staining buffer by centrifugation at 300 $\times$ g for 5 min. The washed hPBMCs were stained with BDCA-3/CD141-PE Mouse IgG1 (Clone 501733), BDCA-1/CD1c-APC Goat IgG, and CD11c-CFS Mouse IgG1 (Clone ICRF 3.9) with the multi-color flow cytometry kit (R&D systems, Inc., Minneapolis, MN, USA) for human myeloid DCs, and IL-3R α /CD123-PerCP mouse IgG1 (Clone 32703, R&D systems, Inc.) for human plasmacytoid DCs. The cells were incubated with each antibody or each corresponding isotype control antibody for 45 min at RT in the dark. After washing with the staining buffer, stained cells were applied to the FACS Aria III cell sorter (BD Biosciences) for RNA sequencing of DCs or to FACSCanto II (BD Biosciences) for analysis of MDDCs. The results were analyzed on a FACSDiva (BD Biosciences). With forward (FSC) and side light scattering (SSC), viable cells were detected, and cell debris were excluded from the analysis. DC subsets stained with anti-BDCA-3 and CD11c antibodies were gated and sorted. The sorted DCs were lysed with QIAzol lysis reagent (QIAGEN, Hilden, Germany) and applied to mRNA sequencing.

Next-generation sequencing (NGS) analysis

Total RNAs from 3 allergic DCs and 3 non-allergic control DCs were isolated and quality controlled. The preparation of whole transcriptome libraries and sequencing were performed by Macrogen, Inc. (Seoul, Korea). The total RNA concentration was measured using Quant-IT RiboGreen (Invitrogen, Carlsbad, CA, USA). The total RNA was verified on the

TapeStation RNA screentape (Agilent Technologies, Santa Clara, CA, USA). Only high-quality RNA samples with RNA integrity number (RIN) over 7.0 were used for RNA library construction. Total RNA (1 µg) was used as input material for library construction. After removal of ribosomal RNA, the RNA fragments underwent reverse transcription using random primers, and 100 nt paired-end sequencing was performed by the Illumina HiSeq2500. The libraries were quantified using qPCR according to the qPCR Quantification Protocol Guide (KAPA BIOSYSTEMS, Wilmington, MA, USA) and qualified using an Agilent Technologies 2100 Bioanalyzer (Agilent Technologies).

The raw reads from the sequencer were preprocessed to remove low quality and adapter sequences, and the processed reads were aligned to the *Homo sapiens* (hg38) using HISAT v2.0.5(1). The reference genome sequence of *Homo sapiens* (hg19) and annotation data were downloaded from the UCSC table browser (<http://genome.uscs.edu>). After alignment, StringTie v1.3.3b was used to assemble aligned reads into transcripts and to estimate their abundance, which provided the relative abundance estimates as fragments per kilobase of exon per million (FPKM) mapped of the transcript and gene expressed in each sample. Normalized FPKM values were used for comparative analysis of differentially expressed genes between samples.

ELISA

Competitive ELISA for cAMP

To measure the concentration of cAMP in DCs from allergic and normal subjects using a cAMP competitive ELISA kit (Thermo Scientific), DCs were treated with a phosphodiesterase (PDE) inhibitor (25 µM rolipram, Sigma-Aldrich) for 30 min, then an adenylyl cyclase (AC) activator (10 µM forskolin, Sigma-Aldrich) and AC substrate (10 µM adenosine, Sigma-Aldrich) for 30 min at 37°C to promote the accumulation of cAMP. To

assess the dependence of CCL2 expression levels on the concentration of cAMP, normal DCs were treated with rolipram and adenosine, but not with forskolin, to moderately increase cAMP levels. For the control, DCs were not treated with any chemicals and incubated for 1 h in a 37°C incubator with 5% CO₂. Chemically treated and non-treated DCs were lysed with HCl, and then the supernatant was analyzed by ELISA. The cell lysates were neutralized, competed with cAMP-alkaline phosphatase conjugate, bound with cAMP antibody, and reacted with p-NPP substrate for color development according to the manufacturer's instructions. After stopping the reaction, the optical density was read at 405 nm. The data were reported as pmol of cAMP per million cells.

Sandwich ELISA for cytokines

To measure the cytokine concentration, medium from the MDDCs-T cell co-culture was applied to the antibody-coated wells. Following incubation at RT, cytokines bound with biotinylated detection antibody forming sandwich and unbound substances were removed by washing. After conjugating avidin-horseradish peroxidase, substrate solution was added and absorbance was measured at 450 nm. Detailed time and concentration for each cytokine ELISA were determined per manufacturer's instructions. ELISA kits for IL-4 (Human Instant ELISA™ Kit, eBioscience, Vienna, Austria), IL-10 and CCL2 (Human ELISA MAX™ Deluxe Sets, BioLegend, Inc., San Diego, CA, USA), and IL-13 and INF-γ (Human LEGEND MAX™ ELISA Kit, BioLegend, Inc.) were used.

Quantitative real-time polymerase chain reaction

Sample preparation

To extract mRNA, DCs from allergic and normal individuals or chemically treated DCs were harvested, resuspended in QIAzol lysis reagent (QIAGEN), and incubated for 5 min at

RT. Mouse nasal mucosa were also lysed with QIAzol reagent and incubated for 5 min at RT. The lysed suspension was mixed with chloroform, and the mixture was shaken briefly and then incubated for 3 min at RT. After centrifugation at 14,000 rpm for 15 min at 4°C, the mRNA was enriched in the upper transparent layer. The upper layer was mixed with isopropanol by inversion and incubated for 5 min on ice. After centrifugation at 14,000 rpm for 10 min at 4°C, precipitates were washed with 75% ethanol in DEPC water and centrifuged at 14,000 rpm for 5 min at 4°C. Then, the precipitates with mRNA were dried for 5 min and resuspended in DEPC water. The cDNA was synthesized from mRNA using a cDNA synthesis master mix (GenDEPOT, Houston, TX, USA).

PCR conditions

Gene expressions in DCs or mouse tissue were measured by quantitative real-time PCR. The prepared cDNA was amplified and quantified using SYBR green master mix (Qiagen) with the following primers: human CCL2, forward 5'-CCC CAG TCA CCT GCT GTT AT-3' and reverse 5'-GAG TTT GGG TTT GCT TGT CC-3'; human CCL7, forward 5'-GAC AAG AAA ACC CAA ACT CCA AAG-3' and reverse 5'-TCA AAA CCC ACC AAA ATC CA-3'; human GAPDH, forward 5'-GAG TCA ACG GAT TTG GTC GT-3' and reverse 5'-TTG ATT TTG GAG GGA TCT CG-3'; mouse IL-4, forward 5'-TCA ACC CCC AGC TAG TTG TC-3' and reverse 5'-TGT TCT TCG TTG CTG TGA GG-3'; mouse IL-5, forward 5'-GAA GTG TGG CGA GGA GAG AC-3' and reverse 5'-GCA CAG TTT TGT GGG GTT TT-3'; mouse IL-13, forward 5'-ATG GCG CTC TGG GTG ACT GCA GTC CTG-3' and reverse 5'-TTA GAA GGG GCC GTG GCG AAA CAG TTG-3'; mouse IFN- γ , forward 5'-ACT GGC AAA AGG ATG GTG AC-3' and reverse 5'-TGA GCT CAT TGA ATG CTT GG-3'; mouse TNF- α , forward 5'-ACA GAA AGC ATG ATC GGC G-3' and reverse 5'-GCC CCC CAT CTT TTG

GG-3'; mouse IL-10, forward 5'-CCA AGC CTT ATC GGA AAT GA-3' and reverse 5'-TTT TCA CAG GGG AGA AAT CG-3'; mouse GAPDH, forward 5'-CAT CCA CTG GTG CTG CCA AGG CTG T-3' and reverse 5'-ACA ACC TGG TCC TCA GTG TAG CCC A-3'. PCRs were performed using a real-time thermal cycler system (TP800/TP860, Takara, Kusatsu, Japan) with 40 cycles of a 2-step reaction consisting of denaturation at 95°C for 15 s, followed by annealing/extension at 60°C for 45 s. Data were analyzed using the $2^{-\Delta\Delta C_t}$ method.

Co-culture with MDDCs and T cells

To examine the effect of cAMP controlled DCs on differentiation of T cells, the immature MDDCs or Der p1-matured MDDCs were treated with 8-(4-Chlorophenylthio)adenosine 3',5'-cyclic monophosphate (CPT, 50 μ M; Sigma-Aldrich, St. Louis, MO, USA), Bindarit (CCL2 inhibitor, 100 μ M; Abcam, Cambridge, UK), or CCL2 (100 ng/mL, PeproTech), and then the MDDCs were co-cultured with autologous CD4⁺ naïve T cells. In 96-well plates, 5×10^4 MDDCs and 2.5×10^5 T cells were co-cultured in 250 μ L culture media for 4 days, and half of the media were refreshed with IL-2 (100 U/mL, PeproTech) containing media. After incubating for 2 days, the T cells in a well were split into 2 wells, and 125 μ L of culture media (IL-2, 50 U/mL) was added to each well. On every other day, half of the culture media was changed with media containing IL-2 (50 U/mL). At day 10, the T cells were stimulated with media containing CD28 antibody (10 μ g/mL, BioLegend) in wells coated with CD3 antibody (10 μ g/mL, BioLegend) for 24 h. Each time the media was refreshed, the culture media was collected for cytokine measurements by ELISA.

Statistical analysis

Statistical analyses were carried out using SPSS for Windows (version 16.0.0; SPSS, Chicago, IL, USA). Data in graphs are expressed as the means $\pm$ SE of at least three independent experiments. The significance of differences between normal and allergic samples was determined using the Student's *t*-test, while other experimental results were analyzed using one-way ANOVA. Differences with p-values <0.05 (*) or <0.01 (**) were considered significant.

REFERENCES

1. Lee J, Kim TH, Murray F, et al. Cyclic AMP concentrations in dendritic cells induce and regulate Th2 immunity and allergic asthma. *Proc Natl Acad Sci U S A*. 2015;112(5):1529-1534.

Supplementary Information

Table S1. Clinical characteristics of the study participants.

	Healthy nasal mucosa	Allergic rhinitis
Characteristics	(n =30)	(n =30)
Female/Male	10/20	9/21
Age (years)	35.2±7.1	35.7±7.3
Rhinitis duration (years)	-	11.5±1.1
Serum total IgE (IU/mL)	31.2±11.3	534.5±194.4
Serum <i>Der p</i> IgE (IU/mL)	0	93.6±9.1

Figure S1. Enhanced allergic symptoms and allergic inflammation in the nasal airway of *Gnas*^{aCD11c} mice.

A) Rubbing and sneezing symptom scores. **, *p* value < 0.01. B) and C) Histological examination of nasal mucosa. Nasal mucosa of a Cre⁻*Gnas*^{fl/fl} control mouse, a two-month-old *Gnas*^{aCD11c} mouse, and a six-month-old *Gnas*^{aCD11c} mouse were examined by hematoxylin and eosin staining, MBP immunohistochemical staining for eosinophil detection, Masson's trichrome staining (MT) for collagen, and Periodic Acid Schiff staining (PAS) for mucus. Number of eosinophils and intensity of collagen staining are increased. Black arrows

in PAS staining indicate infiltration of the mucus into the epithelial layer. Scale bar, 50 μ m. **, p value < 0.01. D) Cytokine profile of the nasal mucosa. Transcriptional expression of Th2 cytokines (IL-4, IL-5, and IL-13), Th1 cytokines (IFN- γ and TNF- α), and Treg cytokine (IL-10) in the nasal lavage fluid. *, p value < 0.05; **, p value < 0.01.

Figure S2. RNA analysis of isolated CD11c+BDCA3+ populations from human peripheral blood mononuclear cells (hPBMCs). Dendritic cells (DCs) were sorted from hPBMCs of healthy controls and patients with AR and analyzed by RNA sequencing. A) Gating of CD11c+BDCA3+ populations (red dots) in the FACS analysis. B) Volcano plot of gene expression levels in AR and healthy control DCs. C) Transcription level of the myeloid DC marker BDCA1. *, p value < 0.05. D) Comparative analysis of chemokine mRNA levels between mouse BMDCs and human DCs. CCL2 was highly expressed in both groups.

Figure S3. Detection of dendritic cells (DCs) in human turbinate. A) Human turbinates from healthy controls and patients with AR were stained with BDCA1 and CD11c antibodies. BDCA1+CD11c+ DCs were increased in the AR turbinate. B) Graphical analysis of BDCA1+CD11c+ DCs in turbinates. **, p value < 0.01.

Figure S4. Evaluation of CCL7 expression in human MDDCs from healthy controls and patients with AR. The transcriptional expression of CCL7 in MDDCs from patients with AR and healthy subjects.

Figure S5. Evaluation of cytokines in the co-culture medium of human MDDCs and T cells. Detection of IL-13, IL-10, and IFN- γ in co-culture media by ELISA. *, p value < 0.05; **, p value < 0.01.

