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In vivo edited eosinophils reconcile antigen specific Th2 response and mitigate airway allergy

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Abstract

Background Improvement is needed in the remedies used to control Th2 polarization. Bioengineering approaches have modified immune cells that have immunosuppressive functions. This study aims to generate modified eosinophils (Meos) in vivo and use Meos to balance Th2 polarization and reduce airway allergy.

Methods A cell editor was constructed. The editor contained a peptide carrier, an anti-siglec F antibody, MHC II, ovalbumin, and LgDNA (DNA extracted from a probiotic, *Lactobacillus rhamnosus GG*). Which was designated as Cedit. Meos are eosinophils modified using Cedits. An airway Th2 polarization mouse model was established used to test the effect of Meos on suppressing airway allergy.

Results The Cedits remained physically and chemically stable in solution (pH7.2) for at least 96 h. Cedits specifically bound to eosinophils, which are designated as Meos. Meos produced programmed death ligand-1 (PD-L1); the latter induced antigen specific $CD4^+$ T cell apoptosis. Administration of Cedits through nasal instillations generated Meos in vivo, which significantly reduced the frequency of antigen specific $CD4^+$ T cells in the airways, and mitigated airway Th2 polarization.

Conclusions We constructed Cedit, which could edit eosinophils into Meos in vivo. Meos could induce antigen specific $CD4^+$ T cell apoptosis, and reconcile airway Th2 polarization.

Keywords Airway, Th2, Eosinophil, Immune regulation, Biomedicine

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Introduction

Th2 polarization indicates that T cells become activated Th2 cells which produced Th2 cytokines [1]. Aggregation of Th2 cells in tissues is a secondary event to Th2 activation and polarization. Consequently, the local tissues are overloaded with Th2 cytokines. The higher concentrations of Th2 cytokines may enhance the production of IgE by plasma cells [2]. IgE makes mast cells sensitized by binding to the IgE high affinity receptors on the cell surface [3]. Sensitized mast cells release significant allergic mediators upon re-exposure to specific antigens. Consequently, allergic reactions are initiated or inflammation reactions are triggered [3]. Furthermore, Th2 polarization is also linked to numerous other immune disorders. For example, rheumatoid arthritis is associated with skewed Th2 polarization in the local tissues [4]. Remedies for reconciling Th2 polarization are currently limited [5]. It is necessary to develop new remedies to regulate Th2 polarization.

Antigen-specific $CD4^+$ T cells express the T cell receptor (TCR) on the cell surface. Thus, the complex of specific antigen and MHC II can specifically bind to or to be bound by the antigen specific $CD4^+$ T cells [6]. Prompted by the event of chimeric antigen receptor T cells (CAR-T cells) [7], we designed and generated the modified eosinophils (Meos) using an in vivo editor designated as Cedit (Chimeric editor). Programmed death ligand-1 (PD-L1) can be produced by LgDNA (DNA extracted from a probiotic, *Lactobacillus rhamnosus GG*) activation of the Meos. The PD-L1 initiated the apoptosis in antigen-specific $CD4^+$ T cells. As a result, experimental airway Th2 polarization and airway allergy were mitigated.

Materials and methods

Reagents

MHC II protein, antibodies (Abs) of H3K9 (Cat#ab8898) and H3 (ab1791) were purchased from abcam (Cambridge, MA). Abs of CD4 (sc-19641; fluorochrome: AF488), CD3 (sc-20047, AF790), IL-4 (sc-32242, AF700), CD11c (sc-1185, AF546), F4/80 (sc-52664, AF648), CD19 (sc-390244, AF594), CD44 (sc-53068, AF790), OVA (sc-65984) and MHC II (sc-59318) were purchased from Santa Cruz Biotech (Santa Cruz, CA). PD1/PD-L1-IN-3 was purchased from Xinyan Bomei Biotech (Xiaan, China). MHC II ELISA kit was purchased from Jianglai Biotech (Shanghai, China). Annexin V kit, OVA (the endotoxin level is less than 0.1 EU/ml as tested using a Thermo Scientific Pierce LAL endotoxin kit), alum, propidium iodide, FITC-labeling kit were purchased from Sigma Aldrich (St. Louis., MO). ELISA kits of EPX (Detection range: 1.25-80 ng/ml. Kemiao Biotech, Wenzhou, China), Ki-67 (Kemiao Biotech, Wenzhou, China. Detection range: 0.312 ng/ml-20 ng/ml), Mcpt1 (Detection range: 78.125-5000pg/ml. MultiSciences, Hangzhou, China), IL-4 (Detection range: 15.6pg/ml~1000pg/ml. MultiSciences, Hangzhou, China), IL-5 (Detection range: 12.8 pg/ml ~ 1000 pg/ml. MultiSciences, Hangzhou, China), IL-13 (Detection range: 7.81 pg/ml −500 pg/ml. MultiSciences, Hangzhou, China), OVA-specific IgE (Detection range: 2.5 ng/ml −700 ng/ml. AmyJet Biotech, Wuhan, China), siglec F (Detection range: 39.1–2500 pg/ml. NovoProtein Inc., Suzhou, China), PD1 (0.094–10 ng/ml. Chuntest BioMart, Shanghai, China), and PD-L1 (Detection range: 156.3-10000 pg/ml. Chuntest BioMart, Shanghai, China).

Mice

The Experimental Animal Center of Guangdong Province in Fushan City provided male BALB/c mice aged 6–8 weeks. Jackson Laboratory (Bar Harbor, ME) provided DO11.10 mice for purchase. Mice were kept in an animal facility that was free of pathogens. Mice were given free access to food and water. The Ethics Committee at Shenzhen University approved the animal experiments with an approval number of 2,023,008. The ARRIVAL Guidelines were followed when conducting the present study.

Preparation of a peptide carrier

The peptide carrier sequence was taken from Wang's report [8], and modified by inserting three proline amino acids into the original sequence to enhance its adhesive ability. The sequence is "Cys-Trp-Pro-Trp-Arg_8-Pro-Cys-Arg_8-Pro-Cys-Arg_8-Cys" and synthesized by Sangon Biotech (Shanghai, China).

The components of Cedit

The Cedit consists of a peptide carrier, an anti-siglec F (Sialic acid-binding, immunoglobulin-like lectin F, siglecf, in short) Ab, MHC II, ovalbumin (OVA), and LgDNA (DNA was extracted from probiotic *Lactobacillus rhamnosus GG*).

Preparation of the cedit

Following the published strategy [8], the Cedits were prepared by mixing the 200 μg/ml of the peptide carrier solution with 100 μg/ml of OVA, 100 μg/ml of MHC II protein, and 50 μg LgDNA. The mixture was incubated for 2 h at room temperature to cross-link disulfides. The mixture's temperature was increased from 4 °C to 95 °C, and then lowered back to 4 °C. The samples were exposed to vibrations at 300 rpm for 5 min. Published procedures [9] were utilized to determine the diameter and size distribution of Cedits through dynamic light scattering (DLS).

Scanning electron microscopy

The Cedits were coated with Pd-Au, and then a scanning electron microscope (TESCAN, Czech Republic) was used to observe and photograph them.

Assessment of the stability of Cedit

Cedit was added to PBS (pH7.2), or SDS solution, or urea solution at a concentration of 1 μg/ml. Samples were taken at 24, 48, and 96 h later. ELISA and DNA spectrometry were used to quantify the components of Cedit.

Preparation of Meos in the in vivo editing approach

Naive mice were treated with nasal instillations containing FITC-Cedit (5 mg/ml). One day later, the mice were sacrificed. Single cells were prepared from the airway tissues, and analyzed by flow cytometry. The $FITC^+$ eosinophils are designated as Meos.

Assessment of the binding between Meos to antigen specific CD4+ T cells

FITC-Meos were isolated from mice described above. $CD4^+$ T cells were isolated from the spleen of DO11.10 mice and BALB/c mice. The two types of cells were mixed in culture medium at a ratio of 1:5 (Meo: T cell). The cells were analyzed by flow cytometry half an hour later. Alternatively, DO11.10 mice and BALB/c mice were treated with nasal instillation containing Cedit. The mice were sacrificed three hours later. Single cells were prepared from the airway tissues of mice, and analyzed by flow cytometry and confocal microscopy.

Confocal microscopy

FITC-Meos cells were mixed with $CD4^+$ T cells (isolated from the spleen of DO11.10 mice and BALB/c mice) in culture medium at a ratio 1:5. Half an hour later, the cells were stained with CD4 Ab and DAPI, and analyzed by confocal microscopy. Isotype IgG was used to stain negative controls.

Flow cytometry

Cells were labeled with fluorescence labeled Abs of interest (Ab types are indicated in the figures) or isotype IgG (negative control). To count apoptotic cells, cells were also stained with Annexin V reagents. For intracellular staining, cells were fixed with 1% paraformaldehyde (containing 0.05% Triton X-100) for 1 h prior to staining. The data were processed using a Flowjo software. Isotype IgG staining data was utilized as gating references.

Real-time quantitative RT-PCR (RT-qPCR)

Cells that were harvested from relevant experiments were used to extract RNA. cDNA was synthesized using a reverse transcription kit (Qiagen) based on the protocol provided by the manufacturer. A SYBR green master mix kit was used to amplify the cDNA using a qPCR device (CFX96, Bio-Rad). The primers used in the study include *Kdm5a* (cctggcagtaggagcaaaag and cgaccacaaaacatgcaaac), *Pdl1* (tgctgcataatcagctacgg and gctggtcacattgagaagca), *PDL1* (cgaagtcatctggacaagca and atttggaggatgtgccagag). The $2^{-\Delta\Delta Ct}$ formula was utilized to calculate the results and display the relative expression (RE) compared to the housekeeping gene *Actb* (agccatgtacgtagccatcc and ctctcagctgtggtggtggtgaa).

Preparation of LgDNA

Lactobacillus rhamnosus GG (ATCC) was cultured using LB medium. A QIAamp Fast DNA Mini Kit (Qiagen) was used to extract the bacteria's DNA. Spectroscopy (MultiskanTM GO, Thermo Fisher Scientific) was used to determine the quantity and purity of LgDNA. The 260/280 nm absorbance ratios were used to evaluate the quality of the extracted DNA. The DNA quantity was determined using the absorbance at 260 nm. The established conversion factor of 50 ng/μl for 1 optical density unit at 260 nm was applied to convert it into ng/μl of double-stranded DNA.

Enzyme-linked immunosorbent assay (ELISA)

ELISA was utilized to measure the amount of proteins of interest in culture supernatant, nasal lavage fluid, and cellular extracts using commercial reagent kits following manufacturer's protocols. The microplate reader is Synergy™ H4 Hybrid Multi-Mode Microplate Reader (Fisher-Thermo Scientific).

Chromatin immunoprecipitation (ChIP)

ChIP assay was conducted following published procedures [10]. Cells were obtained from relevant studies. 1% formalin was used to fix the cells for 15 min. A lysing buffer was used to lyse the cells, followed by sonication to shear the DNA into small pieces. Protein A/G resin was used to precleared the pre-existing immune complexes in samples. Samples were centrifuged at 13,000 *g* for 10 min. The resin was discarded. The supernatant was incubated with the Abs of interest (which are detailed in the figures) overnight. Immune complexes in samples were adsorbed using protein A/G resin, which was then eluted using an eluting buffer. Using a DNA extraction kit, DNA was taken out of the samples and then analyzed by qPCR with primers targeting the *Il10* promoter (ccgggagtgtacccctaca and tcagtttgggtgggaagaac). The fold change was used to present the results against the input. Proteins in samples were quantified using ELISA with commercial reagent kits based on the provided protocol. All the procedures were performed at 4 °C.

Establishment of an airway allergy animal model

Subcutaneous ovalbumin (OVA) mixed in 0.2 mg alum was used to sensitize BALB/c mice on day 1 and day 7,

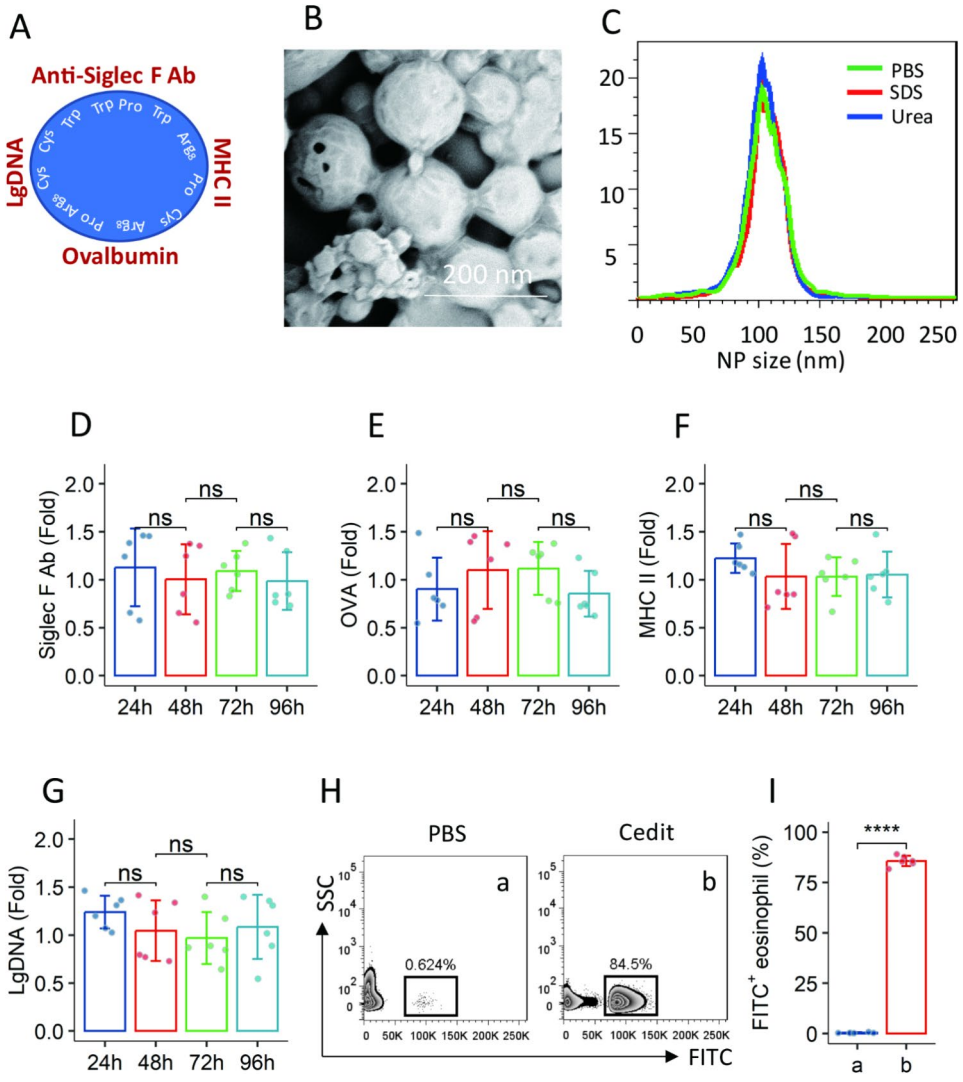

Fig. 1 Characterization of Cedit. **A**, a sketch of Cedit. Bars indicate the counts of FITC$^+$ eosinophils. **B**, an electron photomicrograph of Cedit (the original magnification: ×5 K) NPs. **C**, size distribution of the Cedit NPs after treating with PBS, or SDS (0.05 mM), or urea (0.06 mM). NP size was determined by dynamic light scattering method. **D-G**, Cedits were diluted in PBS (pH7.2) at 1 μg/ml. Samples were taken at indicated timepoints on the X axis. Bars indicate the quantity of indicated molecules in Cedit NPs. H-I, eosinophils were isolated from naïve mouse airway tissues, and exposed to FITC-Cedit or PBS in culture for 30 min. Gated flow cytometry plots show FITC$^+$ eosinophils. The data of bars are presented as mean ± SD. Each dot in bars presents one sample. Statistics: ANOVA + Bonferroni test (**C-F**) or Student's *t*-test (**H**). ns: Not significant. ****$p < 0.0001$. OVA: Ovalbumin. NP: Nanoparticle

respectively. Mice were given daily nasal instillations (20 μl/nose, containing 5 mg OVA/ml) from days 9 to 22 to enhance their immune response. Mice were challenged with a large dose of OVA through nasal instillations (20 μl/nostril, containing 50 mg OVA/ml). Each mouse was monitored for 30 min after the nasal challenge to record nasal scratch (nasal itch) times and sneezing times. Mice were sacrificed by cervical dislocation (use this approach to avoid any possible side effects of anesthesia on the experimental samples). After exposing and opening the trachea, 1 ml of saline was introduced towards the nasal direction. The nasal lavage fluid (NLF) was collected from the nostrils and used in further experiments.

Preparation of single cells from the mouse lungs

To obtain sufficient airway cells for in vitro experiments, we prepared single cells from the lungs of mice. The lungs were excised from mice upon the sacrifice, cut into small pieces, and incubated with collagenase IV (0.5 mg/ml) and DNase I (0.2 mg/ml) at 37 °C for 30 min. Single cells were filtered through a cell strainer, and resuspended in culture medium. The cells were used in other experiments.

Statistical analysis

A Student's *t*-test was employed to determine the difference between data collected from two groups. The difference between data from multiple groups was determined

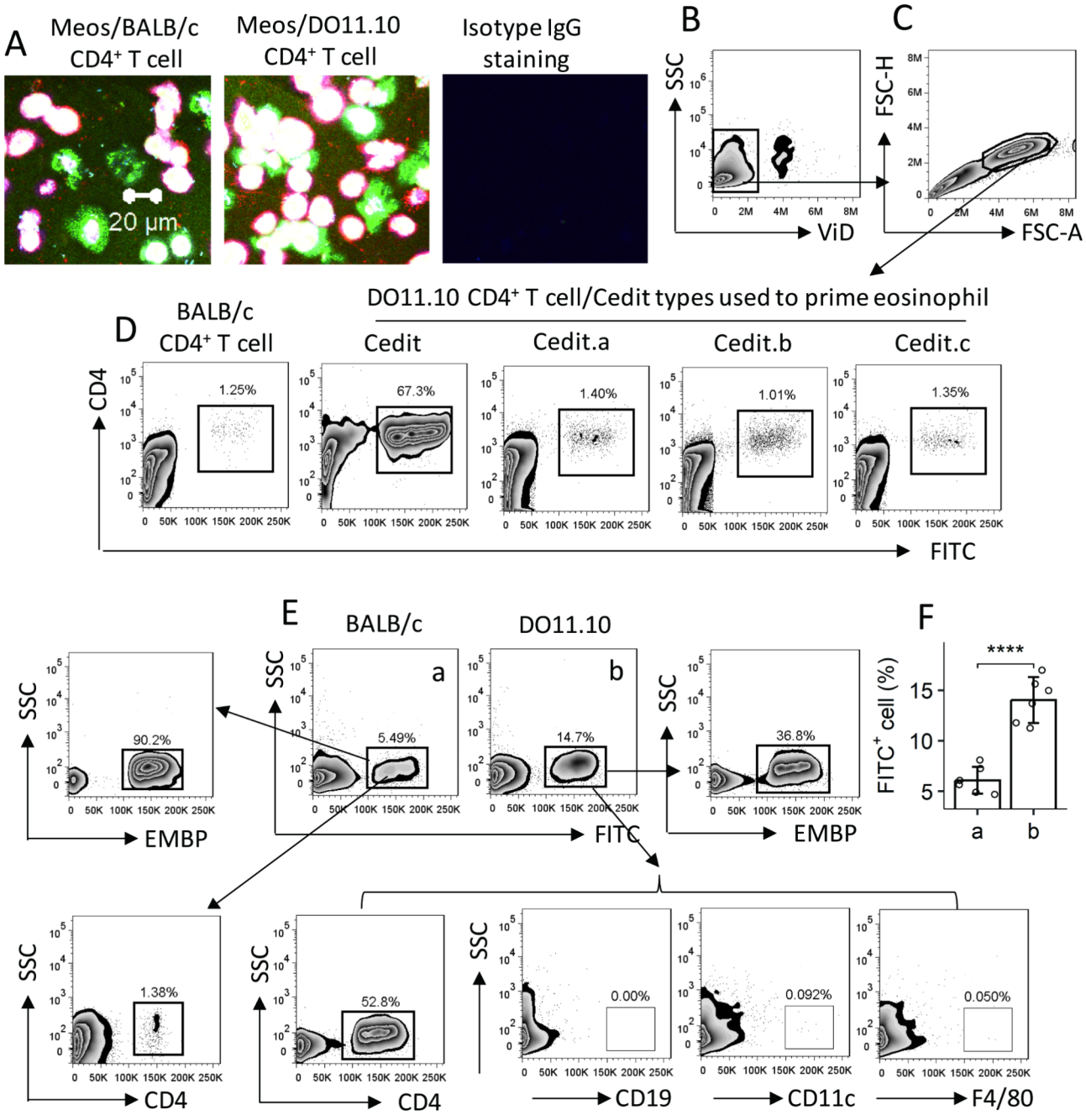

Fig. 2 Moes specifically bind to antigen specific $CD4^+$ T cells. A-D, $CD3^+CD4^+$ T cells were isolated from the spleen of DO11.10 mice and BALB/c mice. The cells were exposed to FITC-Cedit (or control Cedits)-primed eosinophils (Meos) in culture for 30 min. **A**, confocal images (×630) show that Meos (in green) bind to DO11.10 $CD4^+$ T cells (in red). **B**, dead cells were gated out. **C**, adhesive cells were gated out. **D**, $CD4^+FITC^+$ cells were gated. E-F, DO11.10 mice and BALB/c mice received nasal instillations (containing FITC-Cedits). Three hours later, the mice were sacrificed. Single cells were prepared from the airway tissues, and analyzed by flow cytometry. Gated plots are $FITC^+$ cells (cell types are indicated by arrows). Bars indicate the counts of $FITC^+$ cells. The group labels of F are the same as panel **E**. The data of bars are presented as mean ± SD. Each dot in bars presents one sample. Statistics: ANOVA + Dunnett's test (**C**) and Student's *t*-test (**F**). $^{****}p < 0.0001$. ViD: An active amine fluorescent dye used to stain dead cells. Cedit.a, Cedit.b, and Cedit.c are control Cedits, which short of Cedit component OVA, or MHC II, or siglecf Ab, respectively

through one-way ANOVA followed by Bonferroni test. $p < 0.05$ was set as a criterion of significance.

Results

Characterization of Cedit

The structure of Cedit is illustrated as Fig. 1A. Scanning electron microscopy revealed that Cedits are

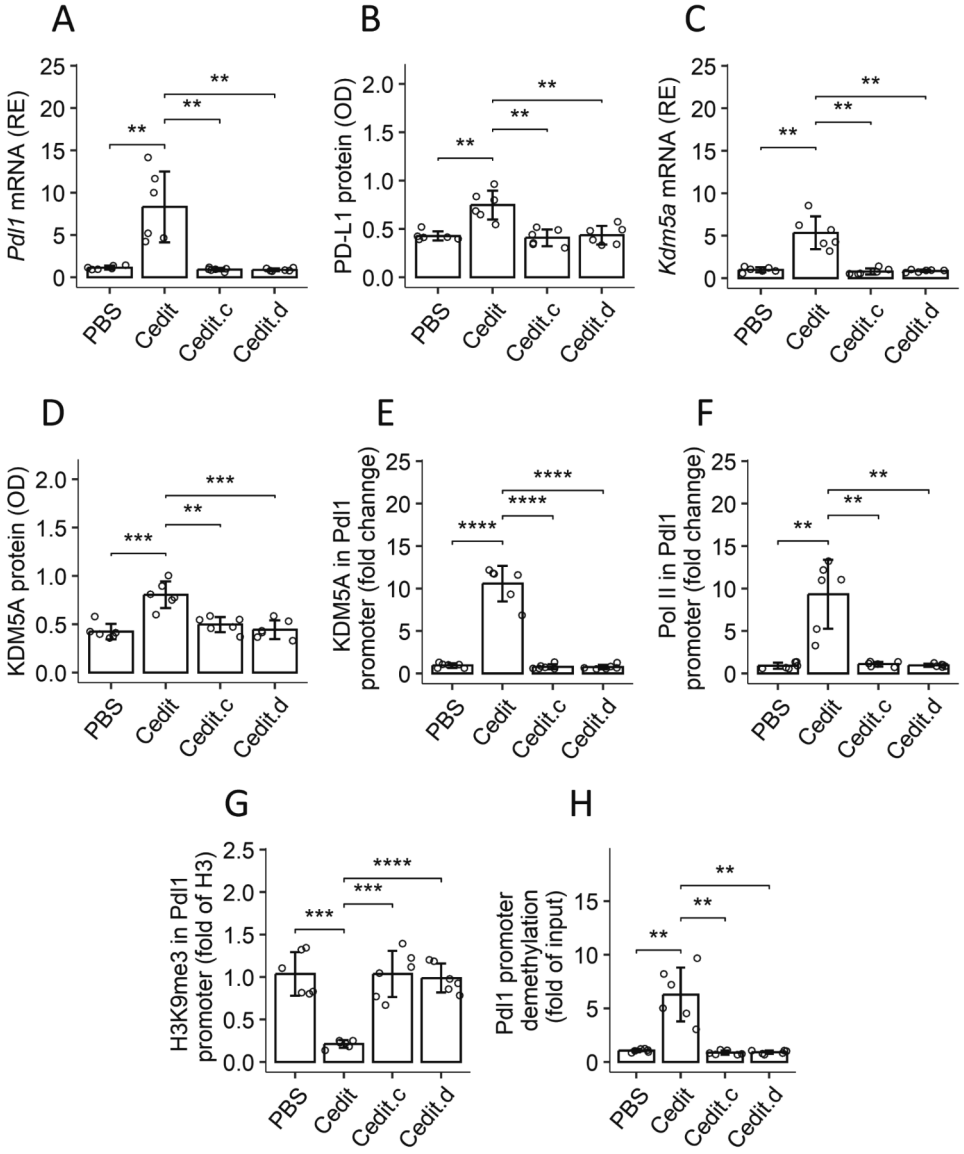

Fig. 3 Cedits induce PD-L1 expression in Meos. Meos were prepared and analyzed using ChIP, ELISA and RT-qPCR. **A**, Pdl1 mRNA quantity. **B**, PD-L1 protein quantity. **C**, Kdm5a mRNA quantity. **D**, KDM5A protein quantity. **E**, KDM5A protein quantity in the Pdl1 promoter. **F**, Pol II quantity in the Pdl1 promoter. **G**, H3K9me3 quantity in the Pdl1 promoter. **H**, demethylated Pdl1 promoter quantity. The data of bars are presented as mean ± SD. Each dot in bars presents one sample. Statistics: ANOVA followed by Bonferroni test. $^{**}p < 0.01$; $^{***}p < 0.001$; $^{****}p < 0.0001$. The Cedit.c and Cedit.d are controls, which short of siglecf Ab or LgDNA, respectively

nanoparticles (NPs) with diameters ranging from 50 to 150 nm, as depicted in Fig. 1B. After treatment with PBS, SDS, or urea, Cedits remain physically stable as their diameter remains 50–150 nm (Fig. 1C). Cedits were also chemically stable in a pH7.2 solution for at least 96 h (Fig. 1D-G). Eosinophils were isolated from the naïve mice and exposed to FITC-Cedits in culture for 30 min. Almost 90% of eosinophils were bound by Cedits according to flow cytometry (Fig. 1H-I). The Cedit-bound eosinophils were designated as Meos.

Meos specifically bind to antigen specific CD4+ T cells

Meos was prepared for an in vitro experiment and showed specific binding to OVA-specific $CD4^+$ T cells, but not to non-specific $CD4^+$ T cells (Fig). 2 A-D), nor control Cedits (short of either OVA, or MHC II, or siglecf Ab). We further treated mice with nasal instillations containing Cedit. Three hours later, single cells were prepared from the airway tissues, and analyzed using flow cytometry. Cedits bound to eosinophils, and then the Moes bound to OVA-specific $CD4^+$ T cells (Fig. 2E-F), but not bound to $CD4^+$ T cells in BALB/c mice, dendritic cells, B cells, or macrophages in the airways of DO11.10

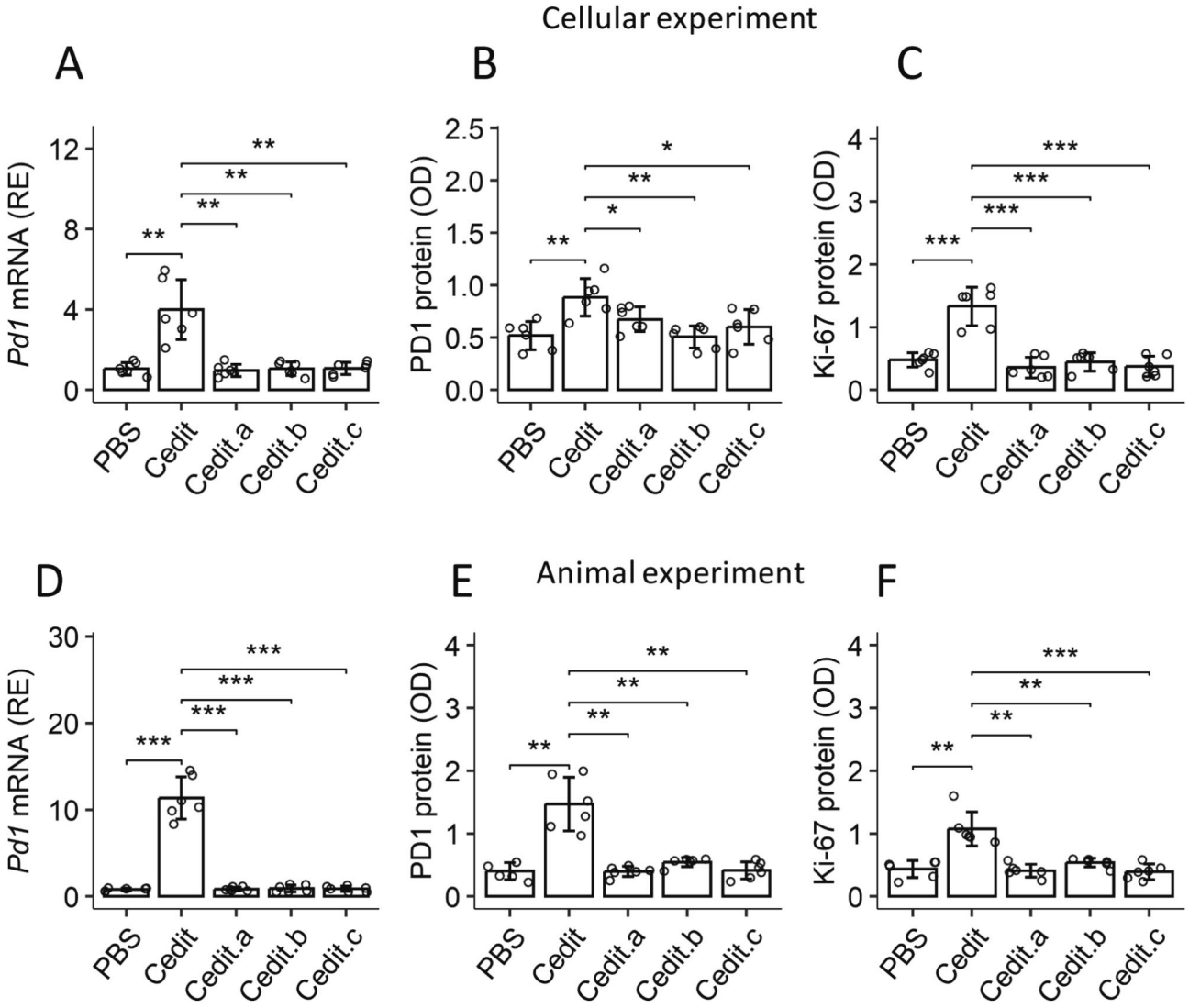

Fig. 4 Meos induce the expression of PD1 in antigen specific $CD4^+$ T cells. **A-C**, Meos were prepared with Cedits as denoted on the X axis of bar graphs. Meos were cocultured with DO11.10 $CD4^+$ T cells for 24 h. Extracts of RNA and proteins were prepared from the cells, and analyzed by RT-qPCR and ELISA. Bars show the quantity of indicated molecules in $CD4^+$ T cells (purified at the end of experiments). **D-F**, DO11.10 mice were treated with Cedits (types of Cedits are denoted on the X axis) by nasal instillations daily for 5 days. $CD4^+$ T cells were isolated from the airway tissues, and analyzed by RT-qPCR and ELISA. Bars show the quantity of indicated molecules in $CD4^+$ T cells. The data of bars are presented as mean ± SD. Each dot in bars presents one sample. Statistics: ANOVA followed by Bonferroni test. $*p < 0.05$; $**p < 0.01$; $***p < 0.001$. The Cedit.a, Cedit.b, and Cedit.c are control Cedits, which short of OVA, or MHC II, or siglecf Ab, respectively

mice (Fig. 2G-H). The results indicate that Cedits can bind to eosinophils; they then bind to antigen-specific CD4 T cells.

Cedits induce the expression of PD-L1 in Meos

We then measured the expression of PD-L1 in Meos. The results showed that elevated expression of PD-L1 was detected in eosinophils after exposure to Cedits in culture for 24 h (A-B). Exposure to control Cedits (which shorted of either siglecf Ab or LgDNA) did not induce the expression of PD-L1 in Meos. We further found that exposure to Cedits increased the amounts of KDM5A and Pol II, while decreasing the amounts of H3K9me3 in the promoter of the Pdl1 gene in eosinophils (Fig. 3C-G). The demethylation levels of the Pdl1 gene promoter also increased (Fig. 3H). It can be concluded from the results that Cedits have the ability to activate the expression of PD-L1 in Meos.

Meos increase the expression of PD1 in antigen specific CD4+ T cells

We prepared $CD4^+$ T cells from the spleen of DO11.10 mice and BALB/c mice. The $CD4^+$ T cells were cocultured with Meos at a ratio of 5:1 for 24 h. We found that

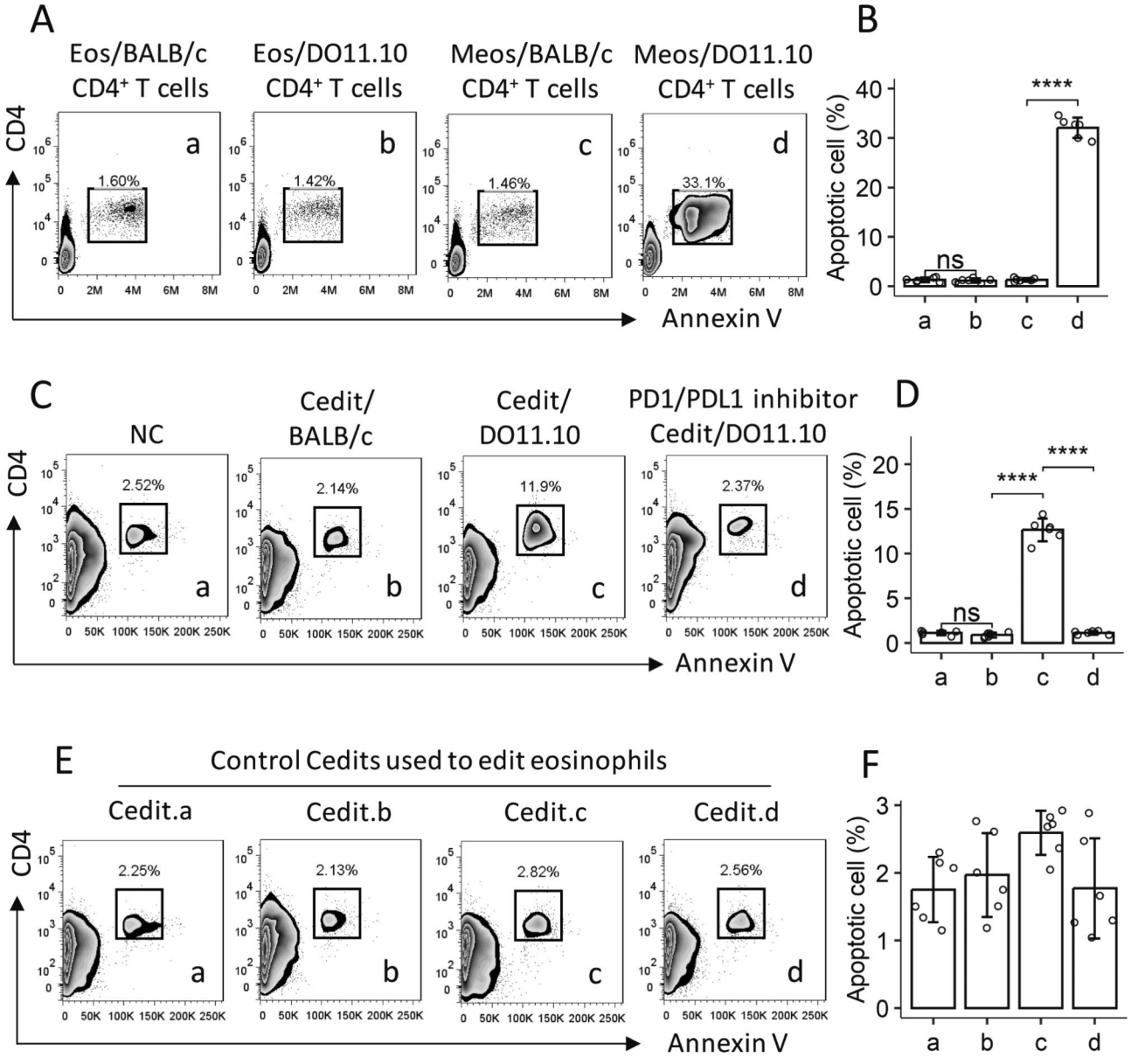

Fig. 5 Meos induce antigen specific CD4$^+$ T cell apoptosis. **A-B**, Cell types in a coculture setting are denoted above each flow cytometry panel. Gated plots are apoptotic cells. Bars show the counts of apoptotic cells. **C-F**, DO11.10 mice and BALB/c mice were treated with indicated Cedit types through nasal instillations daily for 5 day. Single cells were prepared from the airway tissues, and analyzed using flow cytometry. Gated plots are apoptotic cells. Bars show the counts of apoptotic cells. The group labels of bar plots are the same as those in flow cytometry plots on the left side. The data of bars are presented as mean ± SD from 6 samples per group. Statistics: ANOVA + Bonferroni test. ****$p < 0.0001$. ns: Not significant. The Cedit.a, Cedit.b, Cedit.c, and Cedit.d are control Cedits, which short of OVA, or MHC II, or siglecf Ab, or LgDNA, respectively. PD1/PD-L1 inhibitor: PD1/PD-L1-IN-3 (100 nM in nasal instillations)

Meos significantly induced the expression of PD1 in the CD4$^+$ T cells isolated from DO11.10 mice, but not those from BALB/c mice. Meos made from control Cedits (Cedits short of OVA, or MHC II, or LgDNA) did not induce the expression of PD1 in DO11.10 CD4$^+$ T cells (Fig. 4A-C). Alternatively, DO11.10 mice were treated with Cedits through nasal instillations daily for 5 days. We found that Cedits increased the expression of PD1 in airway CD4$^+$ T cells, which was abolished by removing OVA, or MHC II, or siglecf Ab from Cedits (Fig. 4D-F). The results demonstrate that Meos can induce the expression of PD1 in antigen specific CD4$^+$ T cells.

Meos induce antigen specific CD4+ T cell apoptosis

CD4$^+$ T cells were isolated from DO11.10 mice and BALB/c mice, and cultured with Meos at a ratio of 1:5 (Meos: T cells) for 24 h. Apoptotic cells were observed in DO11.10 CD4$^+$ T cells cocultured with Meos, but not

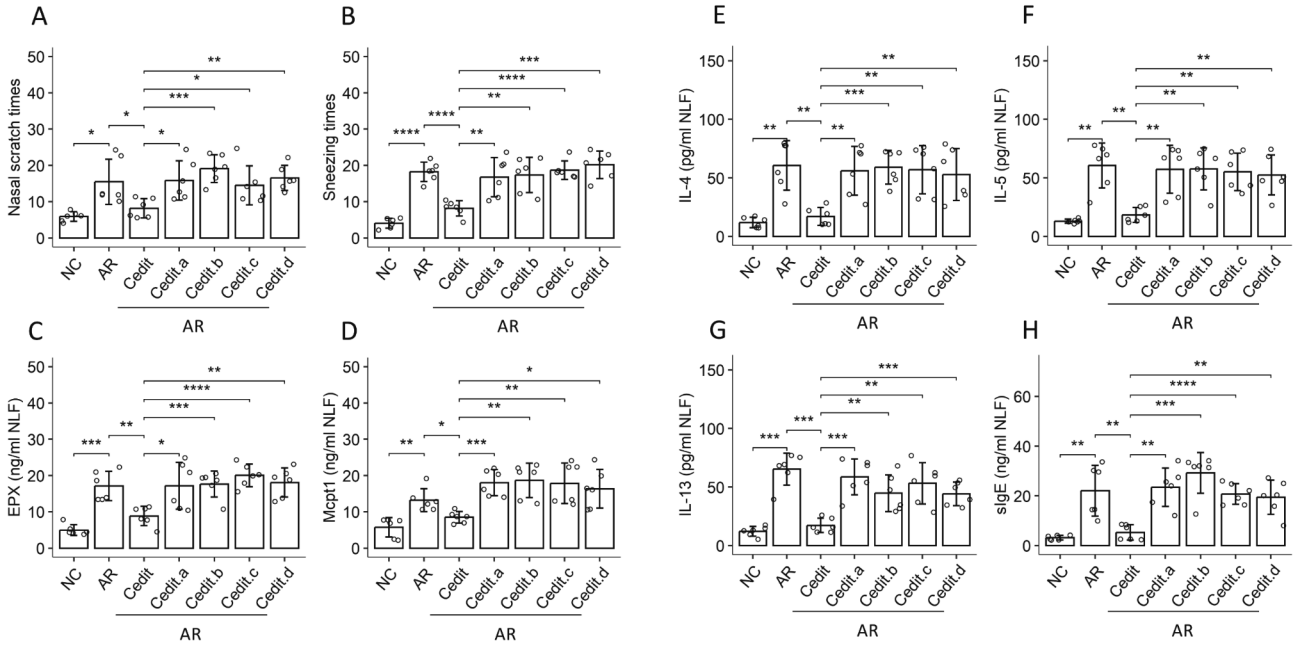

Fig. 6 Administration of Cedit mitigates experimental AR. An AR mouse model was established. Mice were treated with Cedit and control Cedits through nasal instillations daily for 5 days started one day after the completion of sensitization. The bar plots show the amounts of allergic symptoms (**A**, **B**) and indicated molecules in NLF (**C-H**). The data of bars are presented as mean ± SD from 6 samples per group. Each dot in bars presents one sample. Statistics: ANOVA + Bonferroni test. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$. The Cedit.a, Cedit.b, Cedit.c, and Cedit.d are control Cedits, which short of OVA, or MHC II, or siglecf Ab, or LgDNA, respectively. AR: Allergic rhinitis. NC: Naïve control

naïve eosinophils. BALB/c $CD4^+$ T cells were not apoptotic after coculture with either Meos or naïve eosinophils (Fig. 4A-B). Moreover, BALB/c mice and DO11.10 mice were administered Cedits through nasal instillations every day for a period of 5 days. The airway tissues were excised upon the sacrifice. Single cells were prepared from the airway tissues, and analyzed by flow cytometry. We found that administration of Cedits markedly induced DO11.10 $CD4^+$ T cell apoptosis, but not BALB/c $CD4^+$ T cells. The induction of antigen specific $CD4^+$ T cell apoptosis was blocked by the presence of PD1/PD-L1 inhibitor (Fig. 5C-D). Administration of control Cedits (short of either OVA, or MHC II, or siglecf Ab, or LgDNA) did not induce DO11.10 $CD4^+$ T cell apoptosis (Fig. 5E-F). The results demonstrate that Meos can induce antigen specific $CD4^+$ T cell apoptosis.

Administration of Cedits mitigate experimental airway Th2 polarization and allergic rhinitis (AR)

A mouse model of allergic rhinitis (AR) was established. AR mice showed the AR response, including symptoms such as nasal itch and sneezing. (Fig. 6A-B), elevated allergic mediator (Fig. 6C-D) and Th2 cytokine amounts in nasal lavage fluid (NLF) (Fig. 6E-G), and increased amounts of specific IgE (Fig. 6H) in NLF. Mice were treated with Cedit-containing nasal instillations daily for 5 days. The AR response was significantly reduced by the Cedit therapy (Fig. 6A-H). The Cedit treatment significantly decreased the abundance of antigen-specific Th2 cells (Fig. 7). The results demonstrate that the administration of Cedits can alleviate experimental AR.

Discussion

A new method is described in this paper that edits or modifies eosinophils in vivo. This new device is the Cedit. We used Cedits through nasal instillations to edit eosinophils to Meos, which conferred eosinophils the ability to induce apoptosis of airway antigen-specific $CD4^+$ T cells. Antigen specific $CD4^+$ T cells play a critical role in the pathogenesis of airway allergy. As a result, the administration of Cedit effectively mitigated experimental AR.

Referring to the published strategies [8], we constructed a peptide carrier. The reported peptide contains nine amino acids, including 'Cys-Trp-Trp-Arg8-Cys-Arg8-Cys-Arg8-Cys'. To enhance its adhesive property, we added three prolines to modify the peptide to 12 amino acids [11]. All four components were found to be stable in a pH7.2 environment for at least 96 h, which is significantly longer than the effective time of Cedit according to the experimental characterization results. It was observed that Meos were generated in the airway tissues after Cedit was administered for 24 h.

The data show that Cedits bind to eosinophils both in vitro and in vivo. The siglecf Ab component in Cedit was created for this purpose. Eosinophils express siglec F [12]. According to published data, eosinophils have

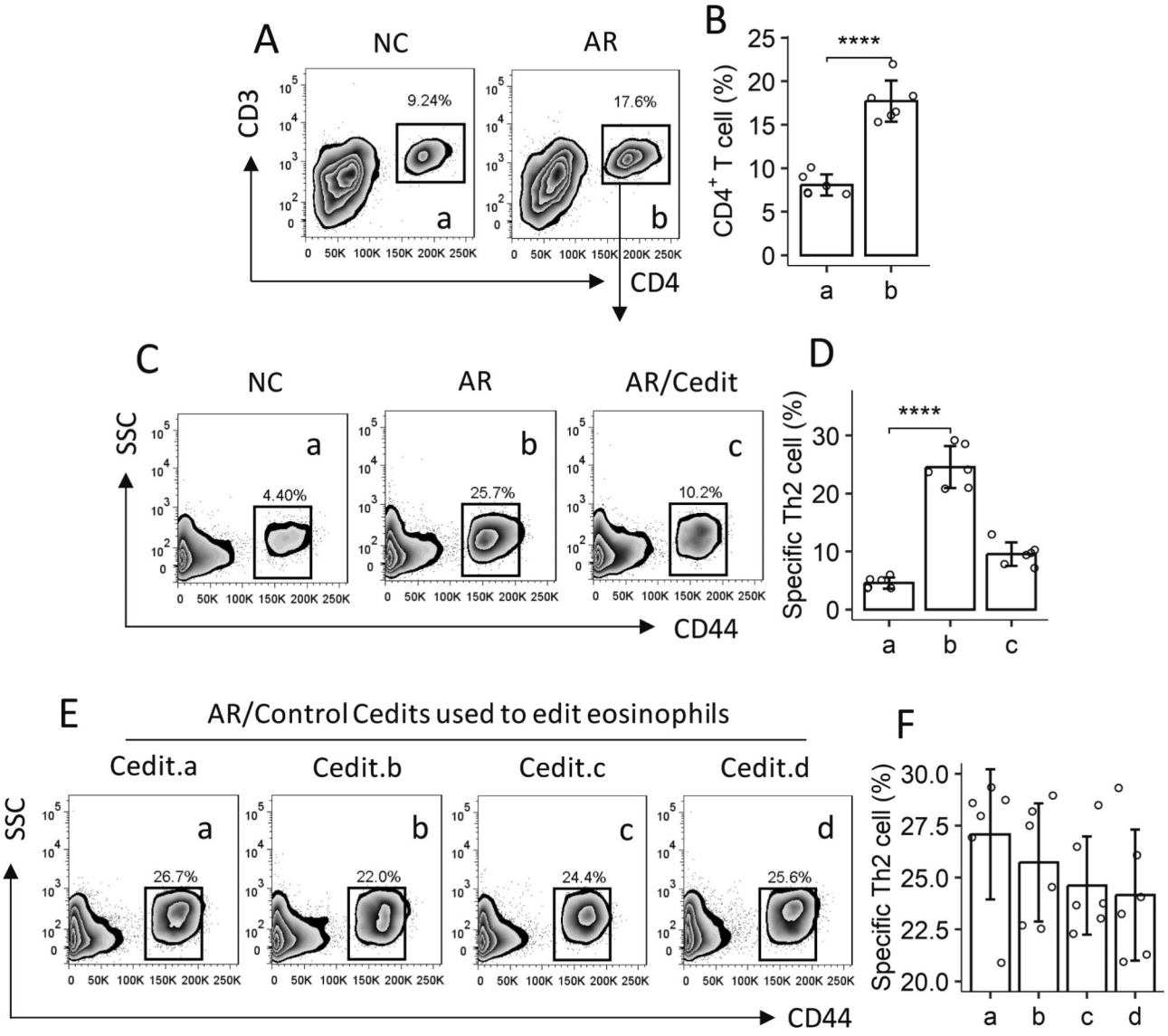

Fig. 7 Administration of Cedit reduces antigen specific CD4$^+$ T cells in the airways of mice with airway allergy. An AR mouse model was established. Mice were treated with Cedit and control Cedits through nasal instillations daily for 5 days started one day after the completion of sensitization. Single cells were prepared from the airway tissues, and analyzed by flow cytometry. **A-B**, gated plots show CD3$^+$CD4$^+$ T cells. Bars show the counts of CD3$^+$CD4$^+$ T cells. **C-F**, gated plots show CD44$^+$ cells in CD3$^+$CD4$^+$ T cells. Bars show the counts of CD44$^+$ cells. The group labels in bar graphs are the same as those in flow cytometry plots on the left side. The data of bars are presented as mean ± SD. Each dot in bars presents one sample. Statistics: Student's t-test (**B**) and ANOVA + Bonferroni test (**D**, **F**). The Cedit.a, Cedit.b, Cedit.c, and Cedit.d are control Cedits, which short of OVA, or MHC II, or siglecf Ab, or LgDNA, respectively. AR: Allergic rhinitis. NC: Naïve control

high levels of PD-L1, which can effectively suppress T cell activities [13]. Current data show that Cedit can bind to eosinophils and induce the production of PD-L1. The airway tissues have a significant number of eosinophils, which can be used as cellular sources to edit Meos.

Our observations indicate that Meos can specifically bind to CD4$^+$ T cells that recognize specific antigens. An OVA molecule is present in Meos because it has been bound by Cedits, which is the underlying mechanism. The OVA-specific T cell receptor (TCR) on the surface of OVA-specific CD4 T cells is able to be specifically recognized by OVA. The binding of specific antigens and antigen-specific T cells has been proven to activate T cells [14]. The activation of CD4$^+$ T cells can lead to the production of PD1. This phenomenon has also been discovered in previous reports. Lactic acid exposure can result in the expression of PD1 in CD4 regulatory T cells [15]. It has been acknowledged that antigen specific CD4$^+$ T cells can be activated by specific antigens [16]. Current data verify the inference by showing that the Cedit-generated Meos induced the expression of PD1 in antigen specific CD4$^+$ T cells, while those control Cedit

(short of OVA, or MHC II, or siglecf Ab, or LgDNA)-generated Meos did not.

We found that administration of Cedits induced antigen specific $CD4^+$ T cell apoptosis. PD-L1 is a transmembrane protein [17]. It binds to PD1 on target cells to suppress the cell proliferation, or inhibit cytokine secretion of the cell, or induce the cell apoptosis [18]. Cancer cells express PD-L1. Immune cells express PD1. Thus, cancer cells can compromise the immune functions in the body [19]. The interaction between PD1/PD-L1 can lead to cancer cells impairing the anti-tumor immune response and escaping immune surveillance (Dermani, 2019 #19). Th2 polarization is the primary cause of allergic disorders. More than needed Th2 cells aggregate in the lesion sites. These Th2 cells produce more than the necessary quantity of Th2 cytokines to induce Th2 pattern inflammation in the tissues [20]. Therefore, generating PD-L1 producing Meos is expected to regulate the activities of skewed Th2 cells. Our data indicate that Meos induce the antigen specific $CD4^+$ T cells apoptosis. As a consequence, the skewed Th2 polarization in the airways was dampened in mice with airway allergy.

This study used LgDNA as one of the components of Cedits. LgDNA is extracted from the probiotic *Lactobacillus rhamnosus* GG. Probiotics have been used as supplements to support immunotherapy or used as a food supplement. The immune regulatory functions of probiotics have been recognized [21]. Although many proposals have been made, the elucidation of mediators from probiotics that fulfill immune regulatory functions is still ongoing. Short-chain fatty acids are believed to involve the immune regulatory activities of probiotics [22]. While short chain fatty acids from probiotics may not be easy to distinguish from those derived from ingested food. It is worth noting that live probiotics are necessary for administration. Some, if not all, probiotics may die in the harsh environment of the digestive tract. DNA can be released from dead probiotics. DNA is widely acknowledged as a powerful regulator for immune response [23]. The probiotic DNA is expected to be involved in the immune regulation. Our data support this inference. Exposure to LgDNA increased the expression of PD-L1 in eosinophils by regulating the methylation status of the *Pdl1* promoter.

Conclusions

Cedits can edit eosinophils in vivo to be the Meos. Meos contact antigen specific $CD4^+$ T cells to induce the cells apoptosis through the interaction of PD1/PD-L1. The data suggest that Cedits has the potential to be developed as a remedy for allergic disorders.

Abbreviations

Meos	Modified eosinophils
LgDNA	DNA extracted from probiotics
PD-L1	Programmed death ligand-1
ELISA	Enzyme-linked immunosorbent assay
ChIP	Chromatin immunoprecipitation
OVA	Ovalbumin
NPs	Nanoparticles

Acknowledgements

NA.

Author contributions

Author contribution: Luo X, Yang J, Zheng H, Zhang Y, Mo L, Huang Q, Wu G, Zhong J and Liu Y performed experiments, analyzed data, and reviewed manuscript. Yang P, Yang G organized the study, supervised experiments. Yang P designed the project and prepared the manuscript.

Data availability

Data are availble upon request.

Declarations

Ethical approval

The Ethics Committee at Shenzhen University approved the animal experiments with an approval number of 2023008. The ARRIVAL Guidelines were followed when conducting the present study.

Competing interests

The authors declare no competing interests.

Received: 10 June 2024 / Accepted: 11 September 2024
Published online: 30 September 2024

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