A target-based discovery from a parasitic helminth as a novel therapeutic approach for autoimmune diseases

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Summary

Background Regulatory T cells (Tregs) can alleviate the development of autoimmune and inflammatory diseases, thereby proposing their role as a new therapeutic strategy. Parasitic helminths have co-evolved with hosts to generate immunological privilege and immune tolerance through inducing Tregs. Thus, constructing a "Tregs-induction"-based discovery pipeline from parasitic helminth is a promising strategy to control autoimmune and inflammatory diseases.

Methods The gel filtration chromatography and reverse-phase high-performance liquid chromatography (RP-HPLC) were used to isolate immunomodulatory components from the egg extracts of *Schistosoma japonicum*. The extracted peptides were evaluated for their effects on Tregs suppressive functions using flow cytometry, ELISA and T cell suppression assay. Finally, we carried out colitis and psoriasis models to evaluate the function of Tregs induced by helminth-derived peptide *in vivo*.

Findings Here, based on target-driven discovery strategy, we successfully identified a small 3 kDa peptide (SjDX5-53) from egg extracts of schistosome, which promoted both human and murine Tregs production. SjDX5-53 presented immunosuppressive function by arresting dendritic cells (DCs) at an immature state and augmenting the proportion and suppressive capacity of Tregs. In mouse models, SjDX5-53 protected mice against autoimmune-related colitis and psoriasis through inducing Tregs and inhibiting inflammatory T-helper (Th) 1 and Th17 responses.

Interpretation SjDX5-53 exhibited the promising therapeutic effects in alleviating the phenotype of immune-related colitis and psoriasis. This study displayed a screening and validation pipeline of the inducer of Tregs from helminth eggs, highlighting the discovery of new biologics inspired by co-evolution of hosts and their parasites.

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Keywords: Helminth-derived peptide; Target-driven discovery strategy; Treg; TolDCs; Inflammation

Introduction

Autoimmune diseases characterized by chronic, systemic, excessive immune activation and inflammation affect approximately 10% of the world's population.[1,2] One possible explanation for the high incidence of autoimmune diseases is the "hygiene hypothesis",[3,4] that is, reduced exposure to parasites because of improved hygiene, which in turn increases the risk of some allergic and autoimmune pathologies.[5] Epidemiological evidences have demonstrated the inverse correlation between the prevalence of parasite infections and the incidence of autoimmune diseases.[6,7] Animal models

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Research in context

Evidence before this study

Functional or numerical Tregs anomalies contribute to the development of autoimmune diseases and inflammatory diseases. Helminths have driven the induction of Tregs that induce a state of hyporesponsiveness or immune suppression within the host. Tregs generated during helminth infection have been associated with suppression of a range of inflammatory conditions such as allergy and autoimmune disease.

Added value of this study

Based on target-driven discovery strategy, we identified a small 3 kDa peptide SjDX5-53 from the egg extracts of schistosome and evaluated the potential efficacy of this peptide to enhance the proportion and suppressive capacity of Tregs in cellular and animal models. Moreover, SjDX5-53 exhibited promising therapeutic effects in ameliorating the development and symptoms of immune-related colitis and psoriasis through inducing tolerance DCs and Treg cells.

Implications of all the available evidence

Combined, we designed a screening and validation pipeline to identify a Tregs inducer against autoimmune diseases, implied the discovery of new biologics inspired by co-evolution of hosts and their parasites.

also support the ability of helminth infections or certain components to prevent autoimmune diseases including type 1 diabetes,[8] multiple sclerosis[9] and colitis.[10] It is well recognized that parasitic helminths have co-evolved with hosts to generate immunological privilege and immune tolerance.[11]

Regulatory T cells (Tregs), a functionally distinct T cell subpopulation are crucial players in the maintenance of immunological self-tolerance and homeostasis.[12] Parasitic helminths[13–15] are thought to promote Tregs to attenuate host's immune attack and prolong their own survival. This immunomodulation by avoiding an excessive activation of the immune system, contributes to host protection against inflammatory disorders. For example, infection with *Heligmosomoides polygyrus* induces Treg cells to prevent streptozotocin-induced diabetes.[16] Cystatin of *Trichinella spiralis* could inhibit Th1 immune response by promoting Treg cells and interleukin (IL)-4 production in 2,4,6-trinitrobenzene sulfonic acid-induced experimental inflammatory bowel disease.[17]

Several clinical trials have been registered to assess the safety or efficacy of probiotic worm therapy in chronic inflammatory conditions and autoimmune diseases. *Necator americanus* larvae was assessed in clinical trials for inflammatory bowel disease,[18] asthma[19] and celiac disease.[20] Although helminth therapy has generated substantial interest in modulating the excessive immune responses, concerns prevail around the implications of pathogenic effects caused by infection with live pathogens, particularly at high doses.[21] Therefore, identification of protective active helminth-derived molecules to substitute treatment with whole worm could circumvent this issue.

Based on target-driven discovery strategy,[22] schistosome eggs considered as the powerful inducer of Tregs were collected for isolation of active components by gel filtration chromatography and RP-HPLC. Following purification, we characterized a peptide named SjDX5-53, which promoted both human and murine Tregs production and enhanced their immunosuppressive function by induction of tolerogenic dendritic cells (tolDCs). SjDX5-53 exhibited promising therapeutic effects by alleviating the phenotype of immune-related colitis as well as imiquimod (IMQ)-induced and spontaneous psoriasis. Hence, development of helminth-derived biologics targeting Tregs will provide new strategies for the treatment of autoimmune diseases.

Methods

Ethics statement

All animal experiments were approved by the Animal Care and Use Committee at the Nanjing Medial University (No. IACUC-2012050, IACUC-2010053 and IACUC-1906032). Human blood collection was conducted according to the clinical protocols approved by the Institutional Review Board of Nanjing Medical University (No. (2021) 812). Informed consent was obtained from all volunteers for the use of human peripheral blood in this study. All the implements were under full compliance with Helsinki declaration.

Mice and parasites

Mice were housed in a specific-pathogen-free (SPF) environment and maintained in sterile isolators with autoclaved food and water under 12-h light and 12-h dark cycle at 24 °C. C57BL/6 and BALB/c mice were obtained from animal facility of Nanjing Medical University. $Rag1^{-/-}$ and $Foxp3^{DTR}$ mice on the C57BL/6J background were kindly provided by Prof. Shuo Yang and Prof. Xiaoming Wang. $Card14^{E138A/+}$ mice were kindly provided by Prof. Xin Lin from Tsinghua University. $TLR2^{-/-}$ mice were kindly gifted from Prof. Liyun Shi from Nanjing University of Chinese Medicine. Mice were matched for age, gender, and body weight, and excluded from the defined experimental conditions if they exhibited rapid body weight reduction, loss of appetite for over 3 days, serious alopecia, weakness and hard to take food or drink water, unexpectable

organ infection and death. Sample sizes were chosen based on the means and variation of preliminary data to achieve at least 80% power and allow for a 5% type I error. Mice were randomized using a random number generator in all experiments, and experiments were conducted with the investigators being blinded as to treatment, group allocation for IHC staining, qPCR, and flow cytometry.

Oncomelania hupensis, infected with the Chinese strain of *Schistosoma japonicum* (*S. japonicum*), were obtained from Jiangsu Institute of Parasitic Diseases (Wuxi, Jiangsu, China). *S. japonicum* eggs were isolated from the livers of infected New Zealand rabbits as described previously.[23]

Cell lines

The 293T cells were grown at 37 °C in DMEM (Gibco, Waltham, USA) supplemented with 10% fetal bovine serum (Gibco), 2 mM L-glutamine and 100 U ml^{-1} penicillin/streptomycin (Gibco) in 5% CO_2. The cell line was validated at Servicebio by short tandem repeat profiling (STR) and mycoplasma testing.

Induction of colitis by adoptive transfer of naïve T cells

Colitis was induced in Rag1$^{-/-}$ mice by adoptive transfer of CD4$^+$CD25$^-$CD62L$^+$ T cells as described previously.[24] In brief, naïve CD4$^+$ T cells were enriched from splenocytes of C57BL/6 mice by MojoSort™ Ms CD4 Naïve T Cell Isolation Kit (cat# 480040, Biolegend, CA, USA). Enriched naïve CD4$^+$ T cells were labeled with FITC-conjugated anti-mouse CD4 (AB_464892), PE-conjugated anti-mouse CD25 (AB_465607), Percp-cy5.5-conjugated anti-mouse CD62L (AB_996667) (all from Invitrogen, Waltham, USA), and CD4$^+$CD25$^-$CD62L$^+$ cells were isolated by cell sorting using FACSAriaII flow cytometer (BD Biosciences, NJ, USA). The Rag1$^{-/-}$ recipients were adoptively transferred with 4×10^5 naïve CD4$^+$ T cells from WT mice, and simultaneously received 2×10^5 Treg cells via tail vein. Mice were monitored for weight and euthanized at 5–6 weeks after transferring.

Psoriasis mouse models

Imiquimod (IMQ)-induced psoriasis-like skin inflammation was achieved in 8-week-old BALB/c mice or C57BL/6 mice by a daily topical dose of IMQ cream (5%) (Aldara; 3M Pharmaceuticals, Minnesota, USA) on the shaved back for 7 consecutive days. To delete Tregs *in vivo*, DT (20 μg/kg, Calbiochem, CA, USA) was injected intraperitoneally (*i.p.*) on day 4 and 6 after starting the IMQ treatment.[25] For therapy, IMQ-applied mice were treated daily with topical Calcipotriol Ointment (40 mg/cm^2) or SjDX5-53. In SjDX5-53 treatment experiment, mice received 0.5 mg/kg or 5 mg/kg SjDX5-53 on the shaved back or intraperitoneally once a day for a week since the day IMQ was applied.

After developing spontaneous psoriasis-like phenotype, 12-week-old Card14$^{E138A/+}$ mice received 0.5 mg/kg SjDX5-53 on the shaved back once a day for a week.

Schistosome egg-derived peptides isolation, purification and sequencing

The crude extracts from eggs of *S. japonicum* were dissolved in 1 × PBS, pH = 6.0. Subsequently, they were loaded on a Protein-Pak 125 Column (Waters, Milford, MA, USA, 10 μm, 19 mm × 300 mm) that was previously equilibrated with the same buffer. Sample fractionation was carried out by eluting the column with a linear gradient of PBS. Elution was performed with a flow rate of 0.5 mL/min at 4 °C, and fractions were collected in each tube. The absorbance of the elution fractions was monitored at both 215 and 280 nm. The fraction from the previous step was resuspended and applied to reverse-phase high-performance liquid chromatography (RP-HPLC) on a C4 column (Waters, 5 μm, 10 mm × 250 mm). Elution was carried out with a linear gradient of 0 %–65% solution B (99.9% acetonitrile, 0.1% TFA) for 70 min at a flow rate of 1.5 mL/min, and the eluted fraction was collected. The primary amino acid sequences were determined by AB Sciex TripleTOF® 5600+ system. The sequence of SjDX5-53 was identified as GQMQQQQRPQQMQPSQQYATNQMTNSR and the control peptide was designed as MYQQSTMG RQANQTPRQSQQPQMQQNQ.

Generation of bone marrow-derived dendritic cells (BMDCs)

Bone marrow cells from C57BL/6 mice were isolated and cultured in DC media (RPMI 1640 supplemented with 10% FBS, 100 U/mL penicillin, 100 mg/mL streptomycin, and 20 ng/mL recombinant mouse GM-CSF (cat# 315-03, PeproTech, NJ, USA). A complete medium change was performed every 2 days. On day 6, the cells were treated with 10 μg/mL SjDX5-53 peptide or control peptide for 48 h. From 24 h of peptides stimulation, BMDCs were stimulated with or without 100 ng/mL LPS (L4524, Merck, Darmstadt, Germany) for the indicated times or for 24 h to generate mature BMDCs.

Human peripheral blood mononuclear cell (PBMC) isolation

Human PBMCs were isolated from heparinized blood within 4.5 h of the blood draw through Ficoll (17144002, Cytiva, MA, USA) density centrifugation at 400 × *g* for 20 min at room temperature as described before.[26] The blood mononuclear cell fraction was recovered and washed in 1 × PBS. 1×10^6 PBMCs were incubated for 72 h in 96-well cell culture plates either alone or in the presence of 1 μg/mL immobilized anti-CD3 mAb (AB_468854) plus 1 μg/mL soluble anti-CD28 mAb (AB_468926), 10 ng/mL IL-2 (200-02) and 5 ng/mL TGF-β (100-21).

T cell suppression assay

To examine whether SjDX5-53-induced Tregs were functionally suppressive, the isolated naive $CD4^+$ T cells were labeled with 5 μM CFSE (65-0850-84, Invitrogen) at 37 °C for 10 min according to the manufacturer's instructions. After PBS washing, the CFSE-labeled $CD4^+$ T cells were seeded onto plates coated with 1 μg/mL anti-CD3 (AB_468847) and anti-CD28 mAbs (AB_468921) in the culture media containing 10% FBS and 1% P/S for 3 days. Tregs were co-cultured with CFSE-labeled responder T cells at various responder: suppressor ratios. The CFSE fluorescence was measured using a flow cytometer.[27]

Enzyme-linked immunoadsorbent assay (ELISA)

Human PBMC or murine splenocytes were treated with medium or peptides component for 72 h. IL-10 (A35590, Invitrogen), IL-4 (A35587, Invitrogen) and IFN-γ (EK180, MultiSciences, Zhejiang, China) protein level measurements were performed following the manufacturer's manual.

To measure IL-10 (BMS614, Invitrogen), TGF-β (1217102, Dakewe, Guangzhou, China), AREG (EMAREG, Invitrogen), and IL-6 (ELM-IL6-1, RayBiotech, GA, USA) production after SjDX5-53 stimulation, culture supernatant of unstimulated and stimulated BMDCs were harvested after 48 h. Level of these cytokines was determined by capture ELISA according to the manufacturer's instructions.

RNA extraction and quantitative PCR

Total RNA from cell cultures or tissues of mice was extracted with RNAiso Plus kits (9109, TaKaRa Biotechnology Co. Ltd., Liaoning, China) according to manufacturer protocol. mRNA was reverse transcribed into first strand cDNA using the PrimeScript RT Master Mix kits (RR036A, TaKaRa). Real-time quantitative PCR was performed in a LightCycler® Real-Time PCR System (Roche, Basel, Switzerland) using SYBR Green Master Mix (Roche) according to the manufacturer's instructions. The cycling parameters were as follows: stage 1, 50 °C for 2 min; stage 2, 95 °C for 10 min; stage 3, 40 cycles of 95 °C for 15 s and 60 °C for 1 min; and the relative expression was calculated with the $2^{-\Delta\Delta ct}$ method. The oligonucleotides used are listed on Supplementary Table S1.

Flow cytometry

For Treg cell staining and analysis, 2×10^6 of murine splenic single cell suspensions were surface-stained with CD4-FITC (AB_464892) and CD25-APC (AB_469365). Then cells were fixed and permeabilized with Cytofix/Cytoperm buffer (BD Pharmingen) and blocked with Fc receptor (Invitrogen). Finally, cells were stained with PE conjugated anti-Foxp3 antibodies (AB_2738006). 2×10^6 human PBMCs were stained with APC-conjugated anti-CD127 (AB_1659670), FITC or PE-cy7-conjugated anti-CD25 (AB_2811739, AB_1257140) and PE or Percp-cy5.5-conjugated anti-CD4 (AB_1582249, AB_1518745), PE-conjugated anti-Foxp3 (AB_1645508) to detect the frequencies of human Tregs.[28]

For Th1/Th17 analysis, 2×10^6 murine splenic cells of single-cell suspension were cultured in complete RPMI 1640 medium (Gibco) and stimulated with 25 ng/mL 12-O-tetradecanoylphorbol-13-acetate (Merck) and 1 μg/mL ionomycin (Merck) in the presence of 0.66 μL/mL Golgistop (BD) at 37 °C in 5% CO_2 for 4–6 h. Cells were stained for surface molecules (CD3e-APC, AB_469315; CD4-FITC, AB_464892), fixed and permeabilized with Cytofix/Cytoperm buffer, then intracellularly stained with IFN-γ-PE (AB_466193) or IL-17A-PE (AB_763582).

The expression of BMDC surface marker was assessed by flow cytometry using cells stained with APC-CD11c (AB_469346), FITC-MHC II (AB_465232), PE-cy7-CD40 (AB_10933422), and PE-CD86 (AB_465767) with Fc blocking reagents.

Histology and immunohistochemistry (IHC)

Tissues samples from each mouse in each group were fixed in 4% formaldehyde, embedded in paraffin, and were cut into 4-μm sections for hematoxylin and eosin (H&E) staining. After hematoxylin staining, images were examined by microscopy (Carl Zeiss, Jena, Germany). For immunohistochemistry, deparaffinized sections were boiled for 15 min in 10 mM sodium citrate buffer (pH 6.0) for antigen retrieval. Endogenous peroxidase activity was inhibited by 3% hydrogen peroxide, and nonspecific binding was blocked using a blocking solution (Beyotime, Shanghai, China). The sections were incubated with anti-Foxp3 mAb (AB_2860568, Abcam, Cambridgeshire, UK) at 4 °C overnight. HRP-conjugated goat anti-rabbit IgG (H + L) were used for secondary Abs. Immunostaining was developed using DAB solution The immunohistochemistry images were taken with Carl Zeiss microscope.

RNA sequencing of BMDCs

In vitro BMDCs culture assays in the presence of SjDX5-53 were carried out. Following 48 h of incubation at 37 °C in 5% CO_2, cells were resuspended in Trizol and submitted for RNA sequencing at SHBIO. RNA was obtained with a RNeasy Micro Kit according to the manufacturer's instructions (QIAGEN, Stockach, Germany) and RNA integrity was assessed with an Agilent 2100 Bioanalyzer (Agilent technologies Santa Clara). Samples underwent VAHTS Universal V6 RNA-seq Library Prep Kit amplification (Vazyme, Nanjing, China) and were sequenced on an Illumina NovaSeq6000. RNA sequencing reads were aligned to the reference mouse genome (GRCm38. p4 (mm10)) using Hisat2. The edgeR package was used to perform differential gene expression analyses between groups. A cutoff of 0.05

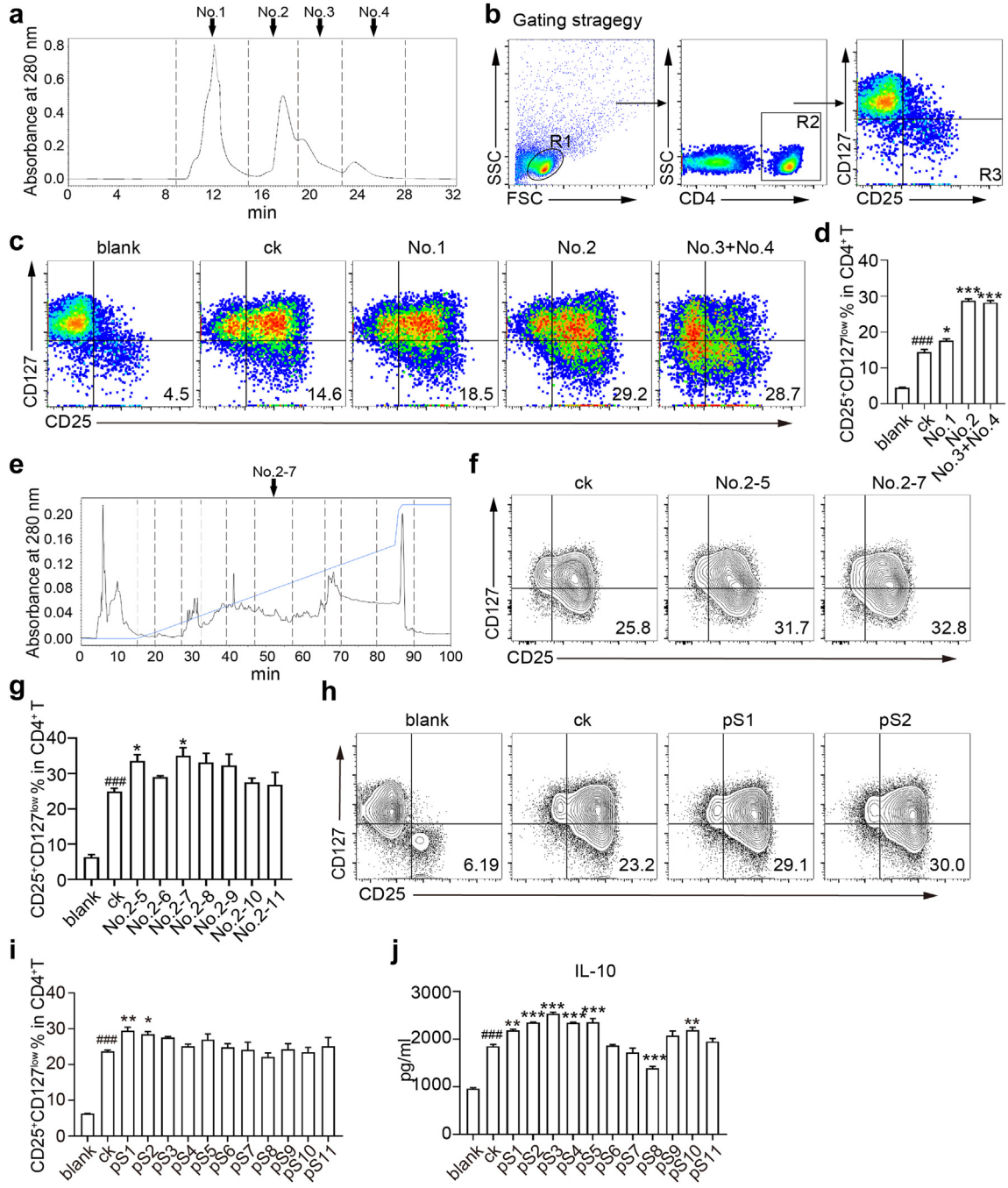

Fig. 1: **Purification of SjDX5-53 from extract of *S. japonicum* eggs.** (**a**) Protein-Pak 125 gel filtration of egg extract from *S. japonicum*. The elution was performed with 1 × PBS, pH 6.0. (**b**) Gating schemes for analysis of the percentages of $CD4^+CD25^+CD127^{low}$ (Treg) cells from R1 and R2. Single-cell suspensions of human PBMC were labeled with CD4-PE, CD25-FITC and CD127-APC for flow cytometric analysis. (**c and d**) Flow cytometric analysis of PBMC from healthy individuals prepared at three days after fractions treatment. Blank: untreated PBMC; ck: PBMC with anti-CD3/anti-CD28/IL-2 stimulation. (**e**) Isolation of fraction 2–7 from the pooled protein fraction by a C4 RP-HPLC column. (**f and g**) FACS analysis of human $CD4^+CD25^+CD127^{low}$ Tregs after treating fractions 2–5 and 2–7. (**h and i**) Flow cytometric analysis of Tregs from human PBMC after exposure to pS1-pS11 from fraction 2–7. (**j**) IL-10 protein level in human PBMC culture supernatants measured by ELISA. Data are presented as mean ± SEM (One-way ANOVA with Tukey's multiple comparisons). #$P < 0.05$, ##$P < 0.01$, ###$P < 0.001$ *vs* blank; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ *vs* ck.

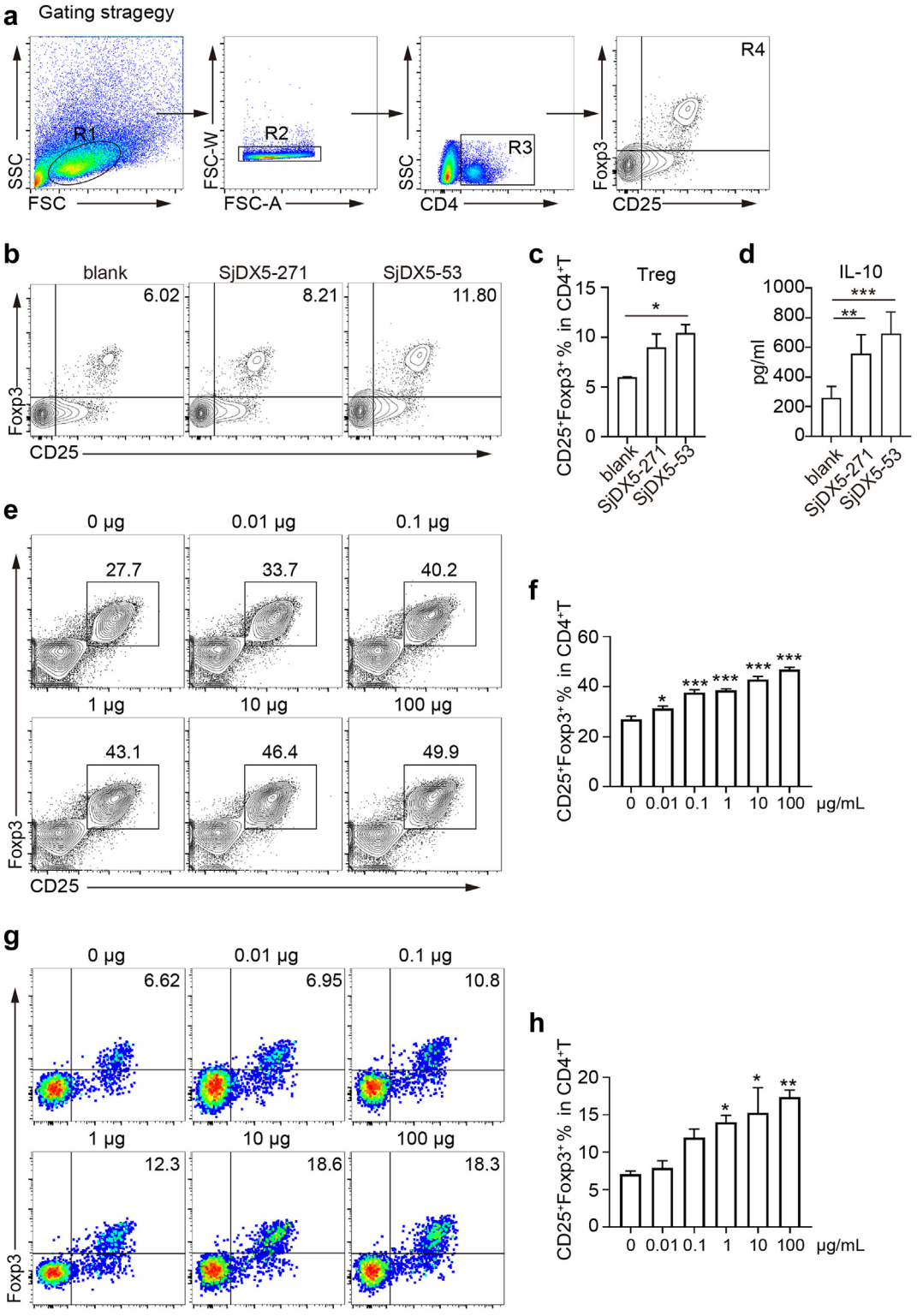

Fig. 2: **SjDX5-53 promoted generation of Treg cells.** (**a**) Gating schemes for analysis of Tregs ($CD4^+CD25^+Foxp3^+$) were gated from R1 and R2. (**b and c**) Splenic cells from mice were stained with CD4-FITC and CD25-APC, and intracellularly stained with Foxp3-PE for detection and calculation of Treg cells. Flow cytometric analysis of proportion of splenic Treg cells after stimulating with SjDX5-53 or SjDX5-271. (**d**) IL-10 protein level in splenocytes culture supernatants measured by ELISA. (**e and f**) Single-cell suspensions of mice splenic cells were labeled

was set on obtained *P*-values to define statistically significant genes for each comparison.

Statistical analysis

Statistical analyses were carried out with the GraphPad Prism 8 software. Data were shown as mean ± standard error of the mean (SEM) from at least three independent experiments. The normal distribution of each data was evaluated for normal distribution using the KolmogorovSmirnov test, Shapiro–Wilk, q-q-plots and histograms test. The significance of difference between two groups was identified using a Student's *t* test. Multiple comparisons were performed by one-way ANOVA with Bonferroni post hoc test for comparison between two groups. P values < 0.05 were considered significant. Significant differences were as follows: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Role of funders

The funders were not involved in the study design, data collection, data analyses, interpretation and writing of report.

Results

Identification of pharmacological peptides from egg extracts of *S. japonicum*

Considering that helminth infection or helminth-derived molecules induce Tregs to prevent autoimmune diseases,[29,30] we tracked the peptides that caused the enhancement of Tregs with various peptide treatments and detected by flow cytometry. As illustrated in Fig. 1a, the supernatant of schistosome eggs extract was divided into 4 fractions by Protein-Pak 125 gel filtration. Fraction 1, 2 and 3 + 4 increased Treg cell frequencies ($CD4^+CD25^+CD127^{low}$)[28] when human peripheral blood mononuclear cell (PBMC) were under Treg cell-polarizing conditions. Among the 4 fractions, a higher proportion of Tregs were induced after stimulation with fraction 2 (Fig. 1b–d). Fraction 2 was further purified by C4 RP-HPLC column as illustrated in Fig. 1e, and the eluted fractions 2–5 and 2–7 had the ability to increase the frequency of human Tregs (Fig. 1f and g). The small peptides were subsequently identified from the fraction 2–7 by liquid chromatography-mass spectrometry (LC-MS). The peptides pS1 and pS2 stimulation showed a significant increase in the Treg cell compartment ($CD4^+CD25^+CD127^{low}$ and $CD4^+CD25^+Foxp3^+$, Fig. 1h and i, Supplementary Fig. S1a–f) with or without Treg cell-polarizing conditions. Moreover, pS1 and pS2 promoted anti-inflammatory cytokine IL-10 secretion (Fig. 1j), but not interferon (IFN)-γ or IL-4 (Supplementary Fig. S1g and h). These two sequences (named SjDX5-271 and SjDX5-53, respectively) were derived from the glutenin high molecular weight subunit DX5 protein of *S. japonicum* (SjDX5) by a search of non-redundant protein databases of the Basic Local Alignment Search Tool (BLAST) server.

SjDX5-53 promotes tregs differentiation and suppressive capability

To ascertain if SjDX5-271 and SjDX5-53 are associated with T cell modulatory effects, we examined the proportion of T cell subsets in splenocytes of mice after peptides treatment. Strikingly, SjDX5-53 stimulation showed a stronger increase in the frequency of forkhead box protein P3 $(Foxp3)^+$ cells in splenocytes (Fig. 2a–c) and the level of IL-10 in culture supernatants (Fig. 2d); whereas the level of IL-4 was not affected (Supplementary Fig. S2b). We observed that Tregs generation was increased in a dose-dependent manner with or without Treg cell-polarizing conditions (Fig. 2e–h). Additionally, SjDX5-53 suppressed the proportion of Th1 and Th17 subsets in splenocytes of mice (Supplementary Fig. S2c–e), and decreased the production of IFN-γ in the supernatant (Supplementary Fig. S2a). The modulatory effects of SjDX5-53 on Treg cells were cell-type specific, as SjDX5-53 had no effect on naïve T cell differentiation into Th1 or Th17 cells as assessed by intracellular cytokine staining of IFN-γ and IL-17 (Supplementary Fig. S2f and g).

To assess the suppressive capacity of Tregs toward responder cells following SjDX5-53 administration, we performed a carboxyfluorescein diacetate succinimidyl ester (CFSE)-based suppression assay of Treg cells (Fig. 3a). The suppressive activity of Treg cells was more intense in SjDX5-53-stimulated Tregs compared to control Tregs sorted from splenocytes (Fig. 3b). We also assessed whether the increased Tregs suppressive function is observed *in vivo* studies when peptide-treated Treg cells are transferred into murine models of autoimmune disease. In the colitis rescue model, 4×10^5 WT naïve $CD4^+$ T cells and 2×10^5 Treg cells harvested from splenocytes with or without SjDX5-53 stimulation were intravenously transferred to $Rag1^{-/-}$ mice (Fig. 3c), and then observed for weight loss and clinical signs of colitis. $Rag1^{-/-}$ mice receiving SjDX5-53-stimulated Tregs showed decelerated pathology, less weight loss, colon shortening, and intestinal wall thickening in colitis mice (Fig. 3d–f), while mice receiving control Tregs showed more prominent colitis

with CD4-FITC, CD25-APC and Foxp3-PE for flow cytometric analysis of Tregs, and the percentage of $CD4^+CD25^+Foxp3^+$ cells were analyzed with anti-CD3/anti-CD28/IL-2/TGF-β stimulation. (**g and h**) Single-cell suspensions of splenic cells of mice were labeled with CD4-FITC, CD25-APC and Foxp3-PE for flow cytometric analysis of Tregs, and the percentage of $CD4^+CD25^+Foxp3^+$ cells were analyzed without anti-CD3/anti-CD28/IL-2/TGF-β stimulation. Data are presented as mean ± SEM (One-way ANOVA with Tukey's multiple comparisons). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

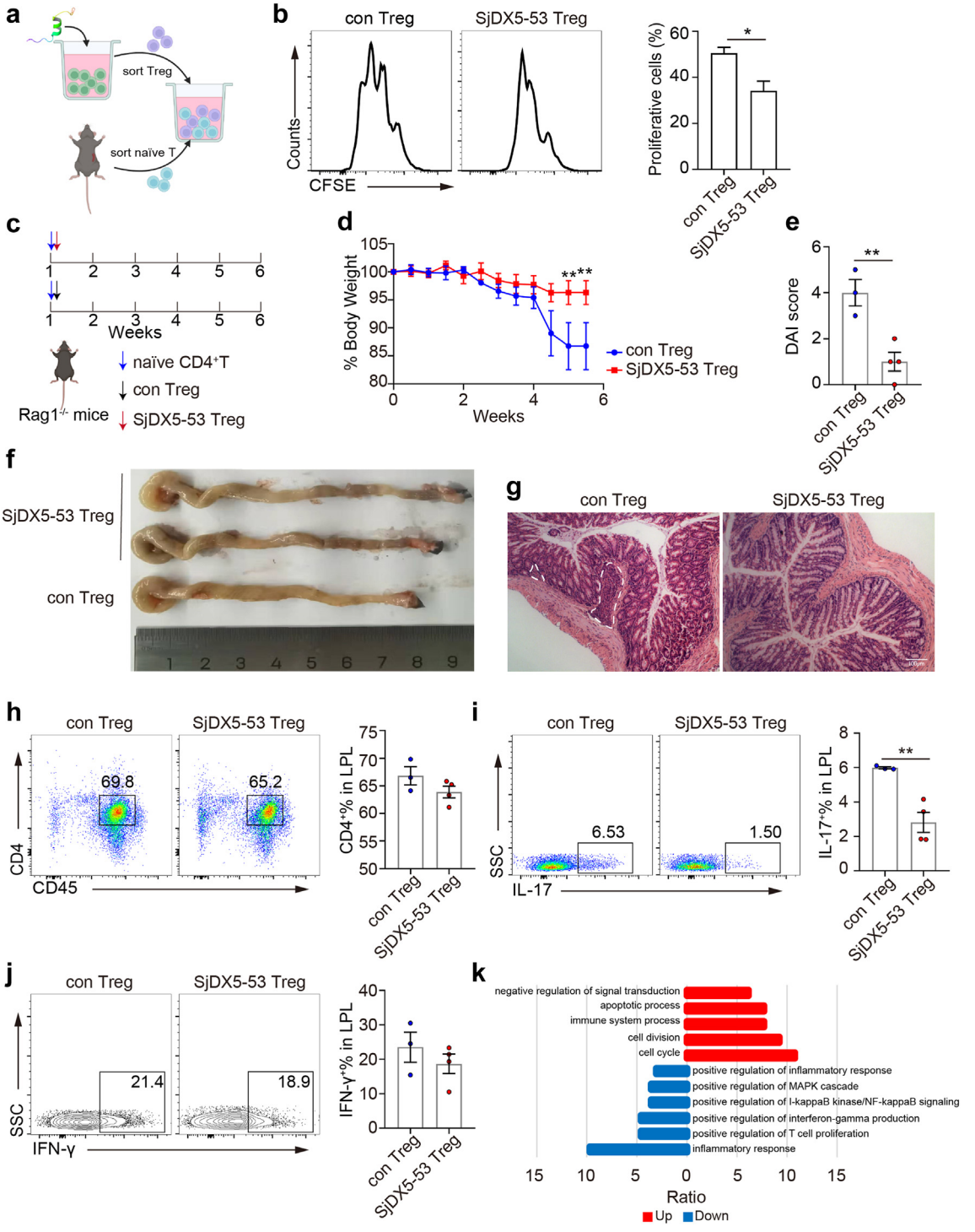

***Fig. 3:* SjDX5-53 induces functional Tregs.** (**a and b**) Naïve $CD4^+$ T cells of mice were labeled with CFSE and mixed with SjDX5-53-treated Treg cells. (**c**) $Rag1^{-/-}$ mice were adoptively transferred i. v. with 4×10^5 WT Tconv and 2×10^5 Treg cells stored from splenocytes with or without SjDX5-53 stimulation (n = 3–4 mice). (**d**) Weight change of $Rag1^{-/-}$ mice during T cell adaptive transfer-induced colitis. (**e**) Disease activity index (DAI) scores of mice. (**f**) Appearance and length of intestinal tissue. (**g**) Hematoxylin and eosin histology of representative colons of colitis are shown. Scale bar, 100 μm. (**h–j**) Flow cytometric analysis and quantification of the frequency of $CD45^+$, Th1, Th17 cells in the colon lamina propria from mice with intestinal inflammation. (**k**) Pathway enrichment analysis of differentially expressed genes in RNA-sequence data. Data are presented as mean ± SEM. (**d**) Two-way ANOVA with Tukey's multiple comparisons. (**e, h–j**) Unpaired two-tailed Student's *t*-test. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

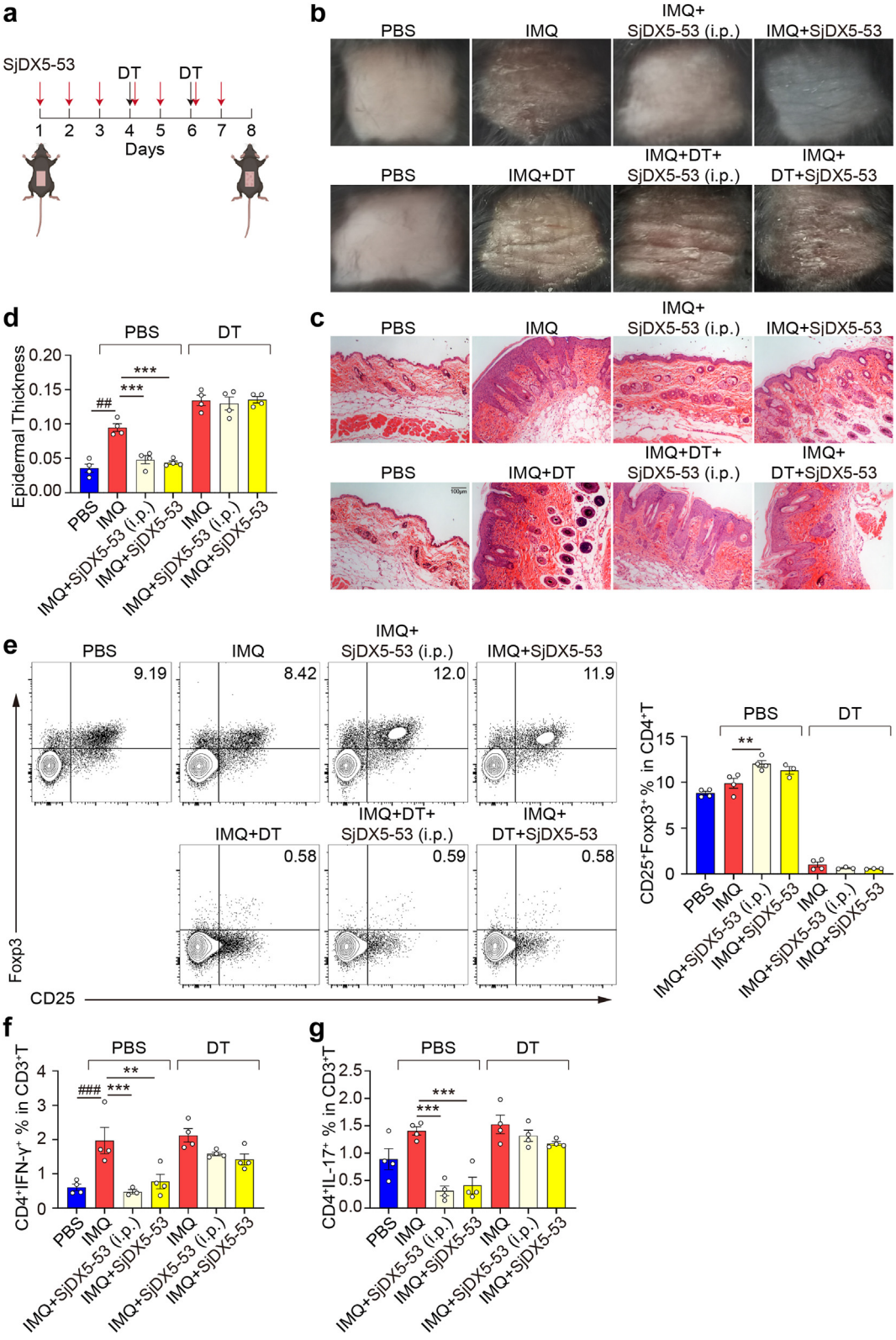

***Fig. 4:* Specific ablation of Foxp3⁺ Treg cells abrogated the effect of SjDX5-53 against psoriasis-like features.** (**a**) Schematic of the experimental design. Foxp3^{DTR} mice or WT mice with IMQ for 7 consecutive days were injected 500 ng diphtheria toxoid (DT) on day 4 and 6

including transmural inflammation, ulceration, reactive epithelial changes and crypt abscesses (Fig. 3g). Additionally, as shown in Fig. 3h–j, SjDX5-53-induced Tregs suppressed the proportion of Th17 cells in the intestinal lamina propria lymphocytes (LPL) in colitis mice. To detect differences in gene expression between control and SjDX5-53-stimulated splenocytes, we analyzed their gene expression profiles by RNA-seq. Intriguingly, gene clusters associated with inflammatory response, regulation of IFN-γ production and regulation of I-kappaB kinase/NF-kappaB signaling were significantly down-regulated in the SjDX5-53-stimulated splenocytes (Fig. 3k and Supplementary Fig. S3). Altogether, peptide SjDX5-53 increased the population and suppressive activity of Treg cells.

SjDX5-53 represses psoriasis-like skin inflammation dependent on tregs

Due to the enhanced suppressive activity of SjDX5-53-treated Treg cells, we hypothesized that SjDX5-53 harbored the therapeutic potential on autoimmune diseases such as psoriasis. To investigate whether SjDX5-53 could suppress inflammatory responses generated in psoriasis, we subjected mice to the IMQ model, and treated with a daily intraperitoneal or topical dose of SjDX5-53 (Fig. 4a). IMQ application to C57BL/6 mice for 7 days induced an exaggerated skin inflammation that was reflected by erythema, scaling and thickening (Fig. 4b–d). Compared with IMQ-treated WT mice, mice receiving treatment with SjDX5-53 showed a striking suppression, characteristic of psoriasis including parakeratosis, acanthosis, elongation of the dermal papillae and skin inflammation (Fig. 4c). In addition, the flow cytometric results showed that both SjDX5-53 injection via intraperitoneal and local administration remarkably increased Treg cells ($CD4^+CD25^+Foxp3^+$; Fig. 4e) in the total $CD4^+$ cell population; and significantly reduced the numbers of pro-inflammatory Th1 ($CD3^+CD4^+IFN\text{-}\gamma^+$; Fig. 4f) and Th17 ($CD3^+CD4^+IL\text{-}17^+$; Fig. 4g) cells.

To determine the importance of Tregs in the protection induced by SjDX5-53 against psoriasis-like inflammation, we treated the $Foxp3^{DTR}$ mice with IMQ for 7 consecutive days and then injected 500 ng diphtheria toxoid (DT) on day 4 and 6 after IMQ treatment initiation[25] simultaneously with or without SjDX5-53 administration (Fig. 4a). After exposure to DT, Treg cells in mice were almost completely absent, demonstrating that Treg cells depletion was highly efficient (Fig. 4e). The results indicated that the protective anti-inflammatory effect of SjDX5-53 against IMQ-induced inflammation was abrogated in the absence of Tregs (Fig. 4b–g). These observations demonstrated that the anti-inflammatory effect of SjDX5-53 was mediated through the activation of Tregs.

Induction of tregs by SjDX-53 requires the participation of DCs

We next assessed whether DCs are required for SjDX5-53-mediated potential of Treg cell induction. SjDX5-53 could not directly increase Treg cell frequencies and immune suppressive function when only naïve T cells are in the culture system. However, SjDX5-53 significantly enhanced Treg cell frequencies when naïve T cells were co-cultured with DCs (Fig. 5a–c). In CFSE-labeled *in vitro* assay, SjDX5-53 treatment did not prevent T cell division when DCs were absent. Rather, compared to unstimulated Tregs (con Treg) or control peptide stimulated Tregs (con peptide Treg), SjDX5-53 treated Tregs (SjDX5-53 Treg) isolated from DCs and T cells co-culture system showed stronger suppressive function against effector T cells proliferation *in vitro* (Fig. 5d–f). This effect was also shown in an *in vivo* assay. We established a classic model of T-cell mediated colitis in Rag1-deficient mice, in which a mixture of effector T cells with unstimulated, control peptide-treated or SjDX5-53-induced Treg cells isolated from DCs and T cells co-culture system was transferred. Compared with con Treg and con peptide Treg cells as donors, mice receiving SjDX5-53-treated Treg cells showed less weight loss and alleviated pathology 3 weeks after adoptive transfer (Fig. 5g and h). Five weeks after cell transfer, the colons of mice treated with control peptide Tregs were shortened and thickened (Fig. 5i). Histological analysis revealed a loss of goblet cells, a transmural thickening of the colon, and a high infiltration of inflammatory cells of mice in con Treg and con peptide Treg groups; while reinfusion with SjDX5-53 Tregs effectively alleviated the pathology of colitis (Fig. 5j). Flow cytometric analysis also confirmed that mice receiving SjDX5-53 Treg cells showed decreased IFN-γ and IL-17 production in colon lamina propria (Fig. 5k and l). Together, these results suggested that DCs are required for mediating the Treg cells-induced suppression by SjDX5-53.

SjDX5-53 induced DCs displays tolerogenic features through TLR2

It was reported that tolDCs could stimulate naive T cells to become Tregs.[31] After 24 h of lipopolysaccharide (LPS)

after IMQ treatment initiation with or without SjDX5-53 administration (n = 4 mice). (**b**) Representative phenotype of SjDX5-53 treated IMQ-induced mice. Experiments were repeated independently for three times. (**c**) Representative images of back stained by hematoxylin and eosin. Scale bar, 100 μm. (**d**) Back thickness of IMQ-induced and SjDX5-53-treated mice was measured. (**e**–**g**) Flow cytometry detection of Treg, Th1 and Th17 cells proportion in splenic suspensions from IMQ-treated mice. Data are presented as mean ± SEM (One-way ANOVA with Tukey's multiple comparisons). #$P < 0.05$, ##$P < 0.01$, ###$P < 0.001$ *vs* PBS; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ *vs* IMQ.

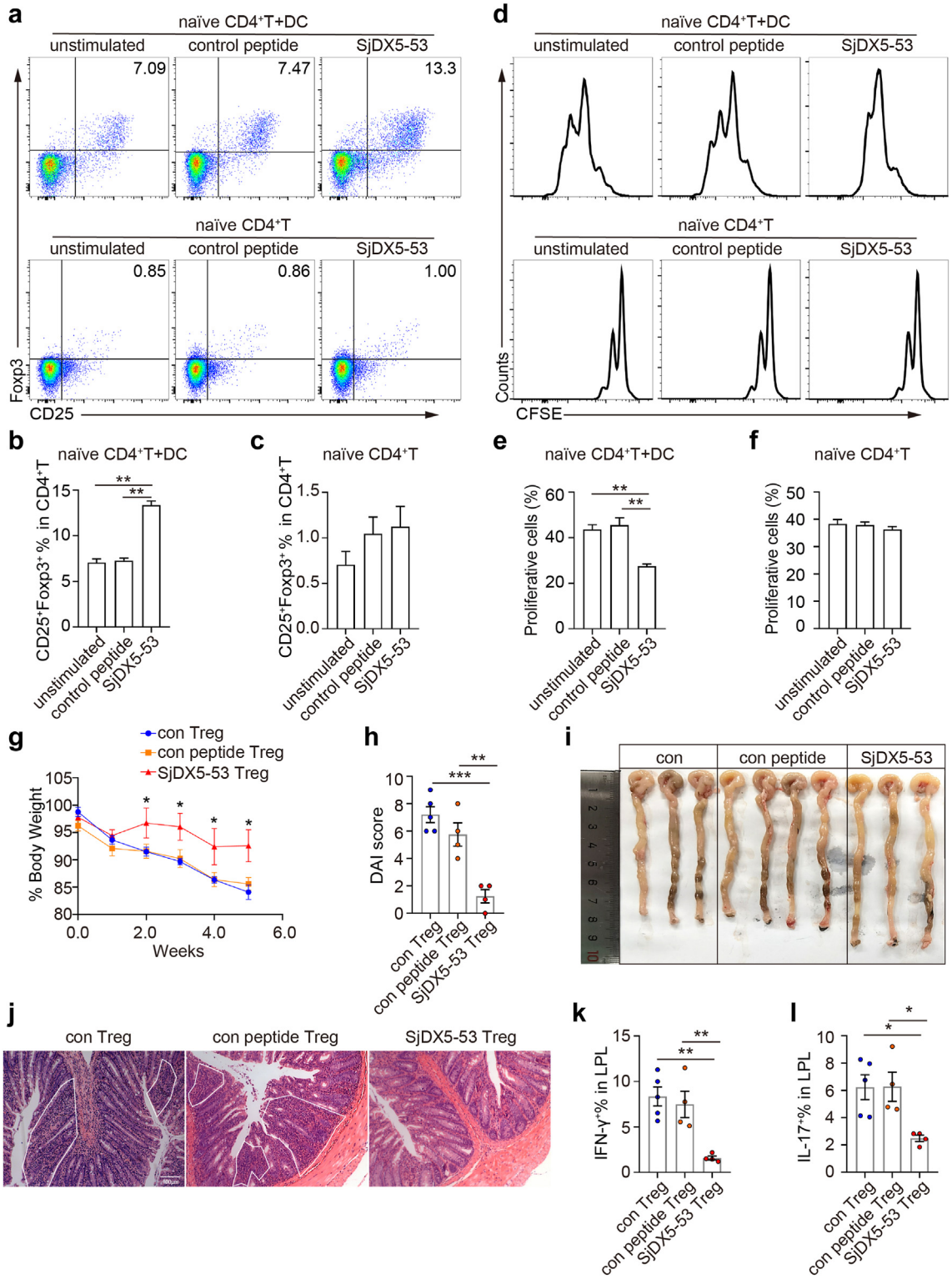

Fig. 5: **SjDX5-53-pulsed BMDCs induced CD4⁺CD25⁺Foxp3⁺ Tregs.** (**a–c**) CD4⁺ T cells were purified from mice and cultured for 3 days with or without BMDCs (2:1) in the present of 10 μg/mL SjDX5-53 or control peptide. $CD4^+CD25^+Foxp3^+$ T cells were analyzed using flow cytometry following double staining for CD25 and Foxp3 expression of cells gated for CD4⁺ expression. (**d–f**) Treg cells were sorted from CD4⁺ T cells

stimulation, SjDX5-53-treated bone marrow-derived dendritic cells (BMDCs) produced significantly lower levels of the pro-inflammatory cytokines IL-6 and higher levels of the anti-inflammatory cytokines IL-10, transforming growth factor beta (TGF-β) and amphiregulin (AREG) (Fig. 6a–d). Supporting these data, SjDX5-53 stimulated BMDCs showed decreased expression of CD86, CD40 and major histocompatibility complex (MHC) II compared to control peptide-treated BMDCs (Fig. 6e–g). Subsequently, for more in-depth analysis, a transcriptome study was conducted to compare gene expression by RNA-seq between LPS-treated BMDCs and LPS + SjDX5-53-treated BMDCs (Supplementary Fig. S4a and b). 594 genes were induced and 166 genes were repressed in LPS + SjDX5-53-treated BMDCs in comparison with LPS-treated BMDCs (FDR < 0.05, logFC > 1.5). Gene ontology (GO) analysis revealed enrichment of functional categories associated with immune system regulation, including negative regulation of IL-17 production and negative regulation of IL-1-mediated signaling pathway ((Supplementary Fig. S4c and d). SjDX5-53-treated BMDCs expressed significantly higher mRNA levels of the classical tolerogenic molecules *Cd274*, *Icosl*, *Ido1*, *Il10*, *Areg* and *Ctla4* than LPS-treated DCs (Fig. 6h).

Toll-like receptor 2 (TLR2) is necessary for priming active Treg[32] and their expansion during schistosomiasis.[33] Subsequently, we sought to decipher the structural basis of the SjDX5-53-TLR2 interaction. We used the ZDOCK and ROSETTA platform to perform docking of the protein structure of TLR2 and SjDX5-53. The predicted binding mode of SjDX5-53 to the TLR2 protein revealed a good shape match between SjDX5-53 and TLR2; Gln2, Arg8, Gln17 and Asn21 residues of SjDX5-53 were involved in forming hydrogen bonds with TLR2 (Fig. 7a). The binding affinity was also calculated by employing the MM/GBSA approach.[34] The predicted total binding free energies ΔG was −47.6 kcal/mol, and this indicated that SjDX5-53 might bind to TLR2 (Supplementary Table S2). To prove that the TLR2 pathway was involved, BMDCs from TLR2-deficient mice were stimulated with SjDX5-53. Compared to control peptide-treated BMDCs, the expression of CD86 was decreased in SjDX5-53-treated DCs; while there was no difference in TLR2$^{-/-}$ DCs with or without SjDX5-53 treatment (Fig. 7b and c). As showed in Fig. 7d and e, the increased proportion of SjDX5-53 induced Tregs was lost in TLR2$^{-/-}$ splenocytes, as well as TLR2$^{-/-}$ DCs and naïve T cells co-cultured system. We then investigated whether TLR2 is essential for SjDX5-53 inhibition of autoimmune disease. We treated WT and TLR2$^{-/-}$ mice with or without SjDX5-53 once per day for 7 consecutive days using IMQ-induced psoriasis-like mouse model. IMQ treated TLR2$^{-/-}$ mice developed severe psoriasis and produced more pro-inflammatory factors than untreated TLR2$^{-/-}$ mice (Fig. 7f–j). Moreover, the deficiency of TLR2 deprived SjDX5-53 peptide of its ameliorative effect and represented similar inflammatory phenotype in skin of IMQ induced-TLR2$^{-/-}$ mice (Fig. 7h). Also, similar levels of Treg, Th1 and Th17 cells proportion were present in control peptide-treated and SjDX5-53-treated TLR2$^{-/-}$ mice with psoriasis (Fig. 7k–m). These results indicated that signaling through TLR2 is required for SjDX5 to induce tolDCs and influence the activation of Tregs.

SjDX5-53 attenuates psoriasis-like phenotype *in vivo*

To rule out the toxicity of SjDX-53, we tested its cytotoxicity and hemolytic activity. Incubation with SjDX5-53 at concentrations ranging from 0 to 100 μg/mL for 24 h did not inhibit the viability of HEK-293T cells (Supplementary Fig. S5a). In the hemolysis assay, SjDX5-53 exhibited lower hemolytic activity against red blood cells (Supplementary Fig. S5b). To investigate the functional relevance of SjDX5-53 in the development of autoimmune disease, we applied low concentration and high concentration of SjDX5-53 locally once per day for 7 consecutive days in the IMQ-induced psoriasis-like mouse model (Fig. 8a). Compared with the IMQ group, the IMQ + SjDX5-53L and IMQ + SjDX5-53H treatment groups both showed the abated psoriasis-like pathological progression and decreased disease severity (Fig. 8b–d). To confirm the reduction of pro-inflammation in skin tissue from the SjDX5-53-treated groups, we analyzed the mRNA expression of cytokines. The results showed that the level of *Il6*, *Tnfa* and *Il1b* from SjDX5-53-treated mice were decreased significantly compared with the IMQ group (Fig. 8b). In addition, SjDX5-53 treatment presented more effective in alleviating the acanthosis, and dermal inflammatory cell infiltration than calcipotriol (Cal) treatment (Fig. 8c and d). Consistently, enhanced Foxp3 (Fig. 8c and e) expression and reduced IFN-γ (Fig. 8f) and IL-17 (Fig. 8g) expression was observed in CD4$^+$ T cells of skin and spleen after SjDX5-53 treatment. These results demonstrated that SjDX5-53 could block the development of IMQ-induced psoriasis.

culture system with or without BMDCs. Naïve CD4$^+$ T cells were labeled with a cell proliferation dye CFSE and cultured for 3 days in the presence of Tregs. (**g**) Rag1$^{-/-}$ mice were adoptively transferred with 4 × 10^5 WT Tconv and 2 × 10^5 Treg cells stored from co-cultured system of T cells and DCs with or without SjDX5-53 stimulation (n = 4–5 mice). Weight change of Rag1$^{-/-}$ mice during T cell adaptive transfer-induced colitis. (**h**) DAI scores of mice. (**i**) Length of colon. (**j**) H&E staining. Scale bar, 100 μm. (**k**, **l**) Flow cytometric analysis and quantification of Th1 and Th17 in the colon lamina propria from mice. Data are presented as mean ± SEM. (**g**) Two-way ANOVA with Tukey's multiple comparisons. (**b–f, k, l**) One-way ANOVA with Tukey's multiple comparisons. *P < 0.05, **P < 0.01, ***P < 0.001.

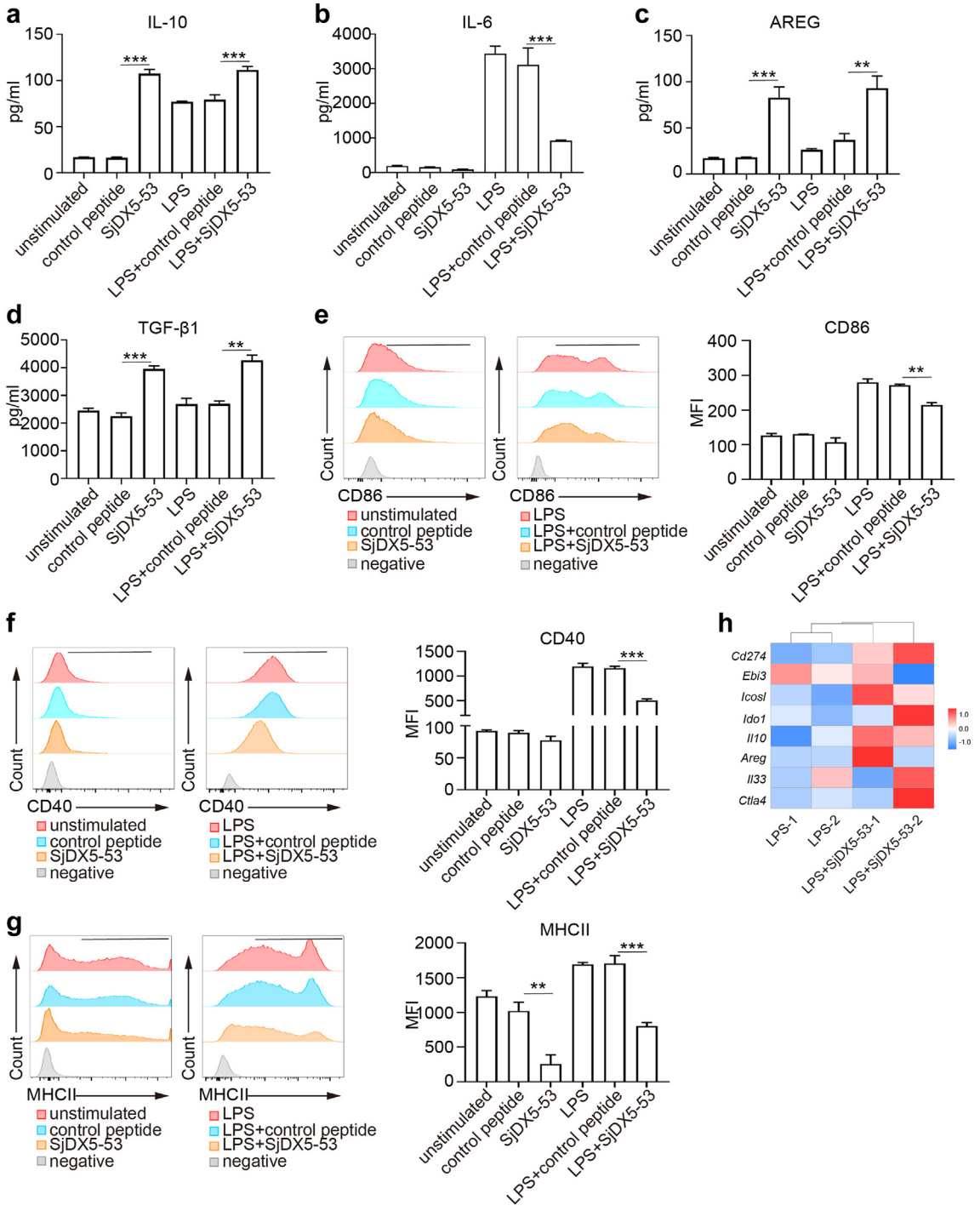

Fig. 6: **SjDX5-53-pulsed BMDCs were associated with tolerogenicity.** (**a–d**) Cytokine or cytokine ratios produced in the presence of medium (Ctrl-) or LPS of conventional BMDCs or SjDX5-53-tolDCs. (**e–g**) Cell surface and co-stimulatory markers of mice BMDCs were analyzed by flow cytometry. (**h**) Heat map analysis of mRNA expression of tolerogenic molecules between LPS and LPS + SjDX5-53-treated BMDCs. Data are presented as mean ± SEM (One-way ANOVA with Tukey's multiple comparisons). *P < 0.05, **P < 0.01, ***P < 0.001.

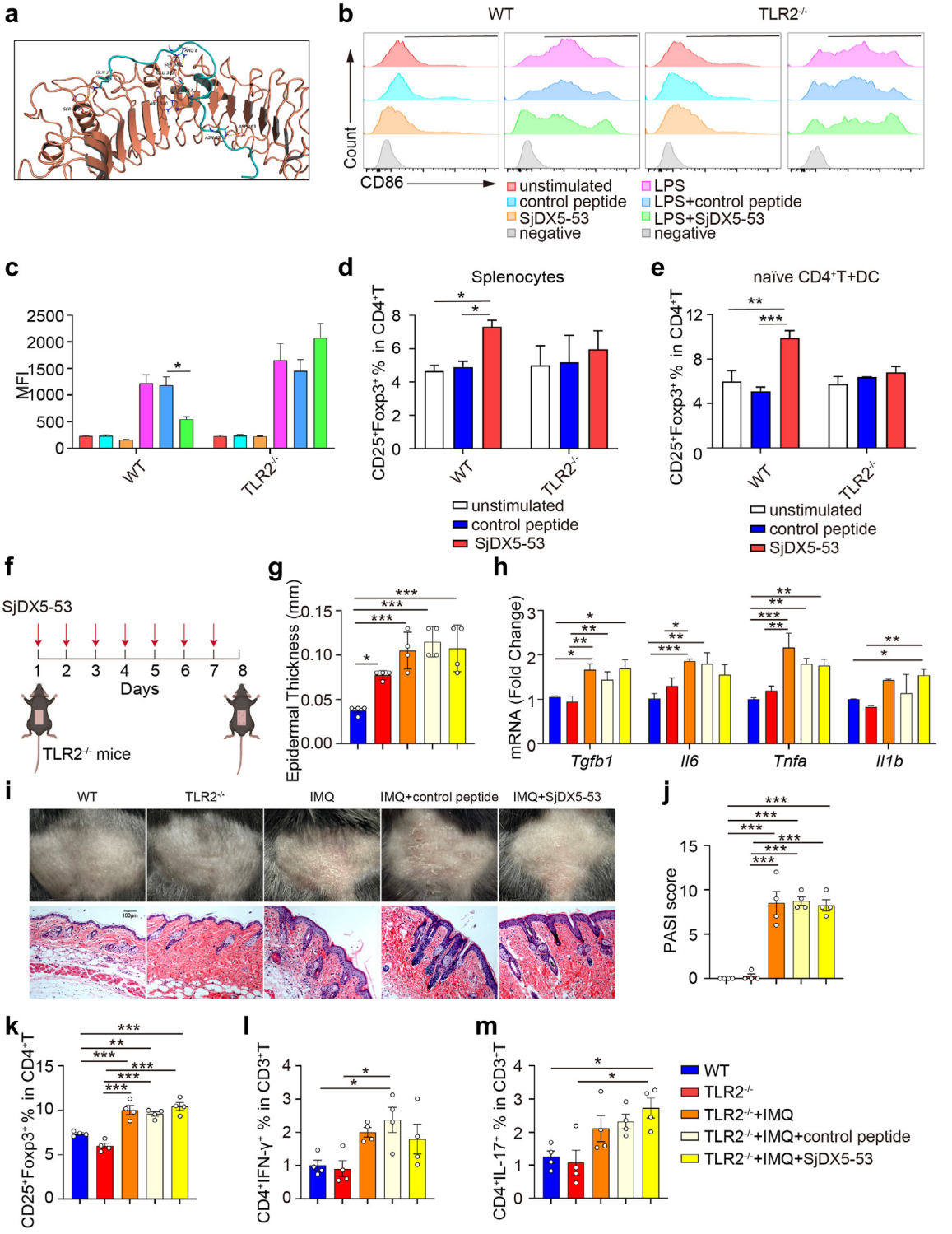

***Fig. 7*: TLR2 was essential for SjDX5-53 inhibition of auto-immune disease.** (**a**) Predicted interaction of protein structures of SjDX5-53 and TLR2 imitated by ZDOCK and ROSETTA. (**b, c**) The expression of CD86 from WT and TLR2$^{-/-}$ BMDCs were analyzed by flow cytometry. (**d**) CD4^{+}CD25^{+}Foxp3^{+} T cells were analyzed using flow cytometry of WT and TLR2$^{-/-}$ splenocytes after SjDX5-53 treatment. (**e**) CD4^{+} T cells were purified from WT mice and cultured for 3 days with WT or TLR2$^{-/-}$ BMDCs (2:1) in the present of 10 μg/mL SjDX5-53 or control peptide. The percentage of CD4^{+}CD25^{+}Foxp3^{+} cells were analyzed by flow cytometry. (**f**) Graphic therapeutic time-line of IMQ-induced modelling of TLR2$^{-/-}$

To further confirm the anti-psoriatic effect of SjDX5-53 *in vivo*, we used Card14$^{E138A/+}$ mouse model with spontaneously producing chronic skin inflammation similar to human psoriasis.[35,36] The Card14$^{E138A/+}$ mice with psoriasis were treated with SjDX5-53 every day (Fig. 8h). After a continuous treatment of SjDX5-53 for 7 days, an obvious improvement of psoriatic symptoms was observed compared with the control group. SjDX5-53 effectively improved erythema, reduced scales and inhibited skin thickening (Fig. 8i and j). Histology showed that SjDX5-53 treatment contributed to a significant alleviation in the psoriatic disease indexes, including hyperkeratosis, focal parakeratosis, epidermal thickness, acanthosis, lack of granular layer, and abundant infiltrated lymphocytes (Fig. 8i). Meanwhile, SjDX5-53 significantly reduced inflammatory cytokines expression (such as *Il6*, *Tnfα* and *Il1b*) in skin lesions (Fig. 8k). Flow cytometry further characterized that the population of Treg cells was significantly increased in SjDX5-53-treated mice compared with PBS control group mice (Fig. 8l). We found that SjDX5-53 could reduce the numbers of Th1 and Th17 compared with those in PBS-treated mice (Fig. 8m and n). Thus, SjDX5-53 alleviated psoriasis-like inflammation and induced Tregs in both the IMQ model and Card14$^{E138A/+}$ mice.

Discussion

During long-term coevolution, both hosts and parasites have applied pressure on each other through complex molecular host-parasite interplay[37] so as to achieve immune homeostasis, allowing the infection to become chronic. Schistosome infection establishes an inherent immune pattern, that is, Th1 response is dominant in the early stage of infection, and when a large number of eggs appear, it shifts to Th2 immune response and gradually develops the robust immune regulation, in which eggs are immunologically unique to induce the potent Th2 response as well as regulatory T cell immune responses.[38,39] Population studies have also shown increased Treg frequencies in schistosomiasis affected individuals.[4] Therefore, it is completely feasible to seek some bioactive components that can induce Treg cells from eggs.

Helminth infection and injection with eggs of helminth were reported to reduce allergic, autoimmune, and inflammatory reactions.[40,41] Many studies focus on higher molecular weight proteins (>5 kDa), and there is a notable absence of research on lower molecular weight products (1–5 kDa).[42] Previously, some helminth-derived proteins with immunomodulatory effects were reported. Macrophage migration inhibitory factor (MIF)-2, isolated from the nematode *Anisakis simplex*, was shown to ameliorate dextran sodium sulfate-induced colitis and allergic airway inflammation through Treg induction.[43,44] Recently, Ryan SM et al. reported a biologics discovery and validation pipeline to generate and screen *in vivo* a recombinant cell-free secretome library of hookworm-derived immunomodulatory proteins, and confirmed 5 hookworm proteins with suppression of inflammatory cytokine secretion by T cells from inflammatory bowel disease (IBD) patient colon biopsies and anti-IBD properties in a mouse model of acute colitis.[45] To our interest, the study of venoms regarding the identification of bioactive peptides[46] suggests that this is a feasible approach to finding the active ingredient in the parasites. Typically, hundreds of venom components were separated using initial purification by RP-HPLC and sometimes orthogonal chromatographic techniques, until the active component was isolated. Although slow, this extremely productive approach led to the discovery of many bioactive peptides and laid a critical foundation for further studies. Thus, using a combination of gel filtration and RP-HPLC, we separated active components from *S. japonicum* eggs and identified a 3223.52 Da peptide, named SjDX5-53, that could significantly promote the differentiation of Tregs in human PBMC. Subsequently, this peptide was proved to increase murine Treg cells and enhance their suppressive function.

The contribution of Tregs in human autoimmune diseases has opened up a new therapeutic avenue. Therapeutic application of adoptively transferring Tregs to treat autoimmune diseases is relatively limited, in part, by the scarcity of Tregs in the peripheral blood.[47] Rather than passive Tregs transfer, some chemical or physical treatments that can induce Tregs production are constantly being explored. Our data showed that the addition of SjDX5-53 markedly up-regulated inducible regulatory T cells (iTreg) generation in human PBMC and murine splenocytes *in vitro*. And SjDX5-53-induced Tregs secreted more IL-10 and significantly inhibited T cell proliferation. Transfer with SjDX5-53-induced Tregs obviously suppressed T-cell mediated colitis and manifested robust immunosuppression. Furthermore, this peptide was directly applied in psoriasis-like mouse models. Psoriasis is characterized by epidermal hyperplasia, increased angiogenesis, and dermal infiltration of inflammatory cells,[48] and its pathogenesis is driven by

mice (n = 4 mice). (**g**) Skin thickness in IMQ-treated TLR2$^{-/-}$ mice in present of SjDX5-53. (**h**) mRNA levels of inflammatory cytokines in back skin were determined by qPCR. (**i**) Representative image of skin damage and Hematoxylin-Erosin (H&E) staining of mice skin. Scale bar, 100 μm. (**j**) Psoriasis area and severity index (PASI) score. (**k–m**) Flow cytometric analyses of Treg, Th1 and Th17 cells from the spleens of TLR2$^{-/-}$ mice after 7 days of SjDX5-53 treatment. Data are presented as mean ± SEM. (**c, d, e, h**) Two-way ANOVA with Tukey's multiple comparisons. (**g, j, k, l, m**) One-way ANOVA with Tukey's multiple comparisons. *P < 0.05, **P < 0.01, ***P < 0.001.

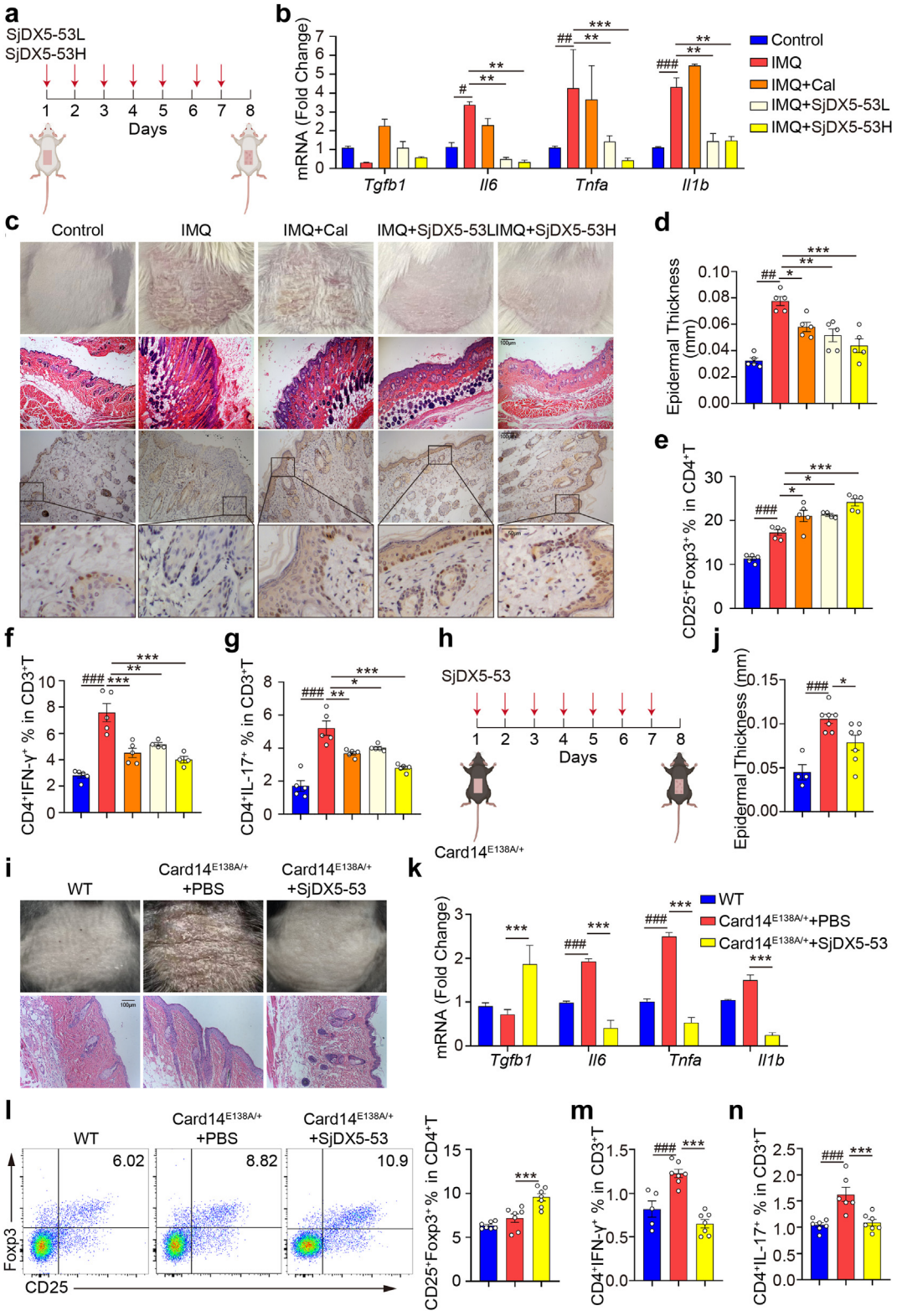

Fig. 8: **Attenuation of IMQ-induced and spontaneous psoriasiform skin symptoms by SjDX5-53.** (**a**) Graphic therapeutic time-line of IMQ-induced modelling. Calcipotriol was used as the positive control (n = 5 mice). (**b**) mRNA levels of inflammatory cytokines in back skin were

the dysfunction of T-cell subsets and the resulting aberrant release of the inflammatory cytokines.[49] At present, some biologics specifically those targeting the inflammatory cytokines or their receptors are substantially more efficacious than conventional systemic immunosuppressive medications in patients with moderate or severe psoriasis.[50] However, lack of adherence to biologics therapy mainly results in loss of response over time and adverse events.[51] In our study, treatment with SjDX5-53, whether intraperitoneal injection or local skin administration, showed a striking suppression of skin lesion, accompanied by remarkably increased Treg cells and significantly reduced the numbers of Th1 and Th17 cells. The protective effect of SjDX5-53 against IMQ-induced and spontaneous inflammation was abrogated when Treg cells were depleted. Thus, the influence of SjDX5-53 peptide treatment on Treg frequency and functionality was important in counteracting psoriasis, as indicated by the alleviation of acanthosis and dermal inflammatory cell infiltration as well as the gradual equilibration of immunity. The limitations of peptide drugs, including low oral bioavailability, low plasma stability and short circulation time, were recognized. Therefore, topical applications may be the most promising route for peptide drugs.[52,53] In addition, due to the common side effects of peptide drugs, cell viability and hemolysis assay were performed to evaluate *in vitro* toxicity of SjDX5-53; meanwhile we did not observe obvious toxicity of this peptide product to mice.[54] Although SjDX5-53 has shown good efficacy and low toxicity in animal models and cells experiments in our study, it is still necessary to assess the safety and efficacy of SjDX5-53 through appropriate clinical trials.

Further, we considered how SjDX5-53 peptide promoted Tregs production. It has been reported that helminth products could induce the tolDCs production in hosts. Excretory-secretory products (ES L1) released by *T. spiralis* muscle larvae induced semimature/tolerogenic status of DCs that shifted the immune response towards a Th2 and regulatory type of T cells. Moreover, induction of tolDCs by ES L1 involved engagement of multiple pattern recognition receptors namely, TLR2, TLR4 and DC-specific ICAM-grabbing nonintegrin (DC-SIGN).[55] In this study, SjDX5-53 treated BMDCs retained the tolerogenic phenotype and function despite of being under inflammatory stimuli. These SjDX5-53-treated tolDCs lacked sufficient co-stimulatory molecular signaling pathways mediated by CD86 and MHC II, and secreted more regulatory cytokines IL-10, AREG and TGF-β, which were essential for FOXP3$^+$ Treg generation[56,57] and regulation of peripheral tolerance. The transcriptomic profile also suggested that SjDX5-53-induced DCs highly expressed *Cd274*, *Icosl*, *Ido1*, *Il10*, *Areg*, and *Ctla4*. Compared to WT BMDCs, TLR2-KO BMDCs treated with SjDX5-53 induced lower ratio of Tregs *in vitro*, suggesting that TLR2 was required for SjDX5-53-induced Treg development and function. Therefore, SjDX5-53 could induce Treg cells with immunosuppressive activity via tolDCs generation.

Of note, immune adaptation between host and parasite has raised the innovative propositions and unveiled the importance of parasite-specific molecules in the protection against autoimmune conditions such as type 1 diabetes mellitus, rheumatoid arthritis, inflammatory bowel diseases as well as psoriasis.[16,58,59] Our encouraging findings starting from the peptides screening with human PBMC to the functional verification in animal models warrant further studies to assess the beneficial effects of SjDX5-53 peptide for clinical translation. With the rapid development of omics technology and large-scale identification technology, as well as the gradually deeper understanding of host-parasite interplay, more parasite-derived modulators will exhibit the potential for clinical applications.

Contributors

M. Ji and R. Lai conceptualized the study. Y. Ni, N. Luan designed the study and performed the experiments. Y. Ni, R. Xiong and Y. Zhu made the Figures, analyzed and interpreted the data. X. Xu, H. Wang, Y. Yang, S. Sun, Lin. Chen, Lu. Chen and M. Hou supervised the research. Manuscript was written and revised by Y. Ni, J.R. Padde, Z. Xu, M. Ji, and R. Lai. R. Lai gave some counseling and assistance in isolation and identification of peptides. K. Yang and C. Yu gave some assistance in the preparation of *S. japonicum* eggs. L. Shi provided suggestion and assistance for animal experiments. All authors read and approved the final version of the manuscript. Y. Ni and M. Ji have accessed and verified the data, and M. Ji was responsible for the decision to submit the manuscript.

Data sharing statement

All data associated with this study are present in the paper or the Supplementary Materials. The RNA-seq sequencing data have been deposited at NCBI Sequence Read Archive (SRA) and are publicly available as of the date of publication. Accession numbers (PRJNA872277).

determined by qPCR. (**c**) Representative image of skin damage, Hematoxylin-Erosin (H&E) staining (scale bar, 100 μm) and Foxp3 immunostaining staining of mice skin on day 8 of experiment (above scale bar, 100 μm; below scale bar, 50 μm). (**d**) Skin thickness in IMQ-treated mice. (**e–g**) Flow cytometric analyses of Treg, Th1 and Th17 cells from the spleens of individual mice after 7 days of IMQ treatment. (**h**) Graphic timeline of therapeutic schedule in Card14$^{E138A/+}$ mice (n = 7). (**i**) Representative image of skin damage and Hematoxylin-Erosin (H&E) staining of mice skin. Scale bar, 100 μm. (**j**) Skin thickness in Card14$^{E138A/+}$ mice after treating with SjDX5-53. (**k**) mRNA levels of inflammatory cytokines in back skin were determined by qPCR. (**l–n**) Flow cytometric analyses of Treg, Th1 and Th17 cells from the spleens of individual mice after 7 days of SjDX5-53 treatment. Data are presented as mean ± SEM. (**b, k**) Two-way ANOVA with Tukey's multiple comparisons. (**d–g, j, l–n**) One-way ANOVA with Tukey's multiple comparisons. #$P < 0.05$, ##$P < 0.01$, ###$P < 0.001$ *vs* Control or WT; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ *vs* IMQ or Card14$^{E138A/+}$+PBS.

Declaration of interests

The protein sequence of SjDX5-53 has been granted patent in China (ZL 2020 1 1256734.8). For patent granted in China, the inventors are M. Ji, R. Lai, Y. Ni, Z. Xu, N. Luan. Other authors have declared that no competing interests exist.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ebiom.2023.104751.

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