

Construction of a chronic spontaneous urticaria-relevant IgE-mediated mouse model based on passive cutaneous anaphylaxis and its transcriptome analysis



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Background: Chronic spontaneous urticaria (CSU) is a common immune-related skin disorder with an unclear pathogenesis and no universally accepted animal model. The passive cutaneous anaphylaxis (PCA) model is the commonly used model in CSU research.

Methods: To propose a modification to the PCA model to improve its alignment with CSU, we established a recurrent PCA (Re-PCA) model via 6 repeated injections of 2,4-dinitrophenyl-human serum albumin (DNP-HSA)-specific IgE and DNP-HSA and compared it to the PCA model. We assessed vascular permeability, scratch frequency scoring, histologic staining, flow cytometry, reverse transcription PCR, enzyme-linked immunosorbent assay, and RNA sequencing to compare phenotypic differences.

Results: The Re-PCA model exhibited more stable and significant skin changes associated with chronic urticaria compared to the PCA model. Lesional tissues of Re-PCA mice showed significantly increased degranulated mast cells and increased immune cell infiltration. Compared to the PCA group, peripheral blood histamine and tryptase levels of Re-PCA mice were significantly elevated. The utilization of histamine receptor blocker, c-Kit- and BTK-targeting drugs abrogated the phenotype of PCA and Re-PCA models. The gene expression profile of the Re-PCA lesions revealed enrichment of more genes and signaling pathways associated with CSU.

Conclusions: Our recurrent IgE-mediated mouse model exhibited greater immunologic and transcriptional relevance to CSU than the commonly used PCA model, which will facilitate research into the pathogenesis of CSU and advance our

understanding of its underlying mechanisms. (*J Allergy Clin Immunol* 2026;158:1032-46.)

Key words: Chronic spontaneous urticaria, IgE, mast cell, mouse model, passive cutaneous anaphylaxis

Chronic spontaneous urticaria (CSU) is a common autoimmune skin disorder characterized by recurrent episodes of wheals and erythema accompanied by marked pruritus, with a disease duration exceeding 6 weeks.¹ The weighted prevalence of CSU in the United States is 0.78%,² while in Europe it is 0.92%.³ Because of its pronounced pruritus and recurrent disease pattern, CSU exhibits significant comorbidity with anxiety, depression, and sleep disorders.⁴ Concurrently, patients with CSU experience markedly impaired quality of life,⁵ imposing substantial health and economic burdens.^{3,5}

The core pathogenesis of CSU involves abnormal mast cell activation triggered by multiple coacting factors, alongside the involvement of various immune components.⁶ IgE cross-links with FcεRIα on mast cell surfaces, multiple antigens bind to the cross-linked FcεRIα, triggering rapid mast cell degranulation and the release of inflammatory mediators.⁷ These mediators—including various cytokines, chemokines, and neuroactive substances—participate in the immunopathogenesis of CSU and contribute to the disease's immune processes.⁷ The mast cell activation mechanism in autoimmune-associated CSU involves IgE autoantibodies or IgG antibodies directed against IgE or the high-affinity IgE receptor (FcεRIα).⁸ Additionally, the coagulation cascade⁹ and endothelial cell activation¹⁰ are also implicated in the pathogenesis of CSU. Beyond conventional antihistamine therapy, omalizumab—an anti-IgE monoclonal antibody—has gained widespread application in treating CSU.⁷ Currently, various targeted therapies including Bruton tyrosine kinase (BTK) inhibitors, c-Kit antagonists, and Mas-related G protein-coupled receptor X2 (MRGPRX2) antagonists are undergoing clinical trials to validate their efficacy for CSU; however, drug resistance and posttreatment relapse remain persistent clinical challenges,¹¹ underscoring the urgent need for further investigation into the pathophysiologic mechanisms underlying this condition.

CSU is a classic representative disease of chronic mast cell activation and recurrent activation. *In vivo* studies are crucial for investigating its pathogenesis. However, to date, there remains no universally accepted animal model for CSU. The most commonly

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Abbreviations used

BTK:	Bruton tyrosine kinase
CSU:	Chronic spontaneous urticaria
CU:	Chronic urticaria
DEG:	Differentially expressed gene
DNP-HSA:	2,4-Dinitrophenyl-human serum albumin
ELISA:	Enzyme-linked immunosorbent assay
GO:	Gene Ontology (geneontology.org)
MRGPRX2:	Mas-related G protein-coupled receptor X2
OVA:	Ovalbumin
PCA:	Passive cutaneous anaphylaxis
Re-PCA:	Recurrent PCA

used model is the passive cutaneous anaphylaxis (PCA) model, which evolved from the Prausnitz-Kustner reaction established by German physicians Carl Prausnitz and Heinz Kustner.¹² In PCA, antigen-specific IgE antibodies are injected into the dermis of a recipient animal. After a 24- to 72-hour latency period, intravenous administration of the corresponding antigen triggers mast cell activation. This leads to the release of mediators such as histamine, resulting in increased vascular permeability. Dyes indicative of vascular permeability (eg, Evans blue) injected alongside the antigen bind to albumin in the exudate, forming blue plaques. Reaction intensity is quantified by measuring plaque diameter or via colorimetry.¹³ This method is now widely used in studies of urticaria (including CSU) and IgE-mediated cutaneous mast cell activation.^{14,15} Additionally, some researchers have used repeated subcutaneous injections of ovalbumin (OVA) to simulate a chronic urticaria (CU) model,¹⁶ as well as repeated subcutaneous injections of substance P followed by intravenous administration of substance P to induce a CU animal model.¹⁷

In patients with CSU, wheals and pruritus exhibit recurrent clinical manifestations. Histopathologic sections of skin tissue reveal immune cell infiltration, including lymphocytes and neutrophils.¹⁸ Among current animal models for CSU, the PCA model reproduces the IgE-mediated mast cell activation mechanism observed in the disease. However, as a single-dose drug-induced model, it suffers from short modeling duration, mild inflammation, and poor stability. Consequently, it inadequately replicates the clinical phenotype, pathogenesis, and gene expression profile of CSU. In the repeated OVA injection-induced CU model, OVA stimulates B cells to produce IgE, which then binds to OVA itself as an antigen. This activates mast cells via the IgE/antigen/FcεR1α pathway, inducing urticaria-like symptoms. However, this model exhibits a prolonged interval between initial OVA stimulation and subsequent B-cell-mediated IgE production,¹⁹ resulting in delayed mast cell activation events. Therefore, this model cannot fully replicate the mechanism of repeated mast cell activation in CSU. For CU models induced by substance P and compound 48/80, the core mechanism relies on the non-IgE pathway, specifically MRGPRB2-mediated mast cell activation.^{20,21} In CSU patients, MRGPRX2 activation due to multiple factors contributes to pathogenesis,²² though the primary mechanism remains closely linked to IgE/antigen-mediated mast cell activation. Thus, this model partially replicates non-IgE-mediated mast cell activation mechanisms in CSU but inadequately reflects the core pathophysiology.

In CSU, mast cells undergo repeated activation, leading to pathologic alterations in the local skin immune microenvironment.⁶ Gene expression profiling of both lesional and perilesional

skin reveals chronic inflammatory changes even in nonlesional skin areas.²³ Among the characteristics of CSU, chronic and persistent skin inflammation is a significant feature that cannot be overlooked. Therefore, on the basis of the mechanism whereby mast cells trigger skin lesions through IgE-mediated classical activation pathways in CSU, we modified the existing PCA model with the aim of establishing a mouse model more relevant to CSU and advancing research in this field.

METHODS

PCA and recurrent PCA in mice

Female C57BL/6J mice, 6 weeks old and specific pathogen-free, were purchased from Guangzhou Ruige Biotechnology (Guangzhou, China). Mice were housed in specific-pathogen-free-grade animal facilities, with no more than 5 mice per cage. Experiments commenced after a 7-day acclimatization period. All animal housing and experimental protocols were approved by the animal ethics review committee of South China Agricultural University (No. 2025D040). On day 0, recurrent PCA (Re-PCA) group mice received bilateral subcutaneous injections of 0.5 μg anti-2,4-dinitrophenyl-human serum albumin (DNP-HSA) IgE (Merck, D8406) in the ears. Twenty-four hours later, the Re-PCA group mice received a tail vein injection of 300 μg DNP-HSA (Bioresearch Technologies, D-5059). This injection regimen was repeated every 7 days. During the sixth injection, 1.6 mg of Evans blue (Merck, E2129) was added to the tail vein injection of DNP-HSA. Simultaneously with the sixth injection in the Re-PCA group, the PCA group was modeled by subcutaneous injection of 0.5 μg anti-DNP-HSA IgE into both ears. Twenty-four hours later, the PCA group received an intravenous tail injection of 300 μg DNP-HSA and 1.6 mg Evans blue. After the tail vein injection, continuous observation of Evans blue leakage from the ears was conducted. Using a micrometer, the thickness of both ears was measured at 7, 15, 30, 45, 60, and 90 minutes after DNP-HSA injection, with the average values recorded. Simultaneously, the number of ear-scratching episodes within 30 minutes was documented. More details are provided in the Methods section in the Online Repository available at www.jacionline.org.

During the 2 days before the sixth injection of anti-DNP-HSA IgE, the mice were administered loratadine (MCE, HY-17043) 0.5 mg once a day via gavage, or barzolvolimab (Topscience, T76919) 0.4 mg once a day via intraperitoneal injection, or remibrutinib (Topscience, T16730) 0.14 mg once a day via intraperitoneal injection. In the PCA and Re-PCA models, after subcutaneous injection of anti-DNP-HSA-IgE, the corresponding control groups were simultaneously administered subcutaneous injections of anti-DNP-HSA-specific IgE 0.5 μg, nonspecific IgE (MCE, HYP77695) 0.5 μg, histamine (MCE, HYB1204) 40 μg, and immunoglobulin (MCE, HY-NP0196) 0.5 μg. The controls in the PCA group received a single injection, while those in the Re-PCA group received 6 repeated injections.

The entire layer of biopsy tissue from the right ear was fixed and embedded for toluidine blue staining. The remaining skin tissue from the right ear was extracted for Evans blue staining at 65°C for 16 hours. After 12 hours, blood was collected from the mouse, and serum was extracted. Serum histamine concentration was measured via enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (Signalway Antibody, EK2448). Serum tryptase concentration was measured via ELISA according to the manufacturer's instructions (Cusabio,

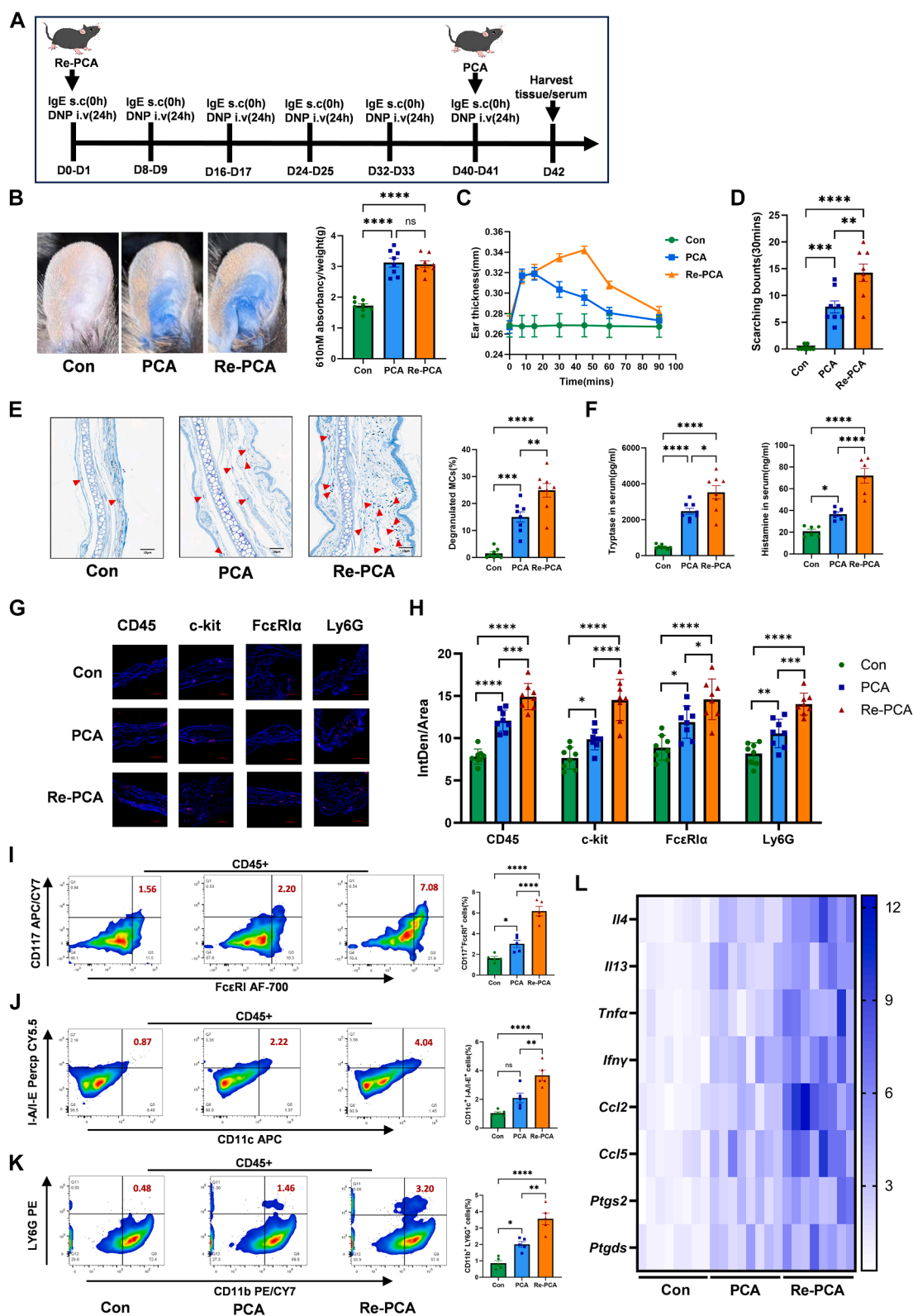


FIG 1. Establishment of Re-PCA model. **(A)** Modeling protocol for CSU-relevant IgE-mediated mouse model. **(B)** Representative images of ears after modeling (*left*) and quantified Evans blue permeability values reflecting vascular permeability changes in modeled ears (*right*) ($n = 8$). **(C)** Dynamic measurement curve of ear thickness 90 minutes after modeling ($n = 8$). **(D)** Count of ear-scratching episodes within 30 minutes after modeling ($n = 8$). **(E)** Representative toluidine blue-stained paraffin section of ear skin after modeling (original magnification 400 $\times$) and percentage of degranulated mast cells in ear skin ($n = 8$). **(F)** Detection of tryptase (*left*) and histamine (*right*) concentration in mouse serum via ELISA. **(G)** Representative image

CSB-E14326m). Portions of the left ear tissue were used for flow cytometry to detect immune cells in the skin. The remaining tissue was frozen in liquid nitrogen and stored at -80°C for subsequent real-time quantitative PCR analysis.

Statistical analysis

Statistical analysis was performed by GraphPad Prism v10.1.2 software. First, data normality was assessed. One-way ANOVA was conducted across multiple groups, followed by the Tukey multiple comparison test or Kruskal-Wallis test, followed by the Dunn multiple comparison test. Sample sizes for each experiment are indicated in the figures. $P < .05$ was considered statistically significant. The significance levels for data were defined as $*P < .05$, $**P < .01$, $***P < .001$, and $****P < .0001$.

RESULTS

Establishment of CSU-relevant IgE-mediated mouse model and comparison with PCA model

We administered $0.5\text{ }\mu\text{g}$ of anti-DNP-HSA-specific IgE via subcutaneous injection into the ear skin of mice. Twenty-four hours later, $300\text{ }\mu\text{g}$ of DNP-HSA was injected intravenously into the tail vein, which was administered weekly for 6 weeks. This model was termed *recurrent PCA* and was compared to the PCA model (Fig 1, A). We observed similar vascular permeability alterations in the Re-PCA group compared to the PCA group (Fig 1, B). After DNP-HSA injection, we measured mouse ear thickness at 0, 15, 30, 45, 60, and 90 minutes to assess ear swelling. We found that within the first 15 minutes, ear swelling in the Re-PCA group was comparable to that in the PCA group. However, swelling continued to increase after 30 minutes, peaking at 45 minutes. Subsequently, swelling levels steadily decreased, returning to baseline values alongside the PCA group by 90 minutes (Fig 1, C). Counting of ear scratching episodes within 30 minutes after model establishment revealed significantly higher scratching frequency in the Re-PCA group compared to the PCA group (Fig 1, D). Skin tissue sections stained with toluidine blue were used to quantify the proportion of degranulated mast cells in the skin. Results showed that the Re-PCA group exhibited a higher proportion of degranulated mast cells, which was further confirmed by quantification of degranulated mast cell numbers (Fig 1, E). Furthermore, we measured serum histamine and tryptase concentrations via ELISA after model establishment. Results showed significantly higher serum histamine and tryptase levels in the Re-PCA group compared to the PCA group (Fig 1, F). To further validate skin immune cell infiltration and activation in the Re-PCA model, we performed Immunofluorescence on modeled skin sections to detect CD45^{+} cells, c-Kit^{+} cells, $\text{Fc}\epsilon\text{RI}\alpha^{+}$ cells, and Ly6G^{+} cells (Fig 1, G). In the Re-PCA group, the infiltration of immune cells,

including these 4 cell types, was significantly increased compared to the PCA group (Fig 1, H). Furthermore, we analyzed the infiltration of mast cells, dendritic cells, neutrophils, and $\text{T}_{\text{H}}1$, $\text{T}_{\text{H}}2$, and $\text{T}_{\text{H}}17$ cells in the modeled skin by flow cytometry (see Fig E1 in the Online Repository available at www.jacionline.org). We confirmed that the Re-PCA group exhibited increased infiltration of these cells (Fig 1, I-K, and see Fig E2, A, in the Online Repository). Additionally, we examined the expression of urticaria-related inflammatory factors in the skin of modeled mice via real-time reverse transcriptase PCR. Results showed that in the Re-PCA model, the expression levels of *Il4*, *Il13*, *Tnfa*, *Ifny*, *Ccl2*, *Ccl5*, *Ptgs2*, and *Ptgds* were significantly higher than in the PCA group (Fig 1, L). On the basis of these findings, we conclude that the Re-PCA model exhibits more stable and significant skin changes associated with CU, as well as significantly higher mediators release levels, greater expression of inflammatory mediators, and increased infiltration of diverse immune cells compared to the PCA model.

Effect of histamine receptor blocker on CSU-relevant IgE-mediated mouse model

Histamine receptor blocker is the first-line treatment for CSU. The effectiveness of histamine receptor blocker on Re-PCA model reflects the association between Re-PCA model and CSU, as well as the role of histamine on the Re-PCA model. Therefore, we used loratadine, a widely clinically deployed histamine receptor blocker, to block the effect of histamine (Fig 2, A). As shown in Fig 2, B-D, the vascular permeability in both PCA and Re-PCA mice was significantly reversed by loratadine, and ear swelling and scratching behavior were also reduced. Toluidine blue stain exhibited decreased proportion of degranulated mast cells after loratadine treatment (Fig 2, E). Consistently, the levels of serum tryptase were also downregulated (Fig 2, F). Flow cytometry analysis revealed a significant decrease of immune cells infiltration in both PCA and Re-PCA lesions after loratadine treatment, including mast cells, dendritic cells, neutrophils, and $\text{T}_{\text{H}}1$, $\text{T}_{\text{H}}2$, and $\text{T}_{\text{H}}17$ cells (Fig 2, G-I, and Fig E2, B-D). Furthermore, the levels of urticaria-related inflammatory factors were significantly downregulated in loratadine-treated groups than in untreated groups (Fig 2, J). These results suggest that both PCA and Re-PCA models primarily induced skin phenotype and immune responses through the effect of histamine.

Effect of c-Kit- and BTK-targeting drugs on CSU-relevant IgE-mediated mouse model

Activation of c-Kit is important for the maintenance of mast cells survive and function. The c-Kit targeting drug has been demonstrated to induce mast cell ablation and exhibited good therapeutic

of CD45 , c-kit , $\text{Fc}\epsilon\text{RI}\alpha$, and Ly6G , and immunofluorescence staining on paraffin-embedded sections of ear skin after modeling; blue fluorescence indicates DAPI, and remaining purple and red fluorescence represents CD45 , c-kit , $\text{Fc}\epsilon\text{RI}\alpha$, and Ly6G , respectively (original magnification $100\times$). (H) Average fluorescence intensity values for CD45 , c-kit , $\text{Fc}\epsilon\text{RI}\alpha$, and Ly6G . (I) Left, Representative flow cytometry image of degranulated mast cells in model skin. Right, Percentage of degranulated mast cells in model skin ($n = 5$). (J) Left, Representative flow cytometry image of dendritic cell subpopulations in modeled skin. Right, Percentage of dendritic cells in modeled skin ($n = 5$). (K) Left, Representative flow cytometry image of neutrophil subpopulations in modeled skin. Right, Percentage of neutrophils in modeled skin ($n = 5$). (L) mRNA expression levels of *Il4*, *Il13*, *Tnfa*, *Ifny*, *Ccl2*, *Ccl5*, *Ptgs2* and *Ptgds* in skin tissue of modeled mice. One-way ANOVAs with Tukey multiple comparisons test were used for (B), (D-F), and (H-K). $*P < .05$, $**P < .01$, $***P < .001$, $****P < .0001$; ns, not significant. DNP, Dinitrophenyl; *IntDen/Area*, integrated density/area.

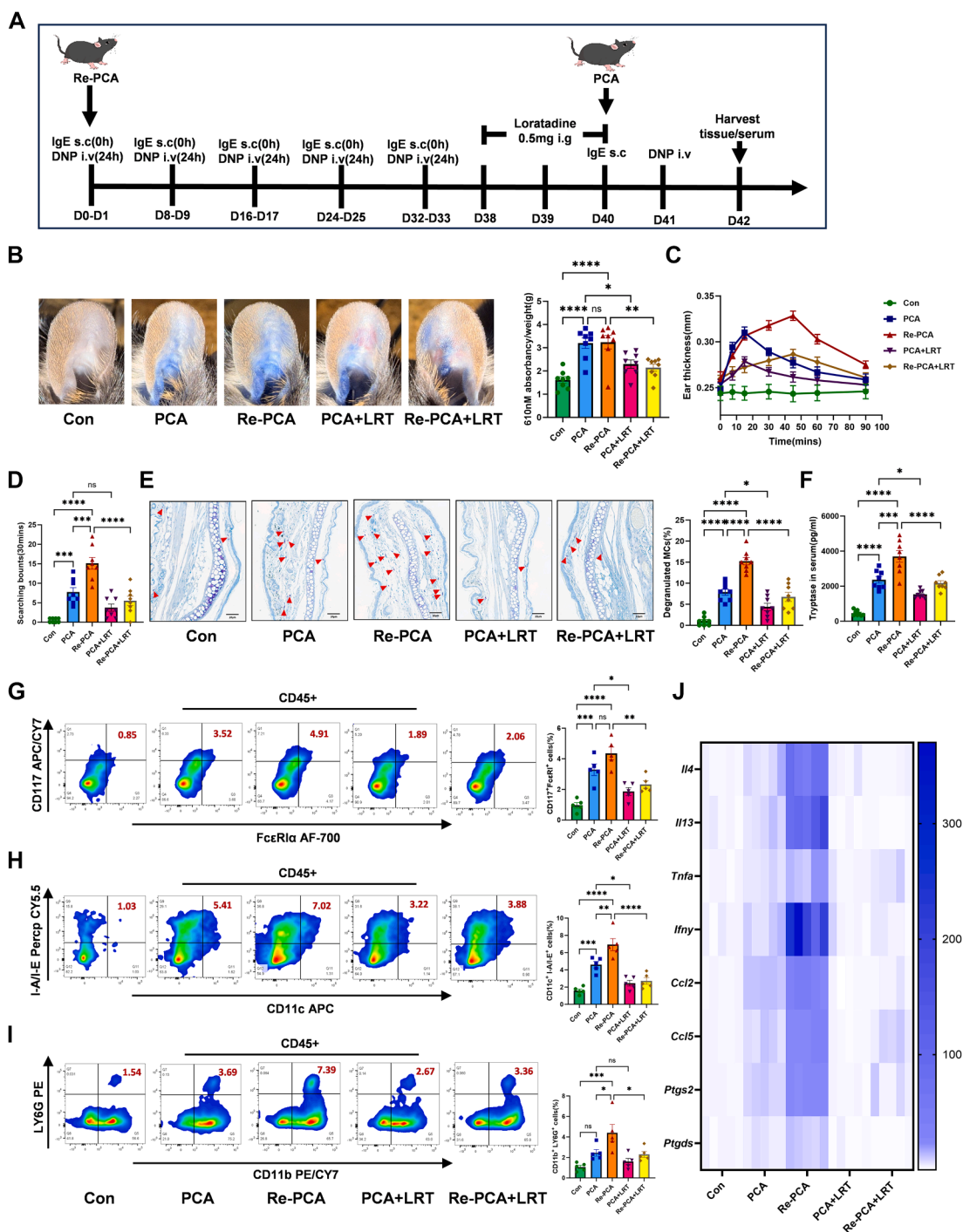


FIG 2. Effect of histamine receptor blocker on CSU-relevant IgE-mediated mouse model. **(A)** Modeling protocol for CSU-relevant IgE-mediated mouse model under loratadine intervention. **(B)** Representative images of ears after modeling (left) and quantified Evans blue permeability values reflecting vascular permeability changes in modeled ears (right) ($n = 8$). **(C)** Dynamic measurement curve of ear thickness 90 minutes after modeling ($n = 8$). **(D)** Count of ear-scratching episodes within 30 minutes after modeling ($n = 8$). **(E)** Representative toluidine blue-stained paraffin section of ear skin after modeling (left) (original magnification $400\times$) and percentage of degranulated mast cells in ear skin (right) ($n = 8$). **(F)** Detection of tryptase concentration in mouse serum via ELISA. **(G)** Left, Representative flow cytometry image of degranulated mast cells in model skin. Right, Percentage of degranulated mast cells in model skin ($n = 5$). **(H)** Left, Representative flow cytometry image of dendritic cell subpopulations in modeled skin. Right, Percentage of dendritic cells in modeled skin ($n = 5$). **(I)** Left, Representative flow cytometry image of neutrophil subpopulations in modeled skin. Right, Percentage of neutrophils in modeled skin ($n = 5$). **(J)** mRNA expression levels of *Il4*, *Il13*, *Tnfa*, *Ifny*, *Ccl2*, *Ccl5*, *Ptgs2* and *Ptgsd* in skin tissue of modeled mice. One-way ANOVAs with Tukey multiple comparisons test were used for **(B)** and **(D-I)**. * $P < .05$, ** $P < .01$, *** $P < .001$, **** $P < .0001$; ns, not significant. DNP, Dinitrophenyl; LRT, loratadine.

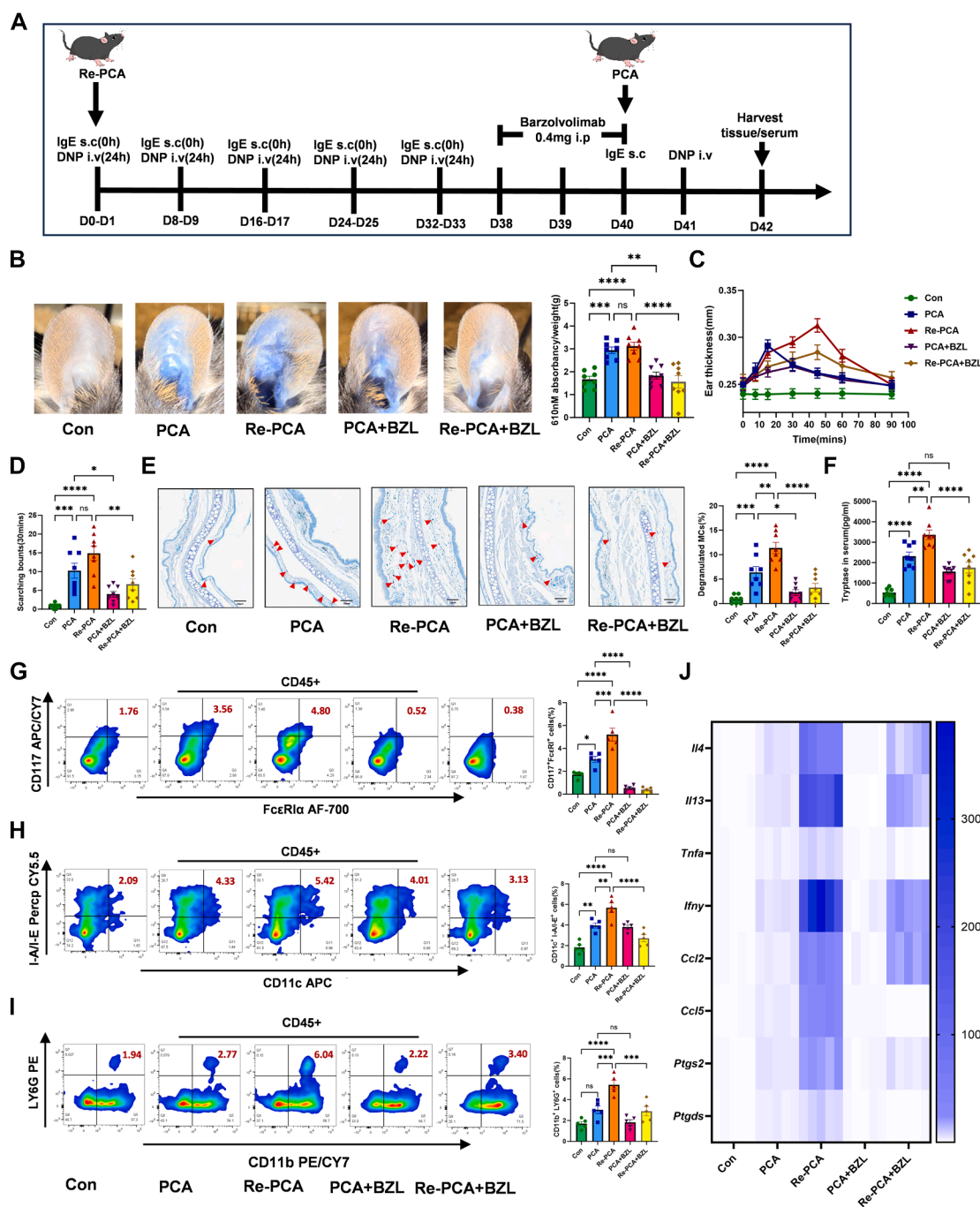


FIG 3. Effect of barzolvolimab on CSU-relevant IgE-mediated mouse model. **(A)** Modeling protocol for CSU-relevant IgE-mediated mouse model under barzolvolimab intervention. **(B)** Representative images of ears after modeling (left) and quantified Evans blue permeability values reflecting vascular permeability changes in modeled ears (right) (n = 8). **(C)** Dynamic measurement curve of ear thickness 90 minutes after modeling (n = 8). **(D)** Count of ear-scratching episodes within 30 minutes after modeling (n = 8). **(E)** Representative toluidine blue-stained paraffin section of ear skin after modeling (left) (original magnification 400×) and percentage of degranulated mast cells in ear skin (right) (n = 8). **(F)** Detection of tryptase concentration in mouse serum via ELISA. **(G)** Left, Representative flow cytometry image of degranulated mast cells in model skin. Right, Percentage of degranulated mast cells in model skin (n = 5). **(H)** Left, Representative flow cytometry image of dendritic cell subpopulations in modeled skin. Right, Percentage of dendritic cells in modeled skin (n = 5). **(I)** Left, Representative flow cytometry image of neutrophil subpopulations in modeled skin (n = 5). Right, Percentage of neutrophils in modeled skin (n = 5). **(J)** mRNA expression levels of *Il4*, *Il13*, *Tnfa*, *Ifny*, *Ccl2*, *Ccl5*, *Ptgs2* and *Ptgsd* in skin tissue of modeled mice. One-way ANOVAs with Tukey multiple comparisons test were used for (B) and (D-I). **P* < .05, ***P* < .01, ****P* < .001, *****P* < .0001; ns, not significant. BZL, Barzolvolimab; DNP, dinitrophenyl.

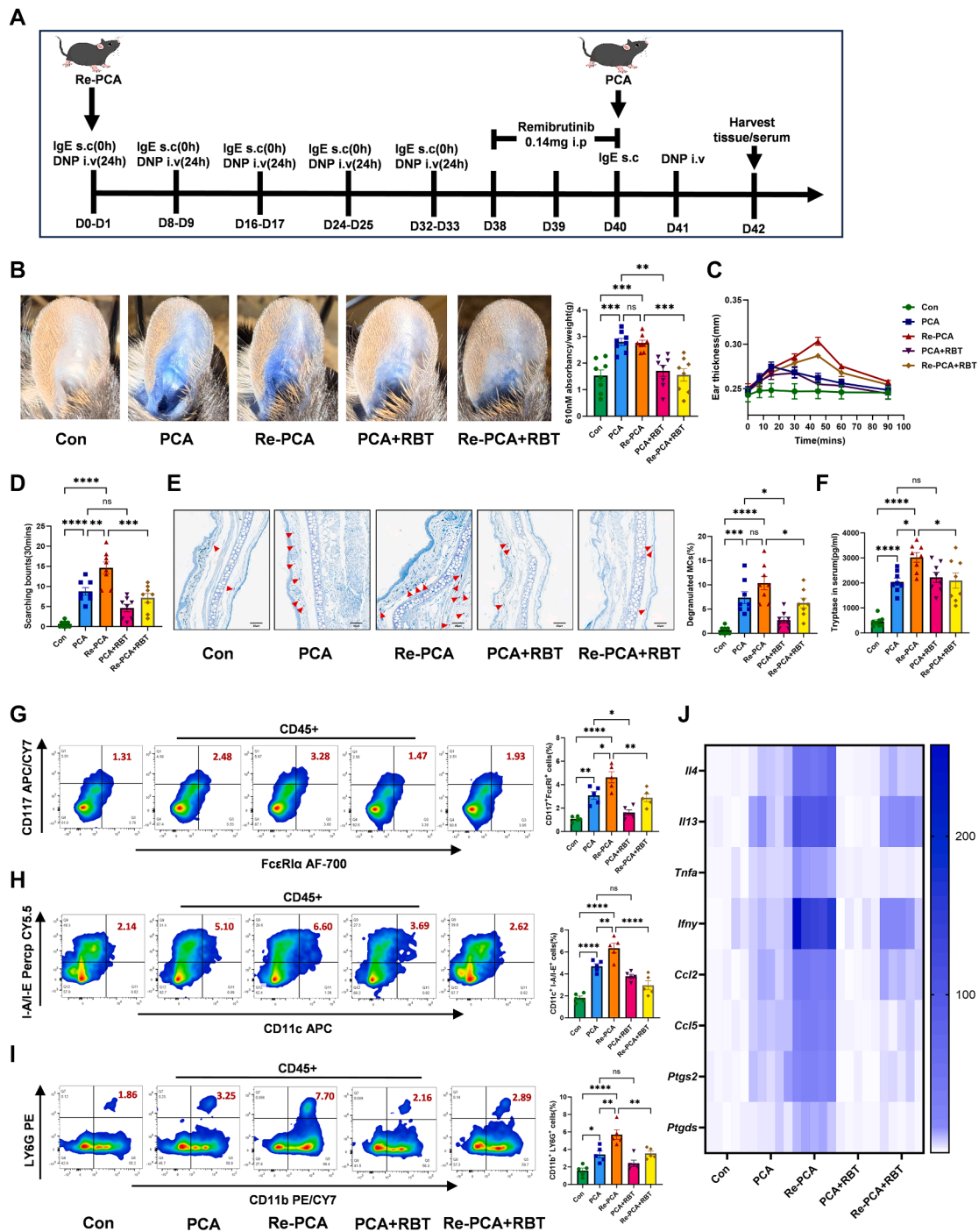


FIG 4. Effect of remibrutinib on CSU-relevant IgE-mediated mouse model. **(A)** Modeling protocol for CSU-relevant IgE-mediated mouse model under remibrutinib intervention. **(B)** Representative images of ears after modeling (*left*) and quantified Evans blue permeability values reflecting vascular permeability changes in modeled ears (*right*) ($n = 8$). **(C)** Dynamic measurement curve of ear thickness 90 minutes after modeling ($n = 8$). **(D)** Count of ear-scratching episodes within 30 minutes after modeling ($n = 8$). **(E)** Representative toluidine blue-stained paraffin section of ear skin after modeling (*left*) (original magnification 400 $\times$) and percentage of degranulated mast cells in ear skin (*right*) ($n = 8$). **(F)** Detection of tryptase concentration in mouse serum via ELISA. **(G)** *Left*, Representative flow cytometry image of degranulated mast cells in model skin. *Right*, Percentage of degranulated mast cells in model skin ($n = 5$). **(H)** *Left*, Representative flow cytometry image of dendritic cell subpopulations in modeled skin. *Right*, Percentage of dendritic cells in modeled skin ($n = 5$). **(I)** *Left*, Representative flow cytometry image of neutrophil subpopulations in modeled skin. *Right*, Percentage of neutrophils in modeled skin ($n = 5$). **(J)** mRNA expression levels of *Il4*, *Il13*, *Tnfa*, *Ifny*, *Ccl2*, *Ccl5*, *Ptgs2* and *Ptgsd* in skin tissue of modeled mice. One-way ANOVAs with Tukey multiple comparisons test were used for **(B)** and **(D-I)**. * $P < .05$, ** $P < .01$, *** $P < .001$, **** $P < .0001$; ns, not significant. DNP, Dinitrophenyl; RBT, remibrutinib.

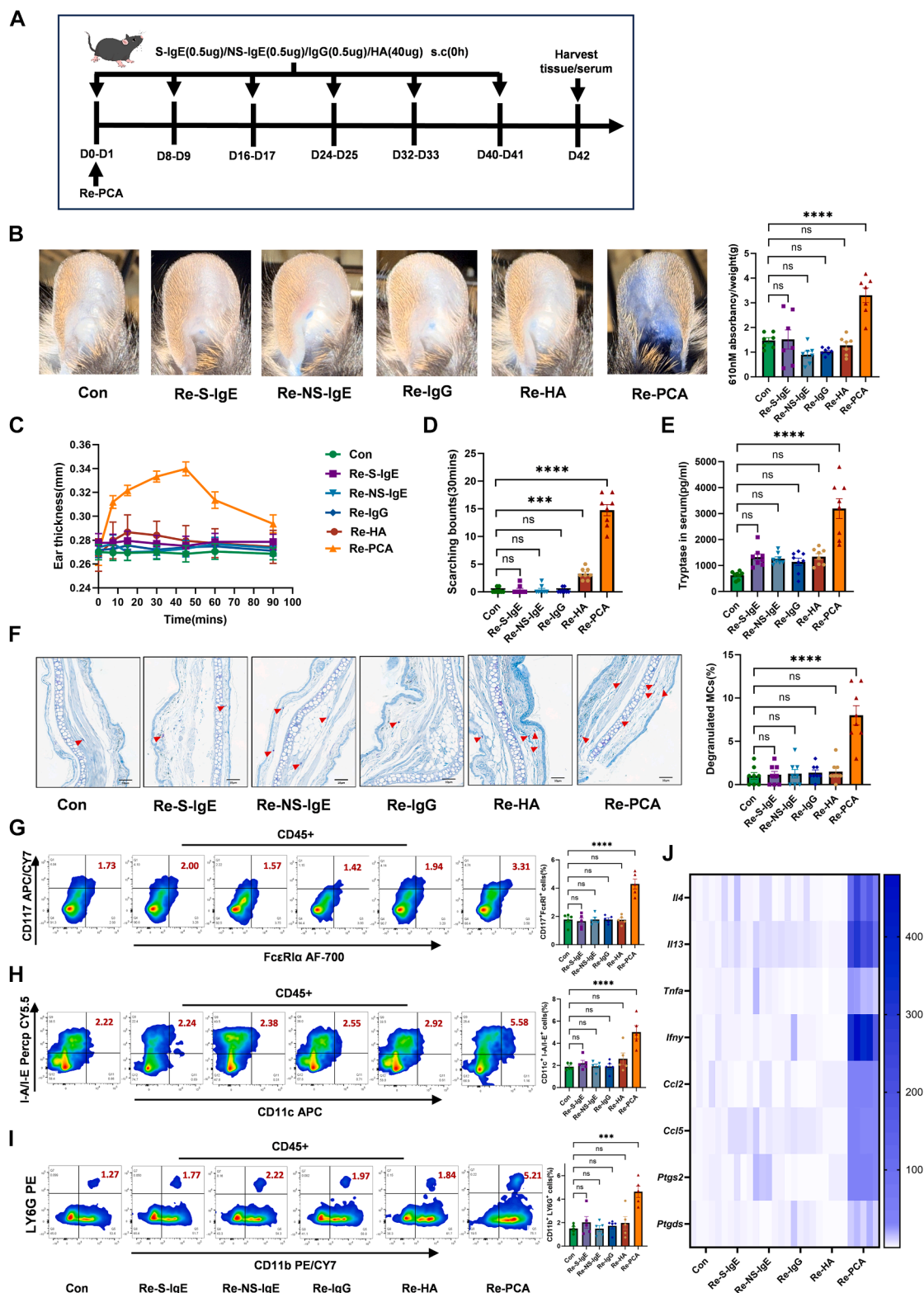


FIG 5. Multiple-injection of anti-DNP-HSA-specific IgE, nonspecific IgE, histamine, and IgG, as control for Re-PCA model. **(A)** Modeling protocol. **(B)** Representative images of ears after modeling (left) and quantified Evans blue permeability values reflecting vascular permeability changes in modeled ears (right) ($n = 8$). **(C)** Dynamic measurement curve of ear thickness 90 minutes after modeling ($n = 8$). **(D)** Count of ear-scratching episodes within 30 minutes after modeling ($n = 8$). **(E)** Detection of tryptase concentration in mouse serum via ELISA. **(F)** Representative toluidine blue-stained paraffin section of ear skin after modeling (left) (original magnification 400 $\times$) and percentage of degranulated mast cells in ear skin (right) ($n = 8$). **(G)** Left, Representative flow cytometry image of degranulated mast cells in model skin. Right, Percentage of degranulated

effects on CU.²⁴ BTK inhibitors are another treatment option for CSU and have been approved for use in adult patients whose disease remains symptomatic despite H₁-antihistamine treatment.²⁵ Therefore, representative c-Kit and BTK inhibitors, barzolvolimab and remibrutinib, were used to investigate the effect of mast cells and BTK signaling on the Re-PCA model, respectively (Fig 3, A, and Fig 4, A). Evans blue distribution on mice ears showed that vascular permeability in both PCA and Re-PCA groups was remarkably reduced after barzolvolimab or remibrutinib administration (Fig 3, B, and Fig 4, B). A consistent reduction in both ear swelling and scratching behavior was observed (Fig 3, C and D, and Fig 4, C and D). As expected, proportion of degranulated mast cells and the level of mast cell–released tryptase in PCA and Re-PCA lesions was significantly decreased by barzolvolimab or remibrutinib treatment (Fig 3, E and F, and Fig 4, E and F). The infiltration of immune cells including mast cells, dendritic cells, neutrophils, and T_H1, T_H2, and T_H17 cells was also downregulated in the barzolvolimab- or remibrutinib-treated groups compared to untreated groups (Fig 3, G-I, Fig 4, G-I, Fig E2, E-G, and see Fig E3, A-C, in the Online Repository available at www.jacionline.org). Moreover, heat maps showed that barzolvolimab or remibrutinib administration significantly reversed the expression levels of CSU-related inflammatory molecules in PCA and Re-PCA models (Fig 3, J, and Fig 4, J). Taken together, these results demonstrate the therapeutic efficacy of c-Kit- and BTK-targeting drugs in Re-PCA model, revealing that mast cells and BTK signaling are crucial for the establishment of the Re-PCA model.

Specificity of CSU-relevant IgE-mediated mouse model

To validate the specificity of Re-PCA and PCA model induced by anti-DNP-HSA-specific IgE and DNP-HSA, we treated mice with anti-DNP-HSA-specific IgE alone, nonspecific IgE, IgG, and histamine as controls. Control groups of the Re-PCA model received a weekly dose of these molecules for 6 consecutive weeks, while controls of PCA model received only a single dose (Fig 5, A, and see Fig E4, A, in the Online Repository available at www.jacionline.org). Evans blue distribution and thickness of mice ears revealed that the administration of anti-DNP-HSA-specific IgE alone, nonspecific IgE, IgG, and histamine did not induce the clinical phenotype related to CSU (Fig 5, B and C, and Fig E4, B and C). Consistently, the counting of ear-scratching episodes of these groups was not significantly different with the untreated control group (Fig 5, D, and Fig E4, D). Furthermore, the concentration of tryptase and the proportion of degranulated mast cells in lesions of anti-DNP-HSA-specific IgE alone-, nonspecific IgE-, IgG-, and histamine-treated mice was comparable to the untreated control group (Fig 5, E and F, and Fig E4, E and F). Results of flow cytometry showed that the infiltration of mast cells, dendritic cells, neutrophils, and

T_H1, T_H2, and T_H17 cells was significantly diminished in these control groups (Fig 5, G-I, Fig E3, D-F, Fig E4, G-I, and see Fig E5 in the Online Repository). Additionally, the transcription levels of CSU-related inflammatory molecules of these control groups were significantly lower than the Re-PCA group and PCA group (Fig 5, J, and Fig E4, J). These results reveal the specificity of Re-PCA and PCA model.

Gene expression characteristics of CSU-relevant IgE-mediated mouse model skin and comparison of intersecting genes with CSU gene expression profiles

To further explore the gene expression characteristics of the Re-PCA model and its differences from the PCA model, we performed RNA sequencing on skin samples from both Re-PCA and PCA models. Public sequencing data from human CSU lesions (GSE72540) were downloaded from the Gene Expression Omnibus (www.ncbi.nlm.nih.gov/geo) database for differential gene analysis and pathway enrichment analysis. Differential genes from the Re-PCA and PCA models were intersected with those from GSE72540 to validate the similarity between the two models and the CSU gene expression profile (see Fig E6, A, in the Online Repository available at www.jacionline.org). Both volcano plots and heat maps revealed that the Re-PCA model exhibited more significantly up- and downregulated differentially expressed genes (DEGs) compared to the PCA model (Fig 6, A and B). Gene Ontology (GO; geneontology.org) enrichment analysis indicated that both Re-PCA and PCA models showed enrichment in multiple signaling pathways related to immune responses, lipid metabolism, and neurotransmitters (Fig 6, C). The Kyoto Encyclopedia of Genes and Genomes (genome.jp/kegg) enrichment analysis revealed that the Re-PCA model showed greater enrichment in pathways associated with CSU and mast cell activation, including the MAPK pathway, PI3K-AKT pathway, Hippo pathway, and inflammatory mediator regulation of TRP channels pathway, consistent with the enrichment results of CSU RNA sequencing of DEGs (Fig 6, D, and see Fig E7, C, in the Online Repository). Protein–protein interaction analysis of the top 15 up- and downregulated DEGs revealed that hub genes in the PCA group primarily belonged to the keratin family, while hub genes in the Re-PCA group included chemokine family members, metalloproteinases, and transcription factors (Fig E6, C). We present the differential gene analysis, pathway enrichment analysis, and protein–protein interaction network analysis results for the human CSU lesion common dataset (Fig E7). Subsequently, we identified genes common to the PCA model and Re-PCA model with the human CSU common dataset gene expression profiles, and we performed GO enrichment and protein–protein interaction network analysis on the two sets of common genes. Results revealed that 90 genes shared

mast cells in model skin (n = 8). (H) Left, Representative flow cytometry image of dendritic cell subpopulations in modeled skin. Right, Percentage of dendritic cells in modeled skin (n = 8). (I) Left, Representative flow cytometry image of neutrophil subpopulations in modeled skin. Right, Percentage of neutrophils in modeled skin (n = 8). (J) mRNA expression levels of *Il4*, *Il13*, *Tnfa*, *Ifny*, *Ccl2*, *Ccl5*, *Ptgs2* and *Ptgs* in skin tissue of modeled mice. One-way ANOVAs with Tukey multiple comparisons test were used for (B) and (D-I). **P* < .05, ***P* < .01, ****P* < .001, *****P* < .0001; ns, not significant. DNP, Dinitrophenyl; Re-HA, multiple injection of histamine; Re-IgG, multiple injection of IgG; Re-NS-IgE, multiple injection of nonspecific IgE; Re-S-IgE, multiple injection of anti-DNP-HSA-specific IgE.

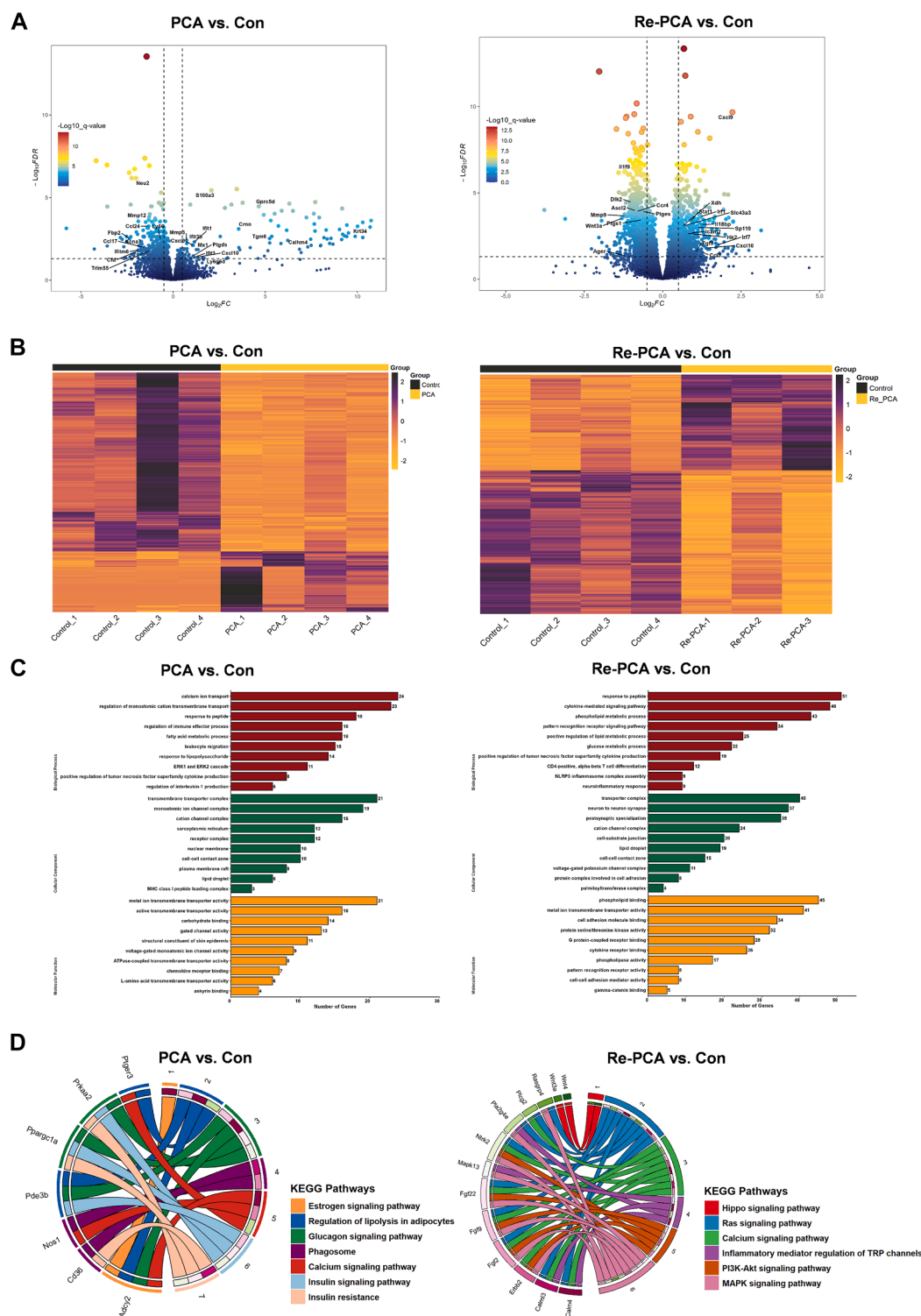
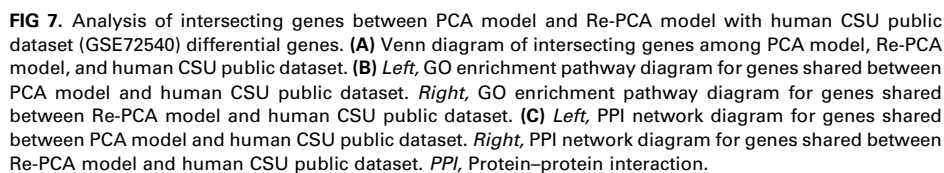


FIG 6. Differential gene expression and pathway enrichment analysis of PCA-modeled skin vs Re-PCA-modeled skin ($P < .05$, $|\log_2FC| > 0.5$). (A) Left, Volcano plot of DEGs between PCA-modeled and unmodeled mouse skin. Right, Volcano plot of DEGs between Re-PCA-modeled and unmodeled mouse skin. (B) Left, Heat map of DEGs in PCA-modeled vs unmodeled mouse skin. Right, Heat map of DEGs in Re-PCA-modeled vs unmodeled mouse skin. (C) Left, GO pathway enrichment diagram of DEGs in PCA-modeled vs unmodeled mouse skin. Right, GO pathway enrichment diagram of DEGs in Re-PCA-modeled vs unmodeled mouse skin. (D) Left, String diagram of KEGG enriched pathways for differential genes in PCA-modeled vs nonmodeled mouse skin. Right, String diagram of KEGG enriched pathways for differential genes in Re-PCA-modeled vs nonmodeled mouse skin. FC, Fold change; KEGG, Kyoto Encyclopedia of Genes and Genomes (genome.jp/kegg).



between the PCA model and the human CSU common dataset, with 62 showing consistent trends (see Table E1 in the Online Repository). Meanwhile, the Re-PCA model shared 390 genes with the human CSU common dataset, including 92 with consistent trends (Fig 7, A, and see Table E2 in the Online Repository). GO pathway enrichment analysis of the two sets of intersecting genes revealed that the Re-PCA model and human CSU common dataset shared more pathways enriched in T cells, monocytes, vascular endothelial growth factor, and neurotrophic factors, with higher enrichment scores. In contrast, the PCA model and human CSU common dataset intersecting genes showed greater enrichment in pathways related to proteins, metal ion transmembrane transport, and endothelial cell differentiation (Fig 7, B). Protein–protein interaction network analysis of the two sets of intersecting genes revealed that genes in the Re-PCA model intersecting with the human CSU common dataset, including *ERBB2* and *IRF7*, exhibited more protein interactions. In contrast, genes in the PCA model intersecting with the human CSU common dataset with more protein interactions were primarily *FLNC* and *ABCC9* (Fig 7, C).

DISCUSSION

Our study established an improved CSU-relevant IgE-mediated mouse model, termed the Re-PCA model, that is based on the PCA model. By observing animal phenotypes including skin vascular permeability, ear swelling, and itching in both PCA and Re-PCA model mice, we confirmed that the Re-PCA model exhibits skin vascular permeability alterations similar to the PCA model, accompanied by more pronounced ear swelling and itching manifestations. Furthermore, we demonstrated that Re-PCA model lesions exhibit more pronounced expression of key CSU inflammatory mediators and immune cell infiltration. Meanwhile, drugs for CSU, including histamine receptor blocker as well as c-Kit- and BTK-targeting drugs, were also effective for Re-PCA mice. Gene expression profiling revealed differential expression of multiple CSU-associated genes, along with significant enrichment of various CSU-related signaling pathways. This model holds significant potential for investigating IgE-mediated mast cell activation mechanisms in CSU. Our findings contribute to a deeper understanding of pathologic alterations in the skin immune microenvironment driven by IgE–mast cell activation pathways in this condition.

CSU is a complex immune-mediated skin disorder. Histologic analysis of CSU skin lesions reveals significant degranulation of cutaneous mast cells, along with perivascular infiltration of immune cells including lymphocytes, neutrophils, eosinophils, and basophils.²⁶ Mast cells and basophils are key effector cells in CSU pathogenesis. Their degranulation releases histamine, tryptase, and multiple inflammatory mediators, leading to local skin vasodilation, increased vascular permeability, and recruitment and activation of various immune cells.²⁷ However, the pathogenesis of CSU remains incompletely understood, primarily as a result of the paucity of a universally accepted animal model. According to the prevailing etiologic theories, over 50% of CSU cases are believed to involve autoimmune mechanisms.²⁸ Among these, 90% of patients exhibit type I autoimmunity mediated by IgE autoantibodies targeting various autoantigens such as thyroid peroxidase,²⁹ IL-24,³⁰ and double-stranded DNA.³¹ Only 10% of patients exhibit type IIb autoimmunity,³² where specific IgG antibodies bind to autoantigens, leading to mast cell activation

and urticaria symptoms.³³ Therefore, we maintain that animal models of CSU that use antigen-specific IgE and antigens exhibit high reproducibility in studying pathogenesis, representing the mechanisms underlying most cases, particularly those mediated by type I autoimmune responses. The PCA model is an IgE-mediated model, and consequently, it is widely utilized in research related to CSU. However, single-dose IgE stimulation of the PCA model is inadequate in representing the characteristics of long-term, repeated immune stimulation in CSU. Therefore, we established the Re-PCA model through recurrent IgE stimulation, which is more relevant to CSU.

Our observation and quantification of urticaria-relevant symptoms in Re-PCA–modeled mice revealed that the Re-PCA model exhibits more pronounced phenotype compared to PCA group. Concurrently, the peak time for ear swelling was delayed in the Re-PCA model compared to the PCA model, which enabled more accurate observation, recording, and intergroup comparison of ear swelling severity after modeling. Regarding itch expression after modeling, the Re-PCA model exhibited significantly more ear-scratching episodes per unit of time compared to the PCA model—a hallmark symptom of CSU. Histologic, immunologic, and molecular analyses of Re-PCA–model mice revealed a higher proportion of degranulated mast cells in the ears than in PCA-model mice. Furthermore, immune cell infiltration—including mast cells, neutrophils, dendritic cells, and T_H1, T_H2, and T_H17 cells—was increased in the Re-PCA group compared to PCA. This provides a reliable *in vivo* experimental foundation for studying the immunologic mechanisms within the skin microenvironment of CSU. Infiltration of multiple immune cells into skin lesions is a common pathologic change in CSU, including CD4⁺ T cells and neutrophils. In autoimmune and nonautoimmune subtypes of CSU, the degree of CD4⁺ T cell infiltration shows no significant difference.^{26,34} Plasma cell-like dendritic cells express FcεRIα on their surface,³⁵ enabling specific binding to IgE. They can present antigens to CD4⁺ T cells via major histocompatibility complex class II molecules and costimulatory molecules, promoting their activation. Additionally, dendritic cells can mediate interactions with mast cells through TNF-α.³⁶ Bioinformatics analysis suggests possible dendritic cell infiltration in the skin lesions of patients with CSU,³⁷ indicating consistency between the Re-PCA model and the immune microenvironment of CSU lesions. Activated mast cells release high levels of IL-4 and IL-13,³⁸ participating in T_H2-like responses.³⁹ In T_H1-type immunity, mast cells may play a promoting role⁴⁰ while also participating in T_H17 responses.^{41,42} Previous studies have confirmed T_H2 cell infiltration in CSU lesions.³⁷ Although definitive evidence regarding the mechanisms of T_H1 and T_H17 immunity in CSU remains elusive, the animal model we established may play a crucial role in future investigations of relevant immunologic mechanisms. Furthermore, serum histamine and tryptase levels in the Re-PCA model were significantly elevated compared to the PCA model. Key inflammatory mediators in CSU—including *Il4*, *Il13*, *Tnfa*, *Ifnγ*, *Ccl2*, *Ccl5*, *Ptgs2*, and *Ptgds*^{6,43–45}—exhibited markedly increased expression in Re-PCA lesions. Therefore, the Re-PCA model provides a more suitable substitute for the pathophysiologic and immunologic characteristics of CSU, making it a reliable disease model for animal-level research on this condition.

Additionally, various treatments related to CSU have also been used to validate the association between the Re-PCA model and CSU. Histamine receptor blockers are recognized as first-line

therapeutic agents for CSU.²⁸ Concurrently, histamine levels in the Re-PCA model were observed to be significantly elevated compared to those in the PCA model. Accordingly, loratadine was used to investigate the role of histamine in the Re-PCA model. As expected, administration of loratadine significantly reversed the phenotype and inflammatory responses in the Re-PCA model. It is worth noting that the disease of a portion of patients shows limited response to histamine receptor blockers. Therefore, drugs such as BTK inhibitors have been approved for clinical use in these patients.²⁵ We further validate the effect of BTK inhibitors on the Re-PCA model, and we found that the urticaria-relevant characteristics in Re-PCA mice were abrogated by BTK inhibitor. In addition, a c-Kit–targeting drug was utilized to further assess the role of mast cells in Re-PCA model. The application of a c-Kit–targeting drug significantly inhibited the phenotype and inflammatory responses induced by Re-PCA. These further demonstrated the association between Re-PCA model and CSU, and confirmed the crucial role of histamine, mast cells, and BTK signaling in Re-PCA model. However, these drugs did not completely abolish the Re-PCA–induced CSU-relevant responses, suggesting that other mediators and cell types also partially contribute to this model, which is consistent with the complex pathogenesis of CSU. In the gene expression profile analysis of skin lesion tissues, we found that the Re-PCA model exhibited a richer distribution of DEGs compared to the PCA model. Among these, key genes associated with CU or CSU, such as *MMP9*,⁴⁶ *PTGS1*,⁴⁷ *IRF1*,⁴⁸ and *CCL5*,⁴⁹ showed significant differential expression. Furthermore, the Re-PCA model enriched pathways including MAPK, PI3K-AKT, inflammatory mediator regulation of TRP channels, and Hippo signaling, which greatly overlapped with pathways enriched in public CSU patient data. The MAPK and PI3K-AKT signaling pathways are key downstream pathways of mast cell activation,^{50,51} and their roles in CSU have been previously reported.^{52,53} In contrast, the PCA model showed no significant enrichment of signaling pathways associated with mast cell activation or CSU. In the protein–protein interaction analysis of the PCA group, hub genes primarily belonged to the keratin family, whereas the Re-PCA group included chemokine families, metalloproteinases, transcription factors, and others. We further discovered that the Re-PCA model shares more overlapping genes with the CSU patient skin lesion gene set. These genes are enriched in signaling pathways associated with CSU, such as T-cell immunity⁵⁴ and altered vascular endothelial function.⁵⁵ In contrast, the PCA model shows no enrichment of relevant signaling pathways in the overlapping genes with the CSU patient skin lesion gene set.

Our research demonstrates that the Re-PCA model performs more closely to the CSU than the PCA model in terms of animal phenotypes, inflammatory responses, immune cell infiltration, and gene expression profiles. However, there are other mouse models that have been used in the investigation of CSU, including the OVA-induced model,⁵¹ MRGPRX2-driven model, autoantibody transfer model, and humanized FcεRI/IgE-induced model. Among these, OVA stimulation requires the process of IgE production,⁵⁶ whereas Re-PCA involves the direct administration of IgE, resulting in a faster immune response. The MRGPRX2-driven model can only induce the MRGPRX2-related pathogenesis, while the IgE-mediated immune responses are regarded as the core pathogenesis of CSU. Furthermore, the study revealed that the IgE-FcεRI–induced model exhibited a stronger correlation with CSU compared to the MRGPRX2-induced model.²¹

The humanized FcεRI/IgE-induced model was established for the research of drugs such as omalizumab that cannot recognize murine IgE,⁵⁷ and the autoantibody transfer model is designed to mimic the spontaneous characteristics of CSU. However, both models have limitations in replicating long-term, recurrent immune stimulation, and they are challenging to establish, which restricts their potential applications. Thus, the Re-PCA model established in this study offers a significant advancement over existing animal models utilized in CSU research because it can replicate long-term, repeated IgE-FcεRI activation.

However, the CSU-relevant IgE-mediated mouse model we established has certain limitations. First, this model can only simulate the pathogenesis of IgE- and antigen-mediated CSU; it is not representative of the IgE autoantibody-mediated and IgG autoantibody-mediated subtypes of CSU. Second, this model also fails to represent CSU pathogenesis mediated by non-IgE pathways, including pathogenesis related to complement components, the coagulation cascade, neuroimmunology, and MRGPRX2. Finally, the model still exhibits certain shortcomings in capturing the spontaneous and chronic characteristics of CSU. Further research remains necessary to improve the model.

In summary, we established a novel IgE-mediated mouse model relevant to CSU that is based on the PCA model. This model exhibits stable urticaria-relevant manifestations and reproduces the pathogenesis of CSU, where repeated activation of IgE and antigen-stimulated mast cells leads to CU-relevant alterations. It demonstrates the pathologic characteristics of CSU in histopathology, immunologic changes, and gene expression profiles. This model holds significant research value for investigating the immunologic mechanisms of CSU, as well as the pathogenic mechanisms associated with multiple key disease-causing genes and signaling pathways, thereby advancing our understanding of the pathogenesis of CSU.

DISCLOSURE STATEMENT

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Key messages

- We established a recurrent IgE-mediated mouse model, the Re-PCA model, which was more relevant to CSU in term of phenotype and inflammatory responses than the commonly used PCA model.
- Drugs effective against CSU, such as histamine receptor blocker, c-Kit inhibitor, and BTK inhibitor, also exhibited significant efficacy in the Re-PCA model.
- Gene expression profiles of the Re-PCA model were more associated with CSU than the PCA model.

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