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Complement C1q essential for aeroallergen sensitization via CSF1R+ conventional dendritic cells type-2

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Abstract

Background: Dendritic cells are heterogeneous, comprising multiple subsets with unique functional specifications. Our previous work has demonstrated that the specific conventional type 2 DC subset, CSF1R^{+}cDC2, plays a critical role in sensing aeroallergen.

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Objective: It remains to be understood how CSF1R$^+$cDC2s recognize inhaled allergens. We sought to elucidate the transcriptomic programs and receptor-ligand interactions essential for this subset's function in allergen sensitization.

Methods: We applied single-cell RNA sequencing to mouse lung DCs. Conventional DC-selective knockout mouse models were employed, and mice were subjected to inhaled allergen sensitization with multiple readouts of asthma pathology. Under the clinical arm of this work, human lung transcriptomic data was integrated with mouse data, and bronchoalveolar lavages were collected from subjects undergoing allergen provocation, with samples assayed for C1q.

Results: We found that C1q is selectively enriched in lung CSF1R$^+$cDC2, but not in other lung cDC2 or cDC1 subsets. Depletion of C1q in conventional DCs significantly attenuates allergen sensing and features of asthma. Additionally, we found that C1q binds directly to human dust mite allergen, and the C1q receptor, CD91 (LRP1), is required for lung CSF1R$^+$cDC2 to recognize C1q-allergen complex and induce allergic lung inflammation. Lastly, C1q is enriched in human bronchoalveolar lavage (BAL) following subsegmental allergen challenge, and human RNA-seq data demonstrate close homology between lung IGSF21$^+$DCs and mouse CSF1R$^+$cDC2s.

Conclusions: We conclude that C1q is secreted from the CSF1R$^+$cDC2 subset among conventional DCs, and our data indicate that the C1q-LRP1 axis represents a candidate for translational therapeutics in the prevention and suppression of allergic lung inflammation.

Capsule Summary:

Under a novel mechanism required for asthmatic sensitization in mice, complement protein C1q, secreted by lung CSF1R$^+$ type-2 conventional dendritic cells, directly binds inhaled allergen and is captured by receptor LRP1.

Keywords

Colony stimulating factor 1 receptor (CSF1R); conventional dendritic cell type 2 (cDC2); Complement 1q (C1q); allergen sensing; allergic asthma

INTRODUCTION

Dendritic cells (DCs) are the major antigen-presenting cells and play a crucial role in the immune response to inhaled allergens by trafficking them to the regional lymph nodes (LNs). There, DCs launch an antigen-specific adaptive immune response involving T and B cells (1–4). DCs, providing the first line of immunological defense, have developmentally evolved into multiple subsets that are found throughout the body (5). DCs are subdivided into plasmacytoid DC (pDC), monocyte-derived DC (moDC), and conventional DC (cDC), the latter of which are further divided into cDC1 and cDC2 (5–7). Conventional DC2 play a critical role in activating Th2 and Th17 immunity against allergens, viruses, fungi, and helminths, whereas cDC1 are specialized to induce Th1 and CD8 T cell activation (5–7). Conventional DC1 are specialized to induce Th1 and CD8 T cell activation, whereas cDC2 play a critical role in activating Th2 and Th17 immunity against allergens, viruses, fungi, and helminths, while recent work has proposed an additional cDC2 subset that is immunoregulatory (8). Depletion of the cDC2 compartment abrogates the development of

Th2 cell immunity in allergic asthma, suggesting an important role for cDC2 in allergic lung disease (1,9–11). We have previously shown that the specific subset of lung CSF1R$^+$cDC2, abundantly located in the alveolar space, initiates the cascade of allergic lung inflammation, delivering “invading” allergens to regional lymph nodes (12,13). This finding is in keeping with the prior observation that specific human *CSF1R* polymorphisms associated with a higher *CSF1R* gene expression predispose to asthma development (14). Despite our novel findings, it remains to be elucidated how the CSF1R$^+$cDC2 subset recognizes and senses inhaled allergen in the airways. As such, we considered that single-cell RNA sequencing (scRNA-seq) might provide comprehensive assessment of the defining transcriptomic programs functioning in CSF1R$^+$cDC2. Given a unique gene expression pattern, we hoped to leverage newly elucidated receptor-ligand interactions, particularly with respect to early events in sensitization, toward novel asthma therapies.

The complement system plays a pivotal role in maintaining tissue homeostasis in the wake of infection, injury, irritants, and apoptotic cell bodies. The classical pathway of complement activation is initiated by the C1 complex and culminates in the rupturing of bacterial cell walls, while in parallel providing chemotactic stimulus for immune cells, and opsonizing antigens for uptake and presentation (15). Inhaled allergens also stimulate the complement system in the lung. For the past several decades, C3a and C5a have been highlighted in asthma research because they stimulate asthma-associated innate immune cells including basophils, mast cells, eosinophils, neutrophils, and macrophages, while also promoting airway remodeling through activation of fibroblasts and airway smooth muscle (16,17). Blockade of C3-C3aR and C5-C5aR showed decreased allergic airway inflammation during the allergic effector response phase in animal models (18–20). Furthermore, genetic studies in human asthmatics strongly supported the association of polymorphisms in complement family genes with asthma susceptibility (21–23). However, despite high expectations, a C5aR antagonist failed in a clinical trial of asthma (24). This unexpected clinical failure prompted us to re-evaluate which complement components might be actionable targets for asthma therapy. The roles of the complement system are reasonably well understood in fully developed allergic inflammation, however, their role in the very early stages of allergic sensitization, when inhaled allergen makes contact with innate immune cells, remains largely unknown.

In the immune microenvironment, DCs and the complement system are essential players for activating innate immunity. Nevertheless, studies investigating how DCs and the complement system are linked are relatively sparse, though the interactions that have been established are intriguing (25). Unlike most complement proteins which are secreted by hepatocytes, C1q is exclusively produced by myeloid cells, including DCs, and not by hepatocytes (26). Thus, the classical pathway of complement activation depends upon myeloid cells for its initiation. Additionally, consistent with overarching interactions between innate immune cells and complement, C3a and C5a promote chemotaxis and maturation of DCs, while C3b, C3d, and C3i serve as opsonins with corresponding receptors expressed by DCs and facilitating antigen uptake (25,27–29). While these phenomena highlight the established role for complement in the recruitment and activation of DCs, there remains a lack of investigation considering such interactions within the context of allergic diseases. Thus, we recognized an important opportunity to clarify the function of the

complement system in specific DC subtypes involved in the pathogenesis of asthma, and in sensitization in particular, given an expected interaction via opsonization of inhaled allergen. Toward this notion, we employ FACS-selective transcriptomics and novel transgenic mice, in addition to functional assays, ultimately identifying a requirement for C1q in allergen uptake by $CSF1R^{+}$cDC2, a necessary step preceding the onset of asthmatic inflammation.

METHODS

Mice

C57BL/6, *Zbtb46-cre*, $Csf1r^{fl/fl}$, and $C1qa^{fl/fl}$ mice were purchased from Jackson Laboratory (Bar Harbor, ME). *Zbtb46-cre* mice were crossbred with either $Csf1r^{fl/fl}$ or $C1qa^{fl/fl}$ to generate *Zbtb46-cre*;$Csf1r^{fl/fl}$ and *Zbtb46-cre*;$C1qa^{fl/fl}$, respectively. All mouse strains were bred in a specific pathogen-free facility maintained by the University of Illinois at Chicago. All mice experiments were approved by the Institutional Animal Care and Use Committee of the University of Illinois at Chicago. Mouse genotypes from tail biopsies were determined using real-time PCR with specific probes designed for each gene (Transnetyx, Cordova, TN). Age & sex-matched 6 to 12-week-old mice were used for the experiments.

SBP-AG Bronchoscopy Protocol

The protocol was approved by the Institutional Review Board of the University of Illinois (Chicago, IL), and an Investigational New Drug (IND) was obtained from the FDA for bronchoscopic administration of dust mite, ragweed, and/or cockroach allergens to volunteers. The details of the protocol were described in our previous publication (30). In brief, we recruited study subjects who are on step 1 asthma therapy according to National Asthma Education and Prevention Program (NAEPP) Asthma Guidelines. After obtaining informed consent, subjects underwent screening for inclusion and exclusion criteria which included skin prick testing for dust mite, short ragweed, and cockroach allergens (Supplemental Tables 1–2). Once the screening was complete, bronchoalveolar lavage (BAL) was performed per standard research guidelines approved by the IRB. Pre-challenge BAL samples were obtained right before sub-segmental broncho-provocation with the identified allergen (SBP-AG). At 48 hours after the SBP-AG, post-challenge BAL samples were obtained, and processed for scRNA-seq as below.

Triple-allergen (DRA-induced) asthma model

We used the previously described DRA-induced mouse asthma model given that it produces highly eosinophilic allergic lung inflammation with high Th2 cytokines which resembles the SBP-AG outcomes (12). In brief, house dust mite, ragweed, and *Aspergillus* were purchased from Greer Lab (Lenoir, NC) and a mixture of 5μg of house dust mite, 50μg of ragweed, and 5μg of *Aspergillus* was insufflated on day 0, 2, 4, 10, 12, and 14. Samples including BAL fluid and lung tissues were collected at designated time points. For rescue experiment, recombinant mouse C1q (RayBiotech, Peachtree Corners, GA) was incubated with DRA for 30 minutes at 37°C and delivered to *Zbtb46-cre*;$C1qa^{fl/fl}$ mice. For blockade experiment, anti-CD91 antibody (ThermoFisher, Waltham, MA) was insufflated with DRA during allergen challenges.

Allergen sensing experiment

Fluorochrome (ZW) was conjugated to the N-terminal proteins in house dust mite to generate the traceable ZW-HDM) which allows us to track allergen uptake in multiple mouse strains as the representative allergen for the DRA as described previously (12,13). At designated time points, lung tissues were collected and the ZW-HDM engulfed by $CSF1R^{+}$cDC2 was measured by flow cytometry. For C1qa rescue experiments, mouse recombinant C1qa (LSBio, Seattle, WA) was used.

FACS analysis

Lung and BAL samples were prepared as described previously (31). Anti-mouse CD45 (APC/Cy7), anti-mouse CD11b & XCR1 (BV510), anti-mouse CD11c (PE/Cy7), anti-mouse Siglec F (PE), anti-mouse CD172a (AF700), anti-mouse CSF1R (PerCP or PE) and anti-mouse CD3, B220, NK1.1, TER119, CD64 (APC) were purchased from BioLegend (San Diego, CA). Samples were run through Gallios flow cytometer and analyzed by Kaluza software (Beckman Coulter, Pasadena, CA).

ELISA

Mouse C1q ELISA kits were purchased from LSBio (Seattle, WA), mouse IL-4 & IL-13 kits from ThermoFisher (Waltham, MA) and mouse IgE kit from BioLegend (San Diego, CA). All procedures are followed by the manufacturer's instructions.

Surface Plasmon Resonance (SPR) binding analysis

The direct binding analyses between allergen and recombinant C1q were performed on a Biacore 8K instrument (Cytiva Healthcare, former GE Healthcare) according to the manufacturer's instructions as described previously (12). In brief, house dust mite (Greer Laboratory, Lenoir, NC) was purchased and immobilized to active flow channels 1 – 3 on a Series S sensor chip CM5 (Cytiva Healthcare) using the standard amine coupling method. Each active channel has its own reference channel as control. BSA (Sigma-Aldrich, St. Louis, MO) or recombinant mouse C1q (RayBiotech, Peachtree Corners, GA) solution (40μM protein in 25mM phosphate, pH 7.4, 137mM NaCl, 2.7mM KCl, 0.05% Tween20) were applied to all channels using single-cycle kinetics at a 25 μL/min flow rate at 25 °C. Sensorgrams were double-referenced with blank channel and zero concentration signals and analyzed using the Biacore Insight evaluation software. Sensorgrams were the best fit with a 1:1 Langmuir model that is described by the following equation, producing two rate constants, k_a, and k_d.

$$A+B \underset{k_{d1}}{\overset{k_{a1}}{\rightleftarrows}} AB \underset{k_{d2}}{\overset{k_{a2}}{\rightleftarrows}} AB^{*}$$

The K_D values were calculated from association rate (k_a) and dissociate rate (k_d) constants. ($K_D = k_d/k_a$)

Lung histology

Histologic tissue preparation and analyses were performed at UIC's Research Histology and Tissue Image Core (RHTIC). Briefly, 10% formalin-fixed and paraffin-embedded sections of lung tissues were used for H&E. The images were taken using an ECHO REVOLVE microscope (ECHO, San Diego, CA).

Single cell RNA-sequencing and data analysis

Mouse lung cDC1 and cDC2 were isolated by MoFlo sorter and all samples were captured with barcoded oligo-dT-containing gel bead using the Chromium controller (10X Genomics) according to the manufacturer's instructions. The library was prepared using 10X Chromium v3.1 kit (10X Genomics) and sequenced on Novaseq 6000 with 2×150nt cycles (UIUC DNA services). Raw reads were demultiplexed, and single-cell gene expression was quantified using CellRanger (10X Genomics). Single cell transcriptomes were analyzed further using Seurat (32). Cells with >=15% mitochondrial expression, unique molecular identifier (UMI) counts <=1000, and <=500 genes were removed. Feature selection was performed to retain the 6000 genes with the most variable expression, controlling for mean expression level. These are likely to be most informative in differentiating cell sub-populations. Normalized gene expression was computed in units of CPM (counts per million) and log-scaled. Dimensionality reduction was performed using PCA (principal component analysis), retaining the top 24 principal components based on statistical significance (JackStraw).

Cell sub-populations were identified upon clustering using the Louvain algorithm with a resolution of 0.5 in Seurat (32). To visualize the cell clusters, t-SNE plot (t-distributed stochastic neighbor embedding) was used. The cellular identity of different sub-populations was determined based on the differential expression of known marker genes using Wilcoxon's rank sum test for each cell cluster versus all other cells. Differential expression analysis result between classical cDC2 (cluster 0) and *Csf1r*^{hi}cDC2 (cluster 8) was displayed using volcano plot with - $\log_{10}$[P] (y-axis) and log2-fold-change (x-axis). Significantly differentially expressed genes (Wilcoxon's rank sum P<0.05 and fold change>1.5) were colored in red and blue for up- and down-regulated genes, respectively. Expression levels of complement genes in each cell clusters was displayed using dot plot showing percentage of expression (size) and average expression levels (color gradient) in each cluster.

Gene Set Enrichment Analysis

The GSEA (48) was performed to assess enrichment of the hallmark gene sets were obtained from MSigDB (49). The cells involved in cluster 0 and cluster 8 were selected and genes were ranked by fold changes between classical cDC2 (cluster 0) and *Csf1r*^{hi}cDC2 (cluster 8). Enrichment score (ES) was computed using a Kolmogorov-Smirnov running sum statistics for each gene set. Significance of the ES was computed using a distribution of null hypothesis which was generated by doing 1,000 random permutations. Finally, the normalized enrichment score (NES) is calculated by dividing positive and negative ES by the mean of positive or negative ES from the null distribution by the permutation. Significantly enriched gene sets were selected with P<0.05 and displayed with NES values as the bar graph.

Integration of single cell transcriptome data from human and mouse lung DC cells

The single cell transcriptome data from human lung DC cells were obtained from freshly resected normal lung tissue that was procured intraoperatively from patients undergoing lobectomy for focal lung tumors in a previously published dataset available through the European Genome-phenome Archive (EGA) under accession EGAS00001004344 (33). The human lung DC cells in this dataset consisted of IGSF21^{+}, EREG^{+}, and TREM2^{+} lung-resident myeloid DC phenotypes at steady state, and canonical myeloid DC1 and DC2 subsets. Give the human DC cell data, mouse orthologs were mapped to human genes to combine expression matrices from human lung DC cells and our mouse DC cells. Unmapped genes were removed for further processing. To reduce data-dependent biases, regularized negative binomial regression for single-cell normalization was applied using sc-transform in the Seurat package (32). Using the combined and bias-corrected expression matrix, dimensionality reduction by PCA and subsequent Uniform Manifold Approximation and Projection (UMAP) embedding were performed to visualize the closeness of cell types between human and mouse. The human and mouse cell type annotation were directly transferred to defined as in the individual data.

Statistical Analysis

A two-sample t-*test* was used for two group comparisons with equal or unequal variance assumptions. To analyze paired samples, the paired *t* test was used. The tests were two-sided, and all our data met the assumptions of the test. Data are presented as mean ± SEM. No statistical methods were used to predetermine sample sizes, but our distribution was assumed to be normal, and variances were assumed to be equal across groups, but this was not formally tested. All analyses were conducted using Prism, GraphPad Software (La Jolla, CA).

Data and material availability

The mouse lung cDC1 and cDC2 raw scRNA-seq data was deposited to the NCBI Gene Expression Omnibus (GEO) and is available as series GSE221208. Materials used in this manuscript could be shared with qualified scientific researchers upon material transfer agreement (MTA).

RESULTS

Lung cDC2 are heterogeneous, expressing a high level of CSF1R

To better understand the characteristics of lung cDCs, we adopted scRNA-seq of this cell type to assess their transcriptional programs. Lung cDC1 (XCR1^{+}) and cDC2 (CD172a^{+}) were sorted from single cell suspension of homogenized lung by surface markers: live CD45^{+}, CD11c^{+}, MHCII^{hi}, lineage^{-} (CD3, B220, NK1.1, TER119, CD64, F4/80), which excludes macrophages and monocyte-derived dendritic cells (Fig. 1A). After sequencing, RNA expression data were visualized by t-stochastic neighbor embedding (tSNE) algorithm for depicting cell groups (clusters) with distinct gene expression profiles (Fig. 1B) (34). Consistent with our prior work, we confirmed that *Csf1r* was selectively expressed by lung cDC2, and not by lung cDC1 (12,13). As expected, the common cDC genes including

Zbtb46 and *Cd24a* were shared by both cDC1 and cDC2. However, cDC subsetspecific genes were exclusively expressed by cDC1 or cDC2, respectively. Lung cDC1 selectively expressed *Xcr1* and *Irf8*, whereas cDC2 exclusively transcribed *Sirpa* (*CD172a*) and *Irf4*, indicating that partitioning of cDC1 and cDC2 was efficient (Fig. 1B & C) (6,7,35,36).

CSF1R expression in cDC2 is required for allergic lung inflammation

We previously reported that airway epithelial cell-derived CSF1 activates CSF1R$^+$cDC2 in sensing aeroallergen and augments CCR7 expression (12,13). To further investigate the function of CSF1R$^+$cDC2, we generated *Zbtb46-cre*;*Csf1r*$^{fl/fl}$ mice (hereafter ***Csf1r*$^{\Delta cDC}$**) by crossbreeding ***Csf1r-flox*** mice (Jackson Lab., Stock No.021024) with a ***Zbtb46-cre*** mouse strain (Jackson Lab., Stock No.028538) (Fig. 2A). Zbtb46 is a cDC-restricted transcription factor that is not expressed by other immune cells such as plasmacytoid DCs, monocytes, or macrophages (37). The *Csf1r*$^{\Delta cDC}$ mice demonstrated a selective reduction of CSF1R$^+$cDC2 in the lung and bronchoalveolar lavage (BAL), compared with littermate controls, while representation in bone marrow and blood was already exceedingly low at baseline (Fig. 2B & C). Functionally, we first evaluated how CSF1R$^+$cDC2 affects sensitization to aeroallergens using fluorescence-conjugated house dust mite (ZW-HDM), as described previously (13). The number of CSF1R$^+$cDC2 with engulfed fluorescent-conjugated allergen was significantly reduced in the mediastinal lymph node of *Csf1r*$^{\Delta cDC}$ mice, compared with littermate control mice (Fig. 2D). Next, we employed an acute asthma model using triple allergens (**D**ust mite, **R**agweed, and ***A**spergillus*; hereafter referred to as the acute **DRA** model), which is a well-established allergic airway inflammation model marked by eosinophilic and Th2 immune responses (13). The majority of BAL cDCs were cDC2 under the DRA model, and total cDC2 cells were significantly reduced in BAL of *Csf1r*$^{\Delta cDC}$ mice, although the proportion of cDC2 was not significantly changed (Fig. 2E). It should be noted that cDC2 outnumbers cDC1 in BAL, both at steady state and under asthmatic conditions as we have shown in the SBP-AG subjects. This is in contrast to whole lung tissue, where populations are relatively equal. Furthermore, while the proportion of cDC1 was somewhat reduced under DRA conditions, compared with steady state, their absolute numbers were actually mildly increased (data not shown). In conjunction with the blunted expansion of the BAL cDC2 population, *Csf1r*$^{\Delta cDC}$ mice exhibited a significant attenuation in eosinophilic allergic lung inflammation compared with their littermates (Fig. 2F–G). Taken together, these data show that CSF1R$^+$cDC2 play a critical role in allergen sensitization and allergic lung inflammation.

C1q family is selectively enriched in CSF1R$^+$cDC2

To elucidate distinct transcriptional programs of DC subsets and gain insight into their biologic functions, we clustered the cells in Figure 1 using the Louvain algorithm revealing nine subpopulations of cDCs (clusters 0–8). Notably, a subset of lung cDC2 (cluster 8) expressed *Csf1r* at markedly elevated levels (Fig. 3A & B). The lung cDC1 cells consisted of four clusters (1, 4, 5, and 6) and the lung cDC2 consisted of 6 clusters (0, 2, 3, 6, 7, and 8). We then compared the distinct pathways and genes between classical cDC2 (cluster 0) and *Csf1r*^{hi}cDC2 (cluster 8), adopting Gene Set Enrichment Analysis (GSEA) (38). Among the pathways highlighted by this tool, we noted that the complement pathway was one of the most enriched pathways in cluster 8 to *Csf1r*^{hi}cDC2 compared with

classical lung cDC2 (Fig. 3C). Of complement components, *C1qa*, *C1qb,* and *C1qc* were highly enriched in *Csf1r*^{hi}cDC2 compared with classical cDC2 (Fig. 3D). Furthermore, upon returning to analysis of all eight clusters, the C1q family was selectively expressed only by *Csf1r*^{hi}cDC2 (Fig. 3E). Other notable findings from our differential expression analysis revealed lysosomal genes (*Lyz1* and *Lyz2*) enriched in *Csf1r*^{hi}cDC2, as well as genes involved in lipid metabolism (*Pltp* and *Lpl*), chemokines *Ccl6* and *Cxcl2*, and immunoregulatory *Retnla* and *Fcrl* family members (39,40). By contrast, classical cDC2 subset expressed *Ccr7*, consistent with a mature phenotype, as well as genes involved in glucose metabolism (*Tbc1d4* and *Basp1*), and CCR4 ligands *Ccl17* and *Ccl22*. To validate our results at the protein level, lung cDC2 cells were divided into CSF1R^{+} and CSF1R^{-} groups by flow cytometry. Consistent with the gene expression level from the scRNA-seq data, the C1q protein level was also selectively high in lung CSF1R^{+}cDC2, compared to lung CSF1R^{-}cDC2 (Fig. 3F).

C1q directly binds HDM allergen

C1q avidly binds PAMPs, DAMPs, irritants, and apoptotic cell bodies, but has never been known to bind allergen (41–44). Thus, to determine whether C1q directly binds to an allergen, we performed a biophysical binding assay, via surface plasmon resonance (SPR). As shown in Figure 3G, HDM extract was immobilized to a chip, and then either purified mouse C1q (32 μg) or control protein (BSA, 32 μg) was analyzed for binding. In physiological buffer, C1q strongly bound to HDM, while the control protein (BSA) demonstrated an unbound reaction. These data indicate that secreted C1q can bind to allergen(s) directly, though further experiments would be required to characterize the structural basis for the observed avidity.

Allergen sensing is suppressed in cDC-specific C1qa-depletion mouse model

As above, scRNA-seq demonstrated high expression of C1q family genes in *Csf1r*^{hi}cDC2 (Fig. 3D). To selectively deplete C1q, we adopted a *C1qa*-flox mouse (Jax.031261) and generated a *Zbtb46cre*;*C1qa*$^{fl/fl}$ (*C1qa*$^{\Delta cDC}$) mouse model by crossbreeding with Zbtb46-cre mice (cDC-specific cre) (Fig. 4A). As expected, the *C1qa*$^{\Delta cDC}$ mouse exhibited significant depletion of C1qa protein in CSF1R^{+}cDC2 cells, compared to *Zbtb46cre* littermate controls (Fig 4B), while the number of CSF1R^{+}cDC2 remained stable (data not shown). Next, *C1qa*$^{\Delta cDC}$ and littermate mice were subjected to fluorochrome-conjugated allergen (ZW-HDM) for evaluating allergen sensing (Fig 4C). The percentage of CSF1R^{+}cDC2 taking up allergen was dramatically diminished in the mediastinal LN of *C1qa*$^{\Delta cDC}$ mice compared to littermate controls (Fig. 4D). Moreover, the relevance of C1q to allergen sensing was further substantiated by the observation that the reduced allergen sensing in *C1qa*$^{\Delta cDC}$ mice was fully rescued by supplementing the recombinant mouse with C1qa protein in a dose-responsive manner (Fig. 4D, the 4th and 5th rows).

Allergic lung inflammation is abrogated in a cDC-specific C1qa-depletion mouse model

To assess the role of CSF1R^{+}cDC2-specific C1q in allergic lung inflammation, *C1qa*$^{\Delta cDC}$ and littermate mice were subjected to the acute DRA model (Fig. 5A). The induced C1qa expression level by DRA challenge was considerably diminished in the BAL of *C1qa*$^{\Delta cDC}$ mice compared to littermates, while the serum C1q level was unchanged (Fig 5B). In

parallel with the reduced C1q in BAL, markers for allergen sensitization and allergic lung inflammation, including BAL eosinophil count, lung histology, serum IgE, and IL-4 & IL-13 production in the regional lymph nodes, were significantly decreased in *C1qa*$^{\Delta cDC}$ mice, compared with littermate controls (Figs. 5C–F). Notably, these attenuated allergic features in the lungs of *C1qa*$^{\Delta cDC}$ mice were reversed by supplementing mice with recombinant C1qa protein in the acute DRA model (Figs. 5C–F).

CD91 (LRP1), a C1q receptor, plays an essential role in allergen engulfment

Recent studies have demonstrated that C1q has the capacity to produce biological signals directly through specific receptors, independently of other complement proteins (45,46). As such, scRNA-seq from lung cDC subsets was further analyzed for expression patterns of established C1q receptors. As depicted in Figure 6A, *C1qbp*, *Cd91,* and *Calreticulin (Calr)* were abundantly expressed in both lung cDC1 and cDC2, while *Lair1*, *Cr2*, and *Cd93* were minimally expressed. To evaluate which C1q receptor is associated with allergen recognition and uptake, we performed an *in vivo* uptake assay using C1q receptor-blocking antibodies and fluorochrome-conjugated house dust mite (ZW-HDM), assessed by measurable fluorescent index, as previously described (12,13). Blocking antibodies against C1qbp, CD91, and calreticulin were administered to wild-type mice through intranasal insufflation. One hour later, ZW-HDM was administered again via intranasal route. Allergen uptake was evaluated in lung CSF1Rc^{+}DC2 by measuring fluorescence. Among receptors assessed, blockade of CD91 (LRP1) by neutralizing antibody demonstrated a dramatic decrease in allergen uptake by CSF1R^{+}cDC2, whereas other receptors appeared to be dispensable for allergen uptake (Fig. 6B). Next, to validate this finding in an asthma model, wild-type mice were subjected to the acute DRA model with or without anti-CD91 blocking antibody. The antibody was insufflated along with DRA allergens during the challenge period and BAL and lung samples were collected on day 3 after the last challenge. We found that features of allergic lung inflammation, including BAL eosinophil count, total & allergen-specific serum IgE, and peri-airway leukocytes on lung histology, were all significantly reduced by treatment with anti-CD91 blocking antibody, compared to IgG treated control mice (Fig. 6C–E). These data indicate that the C1q-LRP1 pathway is essential for sensing aeroallergen via modulating the function of CSF1R^{+}cDC2.

Allergen-sensing transcriptome of human lung IGSF21^{+}DC closely parallels mouse lung CSF1R+cDC2

To validate our results in a human model, we measured the C1q level in human BAL obtained from subjects enrolled in our Subsegmental Bronchoprovocation with Allergen (SBP-AG) protocol in which BAL samples were collected from mild allergic asthma subjects before and after allergen provocation (Supplemental Tables 1–2), as we previously reported (30). Compared to the BAL obtained before allergen challenge, the post-allergen challenged BAL demonstrated a marked increase in C1qa level at 48 hours (Fig. 7A). Next, to identify the human lung DC subset corresponding to the mouse lung CSF1R^{+}cDC2, we analyzed the previously reported lung single-cell transcriptome data from the sections of normal human lung as detailed in the Methods (33). The data analysis revealed novel IGSF21^{+}, EREG^{+}, and TREM2^{+} lung-resident myeloid DC phenotypes at steady state, in addition to canonical myeloid DC1 and DC2 subsets (analogous to murine cDC1 and

cDC2, respectively). Human lung DC data were integrated with our mouse lung cDC data by mapping the mouse orthologs to human genes, with subsequent reduction of data dependent biases (see Methods), to evaluate the linkage between respective steady state cell phenotypes. As depicted in Fig. 7B, human lung cDC2 demonstrate closer transcriptomic homology with mouse lung cDC2 than cDC1, and, furthermore, the transcriptome of the IGSF21$^+$ DC subset overlaps with mouse lung CSF1R$^+$cDC2. Similar to mouse lung cDC2, *CSF1R* was expressed highly in human cDC2 and cDC2-like subsets, with the highest expression in IGSF21$^+$ human lung DCs (Fig. 7C & D). C1q family genes were also highly expressed in IGSF21$^+$ human DC subsets, whereas other lung DC subsets rarely or partially expressed C1q family genes (Fig.7D). Next, we examined the expression of C1q receptors. Among the six known C1q receptors, only *CD93* and *LRP1* transcripts were detected in human DC subsets. In the IGSF21$^+$DC subset, the expression of *LRP1* was higher than that of *CD93* (Fig. 7D). Taken together, we confirm that a subset of human lung DCs exhibit a highly analogous transcriptomic pattern with the mouse CSF1R$^+$ cDC2 subset, expressing *CSF1R*, the C1q family, and its receptor, *LRP1*, at high levels.

DISCUSSION

In this work, we have demonstrated a novel and necessary function for complement protein C1q in the lung resident CSF1R$^+$cDC2 population upstream of allergen sensitization. This requirement exists in tandem with our previously established role for CSF1 signaling, which, in the setting of allergen exposure, is shown to be essential for maturation and lymph node-homing in this DC subset. Notably, C1qa depletion in cDCs effectively recapitulates our previously described CSF1R selective knockout with respect to allergen sensing in the lungs. Under our current model, patrolling CSF1R$^+$cDC2 sample alveolar antigen via C1q-binding at steady state, with uptake mediated by the C1q receptor, LRP1. In response to allergen exposure, CSF1 stimulation subsequently induces DC maturation and migration, with processed allergen presented to T-cells in draining lymph nodes, leading to cytokine release, IgE production, pulmonary eosinophilia, and airway inflammation. Beyond the translational implications of this work, its novelty stems both from the involvement of a previously unrecognized DC subset and additionally from a previously unknown function of the complement system in antigen sampling. Overall, the behavior observed herein is consistent with the accepted function of DCs with respect to antigen uptake and presentation, however, the apparent mechanisms have not been described previously.

It remains unclear whether the CSF1R$^+$cDC2 population represents a distinct DC lineage or if it instead exists as a particular maturation state of classical cDC2. Notably CSF1R$^+$cDC2 ablation does not significantly affect the population size of lung-resident classical cDC2s, suggesting that the former is not a significant precursor differentiating into the latter. The apparent antigen sampling of CSF1R$^+$cDC2 in steady state, along with its RNA expression profile indicating lipid metabolism and low CCR7 level are suggestive of an immature phenotype, and CSF1R engagement may indeed provide stimulus to mature. Previous work does indicate that CSF1R is elevated in a subset of immature cDCs, but is downregulated upon induced maturation (47). Meanwhile, more recent work has suggested the existence of two opposing cDC2 phenotypes, the anti-inflammatory cDC2A and the pro-inflammatory cDC2B (8). Indeed, the cDC2B subset, with its role supported by functional assays of

cytokine production and $CD4^+$T-cell stimulation, is remarkably consistent with the cell type explored herein. When compared with Fig. 3D, transcriptional similarities include *Csf1r*, markers of lipid metabolism and lysosomal activity, and the chemokines *Cxcl2* and *Ccl6.* While this arguably corroborates an immunogenic role for $CSF1R^+$cDC2s in asthma, it is important to note that *C1q* transcript was not addressed in the referenced study, nor does our study assess the immunoregulatory capacity of classical cDC2. Overall, the relationship between C1q and myeloid polarization is highly variable, and likely depends heavily upon the given cytokine milieu and the presence of pathogen or damage associated molecular patterns (48). As such, C1q deficiency is well known to predispose to both autoimmunity as well as opportunistic infection, given decreased capacity to clear apoptotic cells, autoantigen immune complexes, and invading pathogens alike. The majority of studies considering the impact of C1q on leukocyte function have involved macrophages, while data in DCs remains more limited (49). Nevertheless, studies have shown a requirement for C1q in cDC1 cross-presentation of immune complexed antigen to $CD8^+$T cells and in follicular DC presentation to B cells, while moDCs cultured on immobilized C1q demonstrate an inflammatory phenotype by virtue of cytokine production, surface receptor expression, and T cell activation (50–52). Alternatively, a suppressive role for C1q has been demonstrated in apoptotic cell clearance by moDCs, LAIR1 engagement in pDCs, and prion uptake by cDCs, (53–55). Perhaps most germane to our study, exogenous C1q was shown to suppress inflammation in a mouse model of asthma, but, given the doses involved, was likely inhibiting allergen uptake by oversaturating available binding sites (56). Generally, however, comparison to existing studies is complicated by the narrow scope of our work, which focuses on a very specific DC subset.

A striking feature of the $CSF1R^+$cDC2 phenotype is its localization to the alveolar lumen, resulting in marked enrichment in BAL fluid, whereas classical cDC2 are more highly enriched in lung parenchyma. Such positioning is of course consistent with the detection of noninvasive environmental aeroallergens. If, upon stimulation, $CSF1R^+$cDC2 downregulate CSF1R and upregulate CCR7 in the course of maturation, they may more closely resemble classical cDC2, however, their presence in lung tissue at this point would be expected to be transient as they migrate to draining lymph nodes. To this notion, evidence herein of the requirement for this phenotype in antigen transmission to germinal centers and subsequent outgrowth of IgE responses is strongly suggestive migratory capacity. With respect to the localization of C1q, one might expect DCs to express C1q to facilitate the uptake of immune complexes, however, our *in vitro* and *in vivo* data suggests that C1q directly binds allergen in the absence of immunoglobulin and other complement proteins and facilitates allergen uptake prior to sensitization and development of allergen-specific antibodies, respectively. Interestingly, we also find markedly increased soluble C1q protein in both mouse and human BAL following allergen exposure, suggesting release from cDCs by a mechanism that is yet to be determined. Given that we have previously reported commensurate cDC2 recruitment from lung parenchyma, the most likely explanation is an influx of C1qexpressing cDC2s (12,13). Other potential sources of soluble C1q include proliferation of alveolar-resident cDC2s or the release of preformed C1q by these cells. With $CSF1R^+$cDC2 serving as both source and scavenger of C1q-bound antigen, an autocrine mechanism would appear to be implicated.

This work and others are significantly limited by the exceedingly low numbers of DCs that can be collected from BAL and lung tissue for analysis of gene expression profiles and function. As such, we interpret our data with caution, particularly with respect to opaque cellular mechanisms and signal transduction events that might be inferred from the observed phenomena. Nevertheless, the involvement of C1q and CSF1 in allergen sensing is clear, as presented. Perhaps the most glaring question going forward regards the unexpected affinity between C1q and allergen revealed herein. Unfortunately, the structural and proteomic assays required to identify the precise nature of that interaction are beyond the scope of this work, but should be pursued in subsequent studies. Further effort is also needed to clarify the relevance of the C1q-LRP1 signaling axis in human disease, and we have some cause for optimism in replication, given the homology in gene/protein expression profiles of human lung-resident DCs. The UMAP plot in Figure 7B is initially striking for the poor overlap generally between mouse and human DCs, which is expected given known variable expression patterns resulting in even homologous phenotypes being defined by distinct surface markers. Nevertheless, murine *Csf1r*^{hi}cDC2s coincide almost exactly with human IGSF21^{+} DCs. Another outstanding question for both human and murine models entails determining the specific stimulus for CSF1 secretion by airway epithelial cells, which subsequently induces C1q in the featured cDC2 subset. This may involve direct epithelial interaction with allergen, given clear precedent with extensive literature demonstrating the release of IL-33, TSLP, GM-CSF, and many other soluble mediators from allergen-stimulated epithelium (57). One further readily approachable question involves confirming the dependence of our model upon LRP1/CD91 versus other C1q receptors, with tools available for selective knockout in murine cDCs. Analogous work could be pursued in human cells, isolated from BAL, and manipulated *in vitro* with antibody blockade as in this work. Lastly, it would be comparatively straightforward to query GWAS data for polymorphisms in C1q and its receptors for association with asthma phenotypes, as has been demonstrated for CSF1 (14).

Given the burden of disease that asthma continues to impose, and our as yet relatively poor understanding of its pathogenesis, further attention to novel pathways of sensitization is warranted. Although this work primarily addresses mouse models of disease, we additionally demonstrate how the DC subset highlighted herein is highly congruent with phenotypes found in human BAL samples vis-à-vis RNA-seq and high-dimensional flow cytometry (12,13). Furthermore, while the majority of lung resident human DCs generally resemble murine cDC2, the IGSF21^{+} subset additionally expresses high levels of both C1q and LRP1/CD91 implicated in the proposed mechanism of allergen recognition. Thus, as investigators have suspected previously, there is strong evidence that the complement system plays a role in asthma pathogenesis. But whereas complement proteins C3a and C5a have served as prior targets of asthma therapeutics with limited benefit, C1q has never been addressed in this capacity, and furthermore appears to be acting independently of the classical pathway and of other complement proteins more broadly. It is also worth considering that an ideal therapeutic would intervene early in the inflammatory cascade, so the genesis of allergic signaling represents a high priority target. The mechanism we have uncovered herein appears to be situated at such an early stage, prior to sensitization. As such, it is our hope that future interrogation of the role of C1q in respiratory allergen

recognition via LRP1 by lung-resident DCs may lead to practical benefits for asthma patients in the form of targeted therapeutics.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

BAL	bronchoalveolar lavage
BASP	brain acid soluble protein
BSA	bovine serum albumin
CALR	calreticulin
cDC	conventional dendritic cell
CD	cluster of differentiation
CPM	counts per million
CSF	colony-stimulating factor
DAMP	damage-associated molecular pattern
DRA	dust mite, ragweed, aspergillus
ELISA	enzyme-linked immunoassay
EREG	epiregulin
ES	enrichment score
FACS	fluorescence-activated cell sorting
FEV1	forced expiratory volume in 1 second
FCRL	Fc receptor-like
GEO	gene expression omnibus
GM	granulocyte/macrophage
GWAS	genome-wide association study

GSEA	gene set enrichment analysis
HDM	house dust mite
H&E	hematoxylin and eosin stain
IgE	immunoglobulin E
IGSF	immunoglobulin superfamily
IL	interleukin
IND	investigational new drug
IRF	interferon regulatory factor
LN	lymph node
LPL	lipoprotein lipase
LRP	low density lipoprotein receptor-related protein
MAO	monoamine oxidase
MSigDB	molecular signatures database
MTA	material transfer agreement
NAEPP	National Asthma Education and Prevention Program
PAMP	pathogen-associated molecular pattern
PCA	principal component analysis
PCR	polymerase chain reaction
PTLP	phospholipid transfer protein
RETNLA	resistin-like alpha
scRNA-seq	single-cell RNA sequencing
SEM	standard error of the mean
SIRP	signal-regulatory protein
SNE	stochastic neighbor embedding
SPR	surface plasmon resonance
TREM	triggering receptor expressed on myeloid cells
Th	T-helper cell
TSLP	thymic stromal lymphopoietin
UIUC	University of Illinois Urbana-Champaign

UMAP	uniform manifold approximation and projection
ZW	zwitterionic fluorophore

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Key Messages:

- Allergic sensitization depends upon CSF1R^{+}cDC2 secretion of C1q and uptake of complexed allergen-C1q via CD91 (LRP1)
- C1q is secreted into human BAL upon allergen stimulation and directly binds house dust mite allergen
- Lung-derived human IGSF21^{+}DCs are transcriptomically homologous to mouse CSF1R^{+}cDC2s

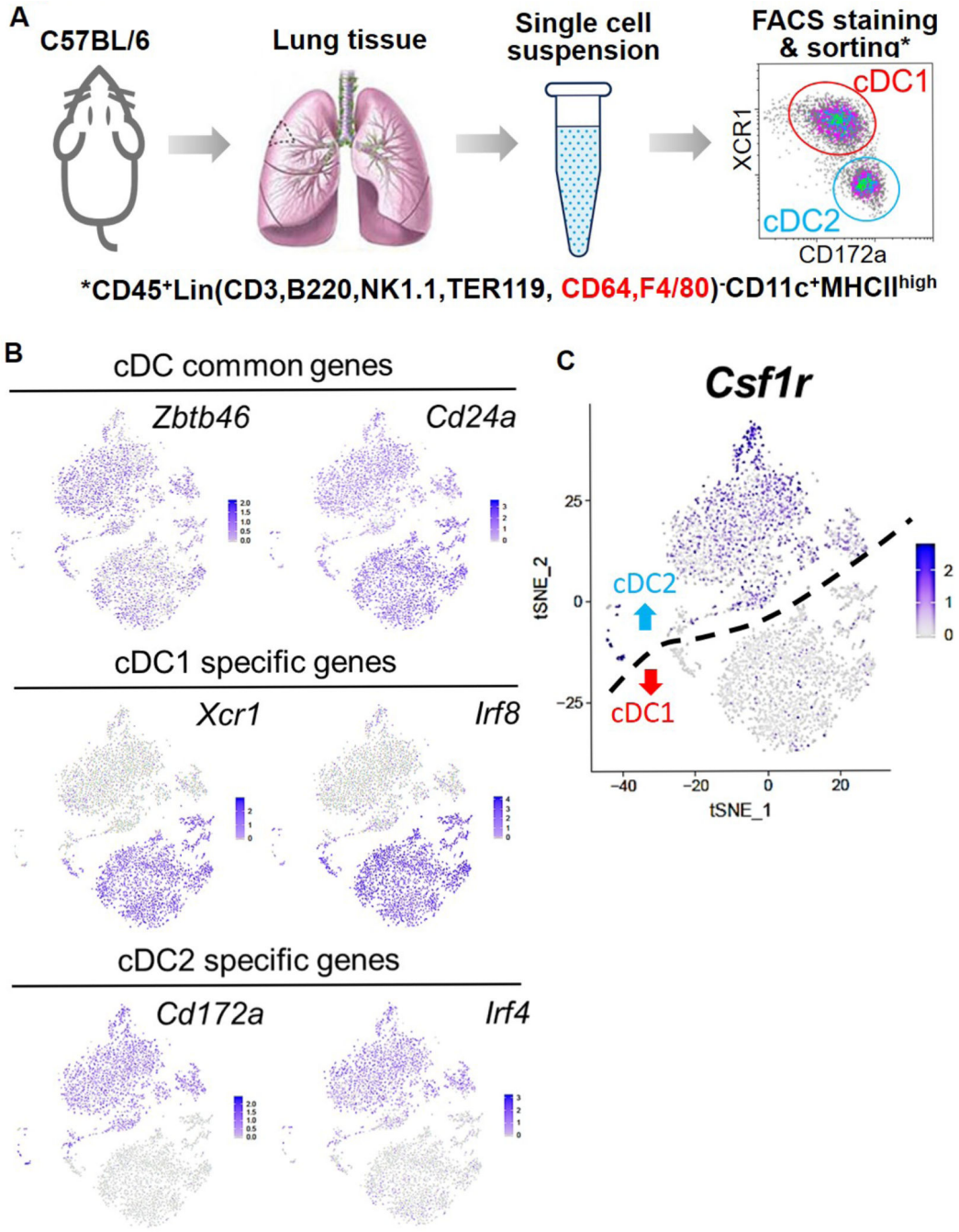

Figure 1. *Csf1r*^{hi}cDC2 subset is identified in a steady state of lung tissue.

(A) Lung cDC1 and cDC2 were sorted using the specific markers for cDC1 and cDC2, including XCR1 and CD172a, respectively, from normal (control) mice as shown in FACS data. ScRNA-seq was performed by 10X Chromium system after sorting each lung for cDC1 and cDC2. The raw data acquired from each cDC1 and cDC2 sample were pooled for analysis, given low cell counts from individual samples.

(B) Feature plots for the representative genes defining cDC1, cDC2, and sub-clusters.

(C) *Csf1r* is selectively expressed by lung cDC2, and not by lung cDC1.

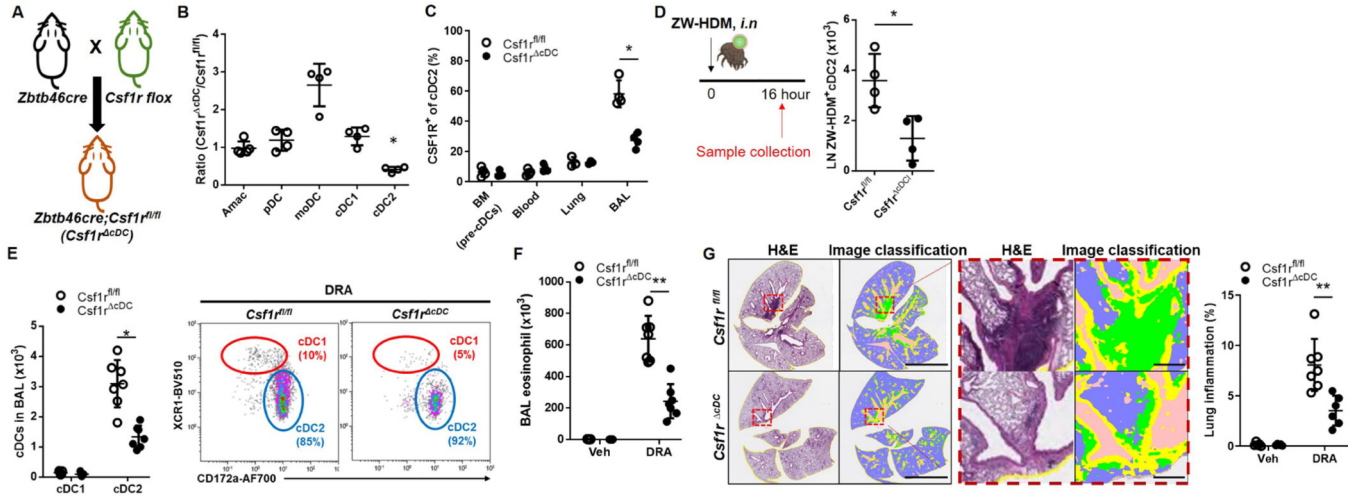

Figure 2. CSF1R^{+}cDC2 is required for allergic lung inflammation

(A) Zbtb46-cre mice were crossbred with *Csf1r*-flox mice to generate a cDC-specific CSF1R depletion mouse model (*Csf1r*$^{\Delta cDC}$).

(B-C) Ratio of DC subtypes in *Csf1r*$^{\Delta cDC}$/littermates (*Csf1r*$^{fl/fl}$) in mouse lung and percentage of *Csf1r*$^{+}$cDC2 in diverse locations in *Csf1r*$^{\Delta cDC}$ and *Csf1r*$^{fl/fl}$ littermate controls (n=3–4). One of three representative experiments is shown.

(D) Fluorochrome-conjugated house dust mite (ZW-HDM) was insufflated to *Csf1r*$^{\Delta cDC}$ and littermate controls and the number of cDC2 with engulfed allergen in regional lymph node was evaluated 16 hours later (n=4). One of two representative experiments is shown.

(E-G) Triple allergens (DRA) were insufflated to *Csf1r*$^{\Delta cDC}$ and littermate controls, and samples were collected 3 days after last challenge (n=5 for Veh & n=7 for DRA). One of three representative experiments is shown.

(E) Flow cytometry analysis was performed in BAL cells after allergen challenges. The percentage and number of cDC1 and cDC2 was evaluated.

(F) The number of BAL eosinophils was examined in *Csf1r*$^{\Delta cDC}$ and *Csf1r*$^{fl/fl}$ littermate controls.

(G) Representative mouse lung pathology (H&E stained) color-coded by green for inflammatory cells, yellow for area of the airway, and purple for void space. Right-sided panels provide higher-magnification views of the left-sided panels.

*p<.05, **<.01

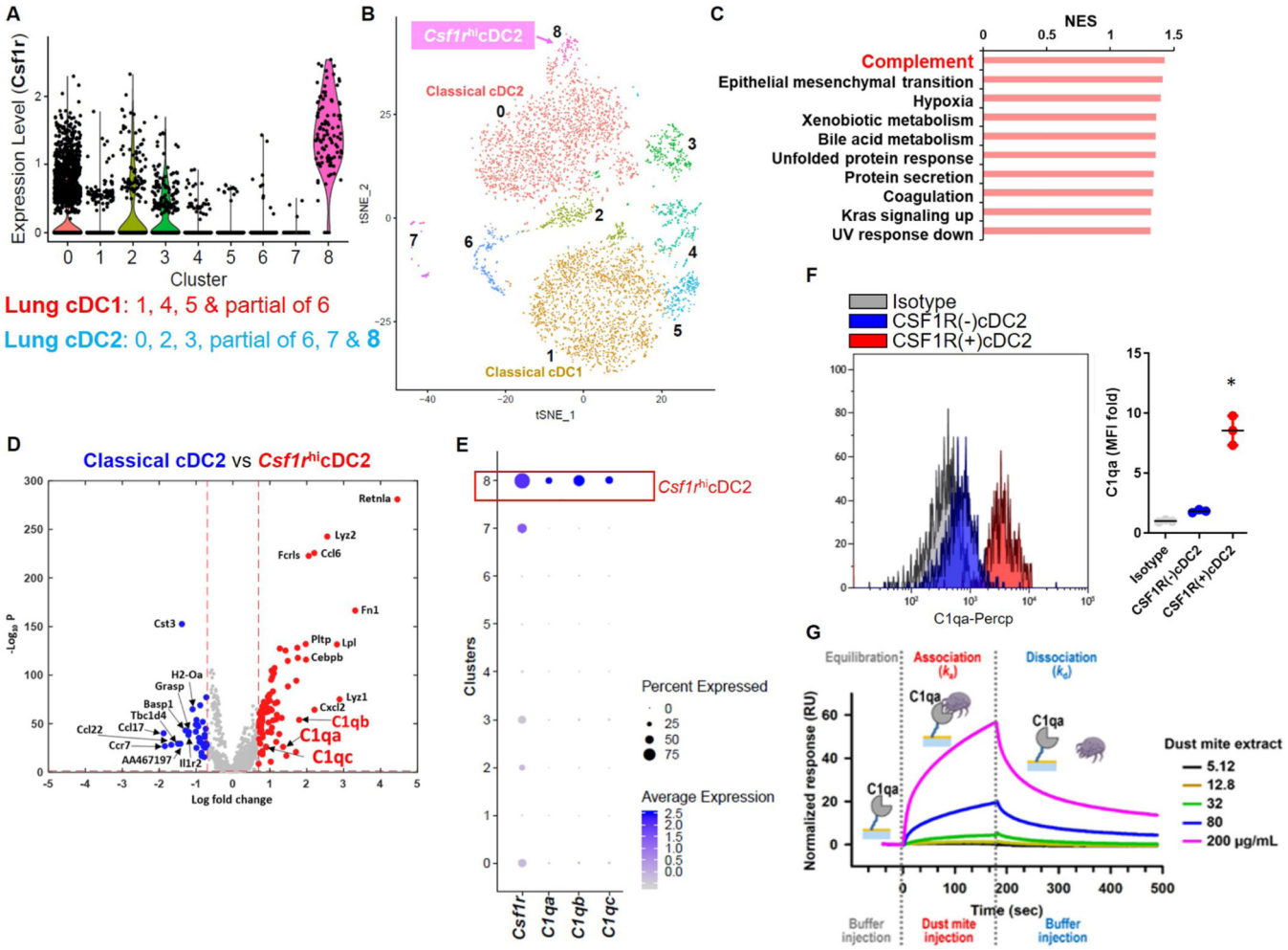

Figure 3. C1q family is selectively enriched in *Csf1r*^{hi}cDC2 subset in steady state

(A) Violin plot for *Csf1r* expression in the cDC clusters indexed by tSNE.

(B) tSNE plot of lung cDC1 and cDC2 subsets according to the transcriptomic landscape.

(C) Pathway analysis was conducted comparing classical cDC2 and *Csf1r*^{hi}cDC2. Among the multiple pathways, the complement pathway was the most enriched pathway in *Csf1r*^{hi}cDC2.

(D) C1q family genes (*C1qa*, *C1qb* and *C1qc*) were significantly expressed in *Csf1r*^{hi}cDC2 subset.

(E) C1q family is selectively enriched in *Csf1r*^{hi}cDC2 subset, but not in lung cDC1 nor other lung cDC2 subsets.

(F) Protein expression of C1qa was higher in CSF1R^{+}cDC2 than CSF1R^{-}cDC2 in healthy mouse lung tissue (n=3). Representative figure of two independent experiments.

(G) SPR analysis demonstrated direct binding of recombinant C1q to an allergen (dust mite extract) in a dose-responsive manner but not to control protein (BSA).

*p<.05

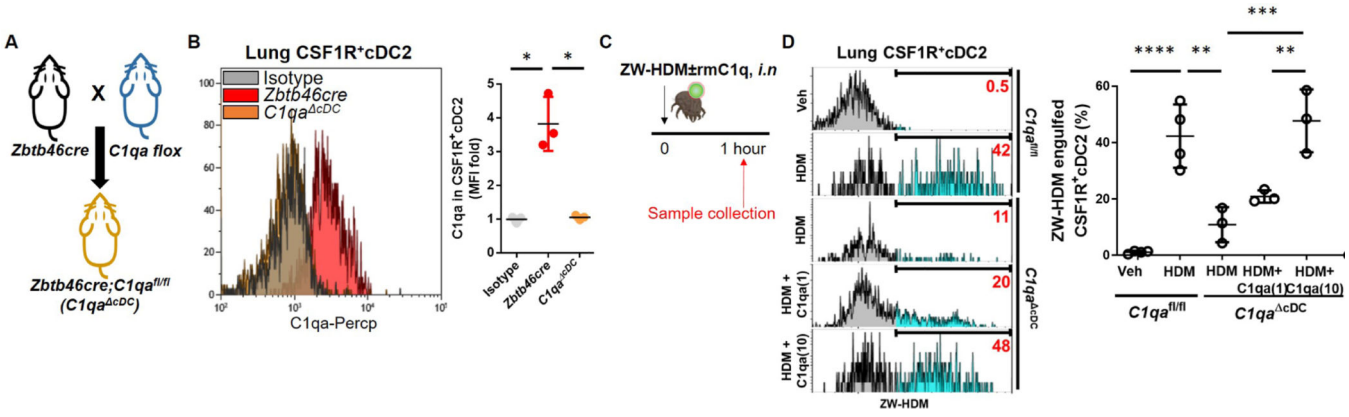

Figure 4. cDC-specific C1qa depletion attenuates allergen sensing in lung CSF1R^{+}cDC2

(A) Conventional DC-specific C1qa depletion model (*C1qa*$^{\Delta cDC}$) was established by crossbreeding Zbtb46-cre and *C1qa*-flox mice.

(B) Protein level of C1qa was determined in Zbtb46-cre (littermate control) and *C1qa*$^{\Delta cDC}$ mice (n=3).

Representative figure of two independent experiments.

(C-D) Allergen recognition was evaluated by measuring the fluorescent activity of the fluorochrome conjugated allergen (ZW-HDM, 100μg) in CSF1R^{+}cDC2 cells collected from the lung at one hour after intranasal challenge with ZW-HDM. For the rescue experiment for C1q depletion, recombinant C1qa (1 or 10μg) was incubated with ZW-HDM at 37°C for 30min and delivered to the *C1qa*$^{\Delta cDC}$ mice (the bottom two panels of Fig. 4D) (n=3–4).

Representative figure of two independent experiments.

*p<.05, **p<.01, ***p<.001, ****p<.0001

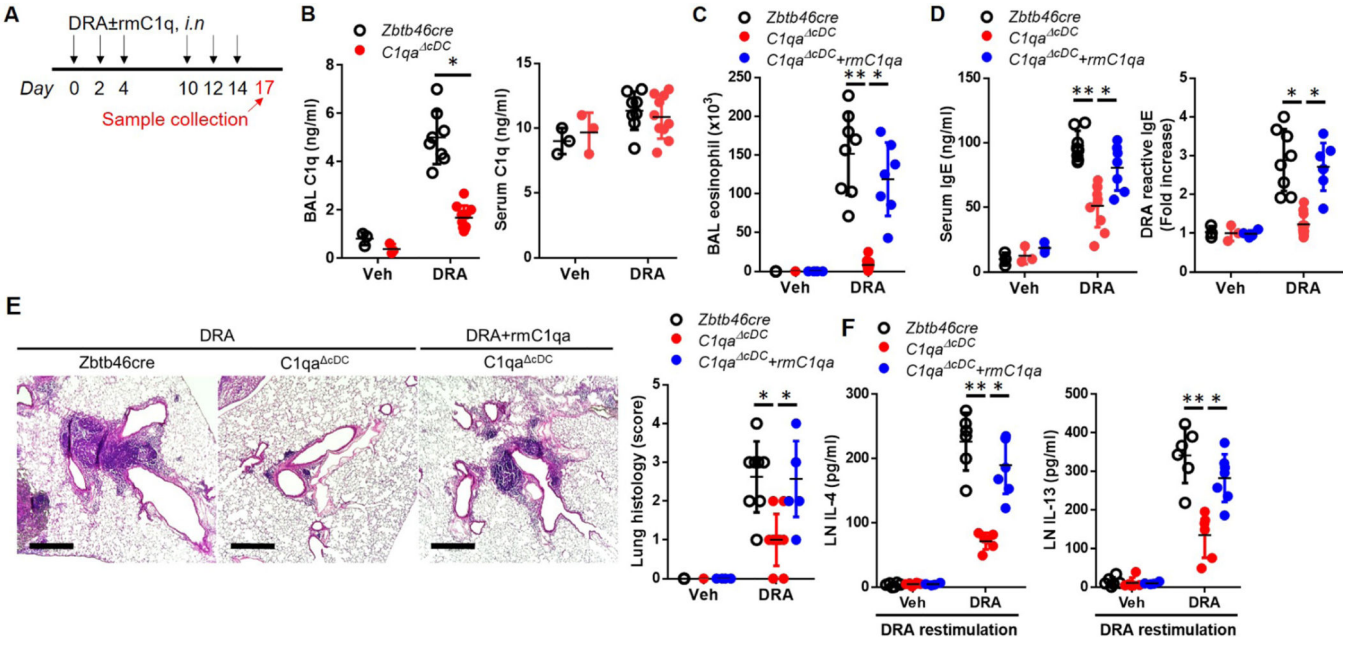

Figure 5. Allergic lung inflammation is attenuated in cDC-specific C1qa-depletion mice

(A-F) Experimental scheme for allergic lung inflammation with or without recombinant mouse C1qa protein (10ng/mouse) (n=3 for Veh, n=7–8 for DRA). One of two representative experiments is shown.

(B) Quantity of C1q was measured in BAL fluid and serum in cDC-specific C1qa-depletion mice and littermates.

(C) The number of BAL eosinophils was measured after cytospin and diff-quik staining.

(D) Total and DRA-reactive IgE were evaluated by ELISA.

(E) Lung histology was prepared by H&E (representative images are presented), and inflammation scores were assessed. Scale bar stands for 520μm.

(F) Mediastinal lymph node (mLN) cells were isolated from each mouse and re-stimulated with DRA for 72-hour *ex vivo* culture in the presence or absence of recombinant C1qa. The Th2 cytokines (IL-4 & IL-13) were measured in culture supernatants.

*p<.05, **p<.01

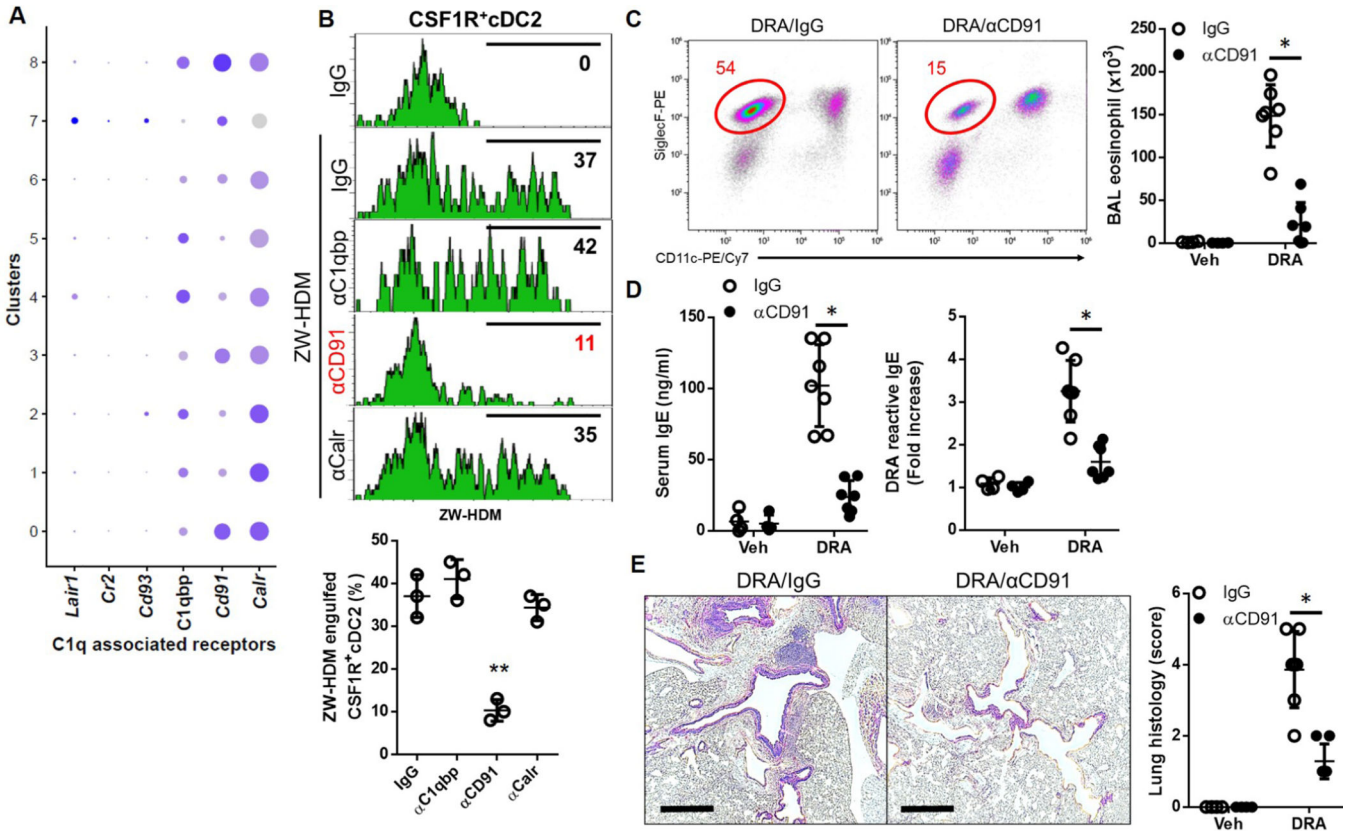

Figure 6. CD91 (LRP1) is required for CSF1R+cDC2 to sense the C1q bound-allergen.

(A) C1q-associated receptors were evaluated in lung cDC1 and lung cDC2 subsets in steady state.

(B) Blocking antibodies against C1qbp, CD91, and calreticulin were administered to wild type mice via intranasal insufflation. One hour later, traceable allergen was administered via intranasal route again, and allergen uptake was determined in CSF1Rc^{+}DC2 by measuring fluorescence. Blockade of CD91 (1μg/mouse) attenuated the allergen uptake in CSF1R$_{+}$cDC2, suggesting a significant role of LRP1 (CD91) in allergen sensing (n=3). One of two representative experiments is shown. IgG denotes isotype controls.

(C-E) Either anti-CD91 (1μg/mouse) blocking or isotype IgG antibody was insufflated with DRA during allergen challenge period (Day 10, 12 and 14) and samples were collected 3 days after last challenge. The features of allergic lung inflammation were reduced by administration of anti-CD91 antibody (n=4 for Veh, n=6–7 for DRA).

One of two representative experiments is shown.

(C) The percentage and number of BAL eosinophils were calculated by flow cytometry analysis after gating CD45^{+}CD11b^{+} cells.

(D) Total and DRA-reactive IgE were measured in sera.

(E) Leukocyte accumulation in lung tissue was assessed on H&E and scored. Scale bar stands for 520μm.

*p<.05, **p<.01

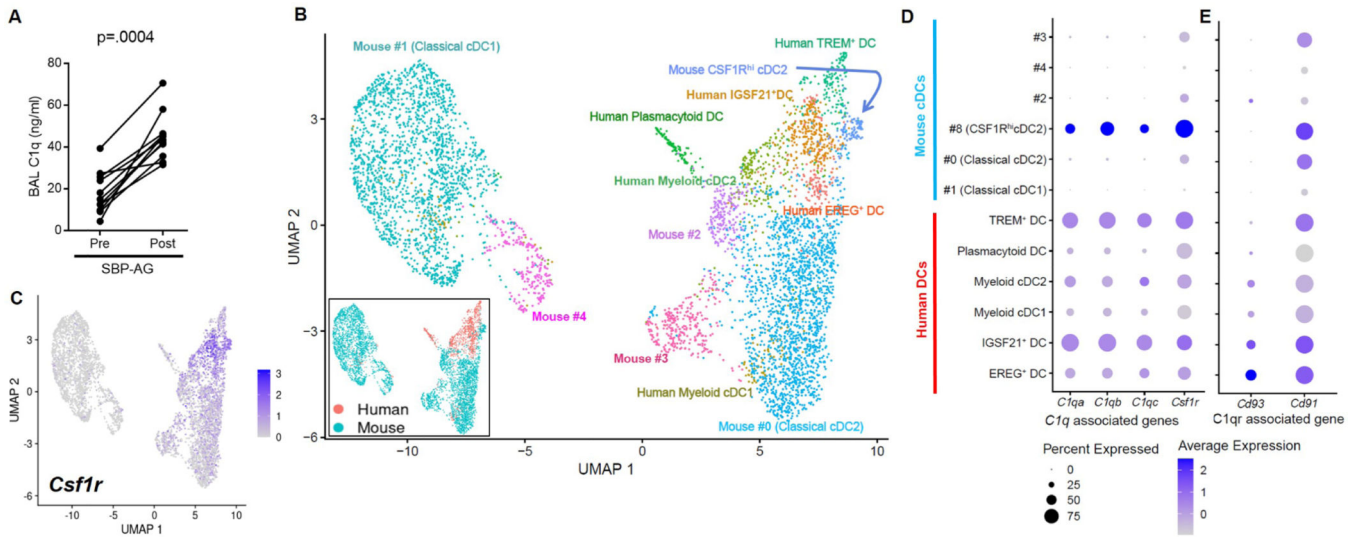

Figure 7. Human lung IGSF21$^+$ DCs display comparable genetic signatures to mouse CSF1R$^+$cDC2 with respect to allergen sensing.

(A) BAL C1q was elevated by bronchoscopic provocation with the sensitized allergen in the patients with mild intermittent asthma (only prescribed as needed bronchodilator) who were enrolled in the SBP-AG protocol.

(B) Among the human lung atlas (75,000 cells), human DCs (1053 cells) were integrated with mouse lung cDCs (5390 cells). Annotation of each human subset was referred from the original article. UMAP plot integrating human lung DC subsets with mouse lung cDCs demonstrates human DC subset transcriptomic homology with mouse lung cDC2.

(C) Feature plot by *Csf1r* transcript level indicates differential expression patterns in DC subsets.

(D-E) Human IGSF21$^+$ DCs showed similar genetic signatures to mouse CSF1R+cDC2 vis-à-vis C1q family and its receptors.