AUTOIMMUNITY

Pathogen-induced tissue-resident memory T_H17 ($T_{RM}17$) cells amplify autoimmune kidney disease

Christian F. Krebs[1,2*†], Daniel Reimers[3†], Yu Zhao[4], Hans-Joachim Paust[1], Patricia Bartsch[1], Sarah Nuñez[5], Mariana V. Rosemblatt[6], Malte Hellmig[1], Christoph Kilian[1], Alina Borchers[1], Leon U. B. Enk[1], Michael Zinke[1], Martina Becker[7], Joanna Schmid[3], Stefanie Klinge[3], Milagros N. Wong[7], Victor G. Puelles[7,8], Constantin Schmidt[3], Tabea Bertram[3], Natascha Stumpf[9], Elion Hoxha[7], Catherine Meyer-Schwesinger[10], Maja T. Lindenmeyer[7], Clemens D. Cohen[7,11], Michael Rink[12], Christian Kurts[9], Sören Franzenburg[13], Friedrich Koch-Nolte[2,3], Jan-Eric Turner[2,7], Jan-Hendrik Riedel[1], Samuel Huber[2,14], Nicola Gagliani[2,14,15], Tobias B. Huber[2,7], Thorsten Wiech[6], Holger Rohde[17], Maria Rosa Bono[18], Stefan Bonn[2,4,19,20], Ulf Panzer[1,2†], Hans-Willi Mittrücker[2,3*†]

Although it is well established that microbial infections predispose to autoimmune diseases, the underlying mechanisms remain poorly understood. After infection, tissue-resident memory T (T_{RM}) cells persist in peripheral organs and provide immune protection against reinfection. However, whether T_{RM} cells participate in responses unrelated to the primary infection, such as autoimmune inflammation, is unknown. By using high-dimensional single-cell analysis, we identified $CD4^+$ T_{RM} cells with a T_H17 signature (termed $T_{RM}17$ cells) in kidneys of patients with ANCA-associated glomerulonephritis. Experimental models demonstrated that renal $T_{RM}17$ cells were induced by pathogens infecting the kidney, such as *Staphylococcus aureus*, *Candida albicans*, and uropathogenic *Escherichia coli*, and persisted after the clearance of infections. Upon induction of experimental glomerulonephritis, these kidney $T_{RM}17$ cells rapidly responded to local proinflammatory cytokines by producing IL-17A and thereby exacerbate renal pathology. Thus, our data show that pathogen-induced $T_{RM}17$ cells have a previously unrecognized function in aggravating autoimmune disease.

INTRODUCTION

Infections have been associated with the development and aggravation of autoimmune diseases (*1*), such as antineutrophil cytoplasmic antibody (ANCA)–associated glomerulonephritis (GN) (*2*), multiple sclerosis (*3*), or inflammatory bowel diseases (IBDs) (*4*). Molecular mimicry, enhanced presentation of autoantigens during infection, and bystander activation of T cells are all considered to be underlying causes. However, the cellular and molecular mechanisms of how infections create a permissive environment for autoimmune diseases in sites like the kidney, brain, or gut are far from being fully understood (*1*, *5*).

GN represents a heterogeneous group of renal autoimmune diseases that are characterized by a pathogenic immune response against renal autoantigens or by manifestations of systemic autoimmunity in the kidney (*6*). GNs are a major cause of chronic kidney diseases, accounting for about 10% of all patients on dialysis. The most severe form, rapidly progressive or crescentic GN, is morphologically defined by extensive glomerular crescent formation, which is formed by proliferation of glomerular parietal epithelial cells and infiltrating leukocytes. This is typically accompanied by tubulointerstitial inflammation and immune cell infiltration. Clinically, crescentic GN is characterized by a nephritic syndrome (hematuria, proteinuria, and often arterial hypertension) rapidly progressing to end-stage renal disease (*7*, *8*). Crescentic GN is a typical feature of renal involvement in ANCA-associated vasculitis and of anti–glomerular basement membrane GN (anti–GBM-GN) (*6*). There is strong evidence for a major role of $CD4^+$ T cells in crescentic GN. GNs are associated with distinct human leukocyte antigen (HLA) class II haplotypes (*7*, *9*, *10*), renal biopsies from patients with crescentic GN show substantial $CD4^+$ T cell infiltration (*11*), and $CD4^+$ T cells reactive to autoantibody targets can be identified during active disease (*7*). Rodent crescentic GN models confirm the role of $CD4^+$ T cells and particularly T_H17 cells in disease development (*12*–*14*). These studies indicate that T cells promote renal damage predominantly by local production of cytokines, such as interleukin-17A (IL-17A), IL-17F, and interferon-γ (IFN-γ) (*6*, *8*, *15*). For example, $CD4^+$ T_H17 cell–derived IL-17A induces the renal expression of CXCL1 and CXCL5 and thereby drives the infiltration of neutrophils and other leukocyte subsets, which promote kidney pathology in crescentic GN (*16*).

[1]III. Department of Medicine, Division of Translational Immunology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany. [2]Hamburg Center for Translational Immunology (HCTI), University Medical Center Hamburg-Eppendorf, Hamburg, Germany. [3]Institute for Immunology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany. [4]Institute of Medical Systems Biology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany. [5]Fundacion Ciencia Vida, Santiago, Chile. [6]Faculty of Medicine and Science, Universidad San Sebastian, Santiago, Chile. [7]III. Department of Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany. [8]Department of Nephrology, Monash Health, and Center for Inflammatory Diseases, Monash University, Melbourne, VIC, Australia. [9]Institutes of Molecular Medicine and Experimental Immunology (IMMEI), Rheinische Friedrich-Wilhelms-Universität, Bonn, Germany. [10]Institute for Cellular and Integrative Physiology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany. [11]Nephrological Center, Medical Clinic and Policlinic IV, University of Munich, Munich, Germany. [12]Department of Urology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany. [13]Institute of Clinical Molecular Biology, Kiel University, Kiel, Germany. [14]I. Department of Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany. [15]Immunology and Allergy Unit, Department of Medicine, Solna, Karolinska Institute and University Hospital, 17176 Stockholm, Sweden. [16]Institute for Pathology, University Medical Center Hamburg-Eppendorf, Germany. [17]Institute for Medical Microbiology, Virology and Hygiene, University Medical Center Hamburg-Eppendorf, Hamburg, Germany. [18]Department of Biology, Faculty of Sciences, Universidad de Chile, Santiago, Chile. [19]German Center for Neurodegenerative Diseases, Tübingen, Germany. [20]Center for Biomedical AI, University Medical Center Hamburg-Eppendorf, Hamburg, Germany.
*Corresponding author. Email: c.krebs@uke.de (C.F.K.); h.mittruecker@uke.de. (H.-W.M.)
†These authors contributed equally to this work.

Tissue-resident memory T (T_{RM}) cells are a recently found distinct memory T cell subset mainly located at barrier sites such as skin or mucosal tissues. T_{RM} cells are defined by their exclusion from circulation and can act as local sentinels for pathogens that have been encountered before (*17*–*20*). In terms of phenotype and expression profile, T_{RM} cells differ substantially from other memory T cell subsets. T_{RM} cells are characterized by high expression of CD69 and down-regulation of the sphingosine 1 phosphate receptor and the transcription factor Krueppel-like factor 2 (KLF2), which is essential for their long-term tissue residency (*21*–*23*). Most studies so far focused on $CD8^+$ T_{RM} cells and their relevance for immediate protection against pathogens upon local reinfection. In contrast, the role of $CD4^+$ T_{RM} cells in peripheral tissues is less well characterized (*24*). Moreover, the implications of T_{RM} cells for the development and progression of autoimmunity, such as GNs, are largely unknown.

Here, we analyzed the composition of human renal T cells in biopsies from patients with ANCA-GN and from healthy kidney tissue from tumor nephrectomies and identified different T cell subsets including $CD4^+$ T_{RM} cells with T_H1 or T_H17 expression signatures. On the basis of this finding, we hypothesized that aberrant activation of pathogen-induced T_{RM} cells promotes renal pathology in patients with GN. To test this, we combined mouse infection models with models for GN and provide evidence that infection-induced $T_{RM}17$ cells can rapidly react to local inflammatory cytokines and aggravate renal autoimmune disease. These results support a new concept for the predisposing role of microbial infections in aggravating T_H17 cell–driven autoimmune diseases.

RESULTS

Human renal tissue displays high abundance of $CD4^+$ T_{RM} cells

T cells are present in the kidney under homeostatic conditions, but there is limited information on the composition and function of these cells (*25*). To elucidate their phenotype and potential function, we performed single-cell RNA sequencing (scRNA-seq) (*26*) in combination with epitope measurement using barcoded antibodies [cellular indexing of transcriptomes and epitopes by sequencing (CITE-seq)] (*27*, *28*) of $CD3^+$ T cells from the human kidney (Fig. 1A). Because biopsies from individuals without kidney diseases were not available, we used tissue from the healthy parts of kidneys after tumor nephrectomy (table S1). We identified 5905 cells and 16,148 genes from three renal samples (Fig. 1B and figs. S1 and S2). Unsupervised clustering analysis revealed 12 distinct populations for renal T cells. CITE-seq considerably improved the analysis by adding important information about CD4, CD8, and CD69 surface expression (Fig. 1C and fig. S2). Clusters C1 to C3 in kidneys (representing 7.5, 11.8, and 14.0% of analyzed cells, respectively) were composed of $CD4^+$ $CD69^{high}$ $CD45RA^{low}$ $CCR7^{low}$ T cells. CD69 expression on $CD4^+$ T cells is indicative of a T_{RM} phenotype of these cells. Comparison of expression profiles of T cells from our datasets with a published signature gene set for human $CD4^+$ T_{RM} cells (*21*) revealed a T_{RM} profile of renal $CD4^+CD69^+$ T cells but not of renal $CD4^+$ $CD69^{low}$ T cells or blood T cells (Fig. 1D). The expression profiles of $CD69^{high}$ clusters (C1 to C3) included genes associated with T_{RM} cells, e.g., high expression of *CD69*, *RGS1*, or *CXCR6* and low expression of *KLF2* or *SELL* (Fig. 1E) (*21*). Flow cytometry confirmed the presence of renal $CD45RA^-CCR7^-CD69^+$ $CD4^+$ T_{RM} cells in the kidney (Fig. 1F).

The clusters of $CD4^+$ T cells from renal tissue (C1 to C6) were analyzed for the expression of sets of signature genes of T_H17, T_H1, T_H2, T_H0, and regulatory T (T_{reg}) cells (Fig. 1G) (*29*). $CD4^+CD69^{high}$ $CCR6^{high}$ T cells of C1 presented with a T_H17 signature, cells in C2 expressed signature genes of T_H1 and T_H2 cells, and cells in C3 expressed signature genes of T_H1 cells. $CD45RA^+$ cells in C5 expressed *CCR7*, *SELL*, and *LEF1* and most likely presented naïve cells, and cells in C6 were T_{reg} cells. Last, we detected IL-17A and IFN-γ production in a subset of these $CD4^+$ T cells after phorbol 12-myristate 13-acetate/ionomycin stimulation (fig. S2D).

In conclusion, our results demonstrate that, under homeostatic conditions, human kidneys harbor a substantial population of $CD4^+$ T_{RM} cells, including major subsets with a T_H1 and T_H17 cell phenotype ($T_{RM}1$ and $T_{RM}17$ cells).

T_{RM} cells are enriched in patients with GN

To address a potential contribution of T_{RM} cells to autoimmune kidney diseases, we analyzed renal expression levels of $CD4^+$ T_{RM} cell–associated genes in different glomerular diseases using datasets from the European Renal cDNA Bank database (*30*). Diseases included minimal change disease, systemic lupus erythematosus, IgA (immunoglobulin A) nephritis, focal segmental glomerulosclerosis, membranous GN, and ANCA-associated GN. Expression levels of the T_{RM} cell–associated genes were highest in the glomeruli of patients with ANCA-associated GN (Fig. 2A). Renal biopsies from patients with active ANCA-GN were further studied by scRNA-seq and CITE-seq. To this end, 6829 cells and 15,360 genes from six samples could be analyzed (table S2). T cells from the kidney are distributed to 10 clusters that differed from those observed in matched blood from these patients (Fig. 2B and figs. S3 and S4). T_{RM} cell–associated genes were enriched in renal $CD69^+$ T cells of clusters C1 to C3, representing 3.4, 17.4, and 13.4% of analyzed cells (fig. S4, C and D). In line with observations under control conditions, signature gene sets for T_H cell subtypes could be associated with distinct clusters of $CD69^+$ T cells. T_H17 signature genes were enriched in C1, and T_H1 and T_H2 signature genes were enriched in C2 and C3 (Fig. 2C). $CD4^+$ $CD69^+$ cells were also detected by immunofluorescence histology in the renal cortex of patients with ANCA-GN (Fig. 2D). Flow cytometry confirmed the presence of renal $CD45RA^-CCR7^-$ $CD69^+$ $CD4^+$ T_{RM} cells (Fig. 2, E and F). We observed limited expression of CD103 on these cells, consistent with a restriction of CD103 to mucosal T_{RM} cells (*31*). To further characterize these renal T cells, we made use of chemokine receptor staining. $CD69^+$ $CD4^+$ T_{RM} cells included $CXCR3^+$ and $CCR6^+$ cells representing T_H1 and T_H17 cells, respectively, as well as a large proportion of $CXCR3^+$ $CCR6^+$ cells, which have been described as T_H1^*/exT_H17 cells (*32*) (Fig. 2, G and H).

In comparison with healthy renal tissue, biopsies from patients with ANCA-GN contained substantially increased numbers of $CD45RA^-CCR7^-CD69^+$ $CD4^+$ T_{RM} cells (Fig. 2I). To compare the expression profiles of T cells from renal biopsies of ANCA-GN and control biopsies derived from nephrectomy patients, scRNA-seq and CITE-seq datasets from both groups were combined, and unsupervised clustering was performed (Fig. 2J). T cells separated into 11 clusters, and T cells from both groups of samples were found in all clusters (Fig. 2K). $CD69^+$ $CD4^+$ T cells were allocated to C1 ($CD4^+CD69^{high}CCR6^{high}$) and C2 ($CD4^+CD69^{high}$). For pathway analysis of differentially expressed genes of $CD69^+CD4^+$ T cells (C1 and C2) from control and ANCA-GN samples, we used a web-based

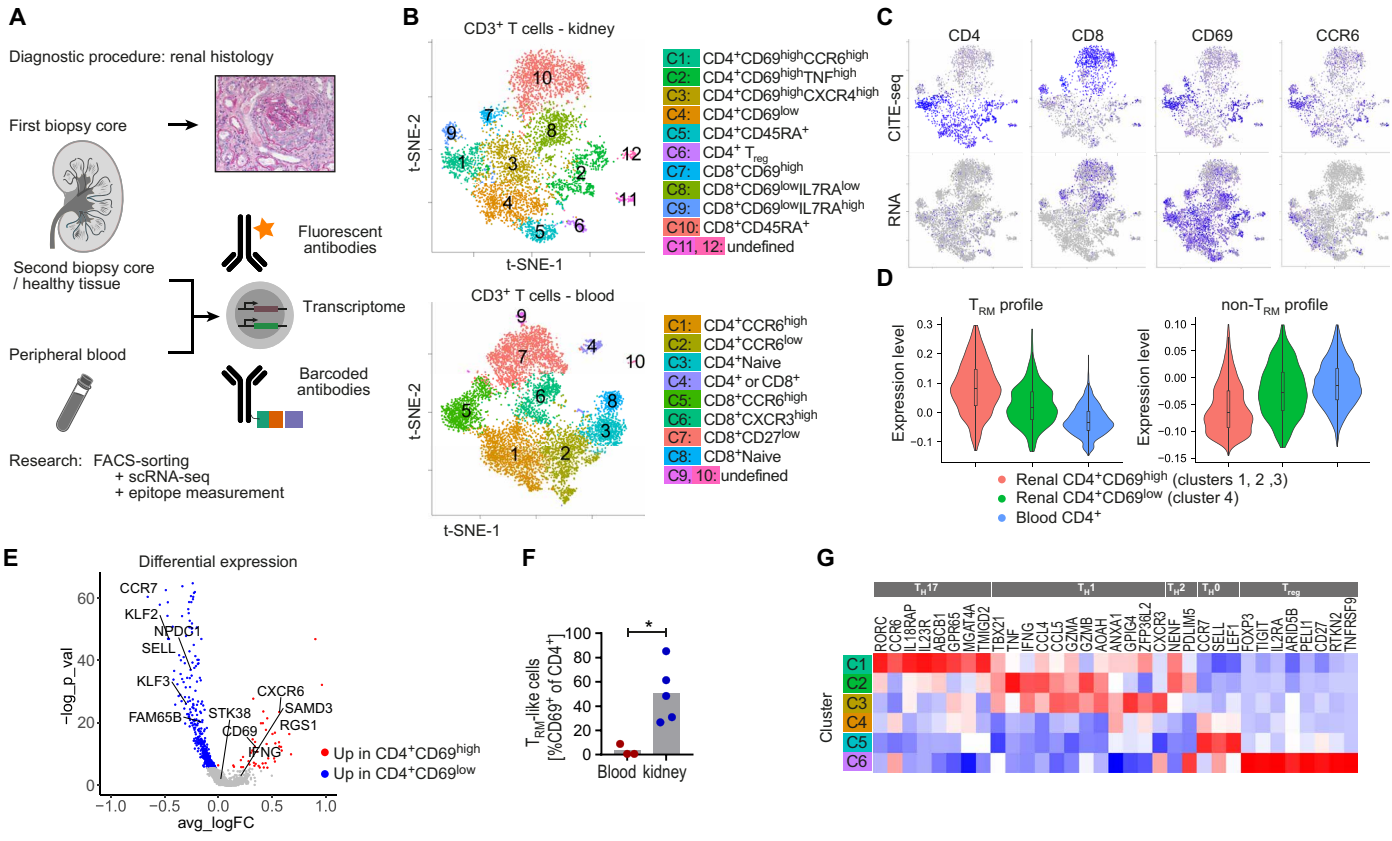

Fig. 1. Human renal tissue displays high abundance of tissue-resident memory T cells. (**A**) Handling of peripheral blood, renal biopsies, and healthy tissue from tumor nephrectomies for the diagnostic procedure and high-dimensional profiling by scRNA-seq including epitope measurement and FACS (fluorescence-activated cell sorting). (**B** to **G**) FACS-sorted CD3$^+$ T cells from peripheral blood and healthy tissue sections from tumor nephrectomy of the same patients were processed for scRNA-seq after labeling with barcoded antibodies (CITE-seq). (B) t-SNE plots of unsupervised clustering of T cells from kidney (top) and blood (bottom). (C) mRNA and protein (CITE) expression profiles of CD4, CD8α, CD69, and CCR6 in t-SNE plots from renal T cells. Clustering revealed three populations of CD69$^+$ renal CD4$^+$ T cells including a cluster of CCR6^{high} CD69^{high} cells. (D to F) Analysis of cells from renal CD4$^+$ CD69^{high}, renal CD4$^+$ CD69^{low}, and blood CD4$^+$ clusters for markers of tissue residency. (D) Comparison of expression profiles of cells with published expression signatures of human CD4$^+$ T_{RM} cells. (E) Volcano plot showing the differential RNA expression profiles of renal CD4$^+$CD45RA^{low}CD69^{high} (clusters C1, C2, and C3; red) versus CD4$^+$CD45RA^{low}CD69^{low} (cluster C4, blue) T cells. (F) Frequencies of CD69$^+$CD4$^+$CD45RA$^-$CCR7$^-$ T cells in blood and kidney samples identified by FACS. *$P < 0.05$ (unpaired t test). (G) Identification of CD4$^+$ T cell subsets in clusters referring to (B). Clusters C1 to C6 were analyzed for T_H17, T_H1, T_H2, T_H0, and T_{reg} cell signatures. The heat map indicates the relative expression of individual genes in individual clusters (red: high expression; blue: low expression).

enrichment analysis tool (Enrichr) (*33*). This revealed up-regulation of genes involved in proliferation, cell activation, and cytokine signaling pathways in T cells from patients with ANCA-GN (Fig. 2L). Scoring of renal biopsies from patients with active ANCA-GN further revealed that increased numbers of CD69$^+$ mononuclear cells correlated with reduced renal filtration rate, as indicated by high serum creatinine concentration (Fig. 2M and table S3). Together, these observations show that kidneys from ANCA-GN show massive accumulation of CD4$^+$ T_{RM} cells including cells with the phenotype and expression signature of T_H1 and T_H17 cells compared with control samples, and the number of CD69$^+$ renal cells in acute disease correlates directly with impairment of kidney function.

Systemic *Staphylococcus aureus* infection induces renal CD4$^+$ T_H17 cells

To test whether CD4$^+$ T_{RM} cells influence renal autoimmune disease, we combined mouse infection models with models for crescentic GN. Previous findings from mouse models and human studies suggest that T_H17 cells play a role in crescentic GN (*8*). Kidneys of healthy specific pathogen-free mice contain only low frequencies of T_H17 cells (*11*). *S. aureus* infection induces and is controlled by T_H17 cell responses (*32*, *34*) and has been associated with relapses of granulomatosis with polyangiitis, a form of ANCA-associated vasculitis (*35*). Intravenous infection of mice with *S. aureus* induced high bacterial titers transiently in the kidney but not in other lymphoid and non-lymphoid tissues (Fig. 3A). Mice were treated with ampicillin to curb chronic infection (Fig. 3, B and C), and mice had completely recovered from infection and showed normal renal function and morphology by day 30 (Fig. 3, D and E). Recovered mice had normal urinary albumin and blood urea concentrations and showed normal kidney pathology. No electron-dense depositions along the GBM were detected, excluding postinfectious GN that is observed occasionally after bacterial infections (Fig. 3E). Renal T cells were isolated after intravenous injection of fluorochrome-labeled anti-CD45 monoclonal antibody (mAb) to identify vascular cells and polyclonally stimulated to induce cytokine expression. Kidneys of naïve mice contained only marginal frequencies of intraparenchymal (CD45iv$^-$) IL-17A–secreting CD4$^+$ T_H17 cells but a substantial population of IFN-γ–secreting T_H1

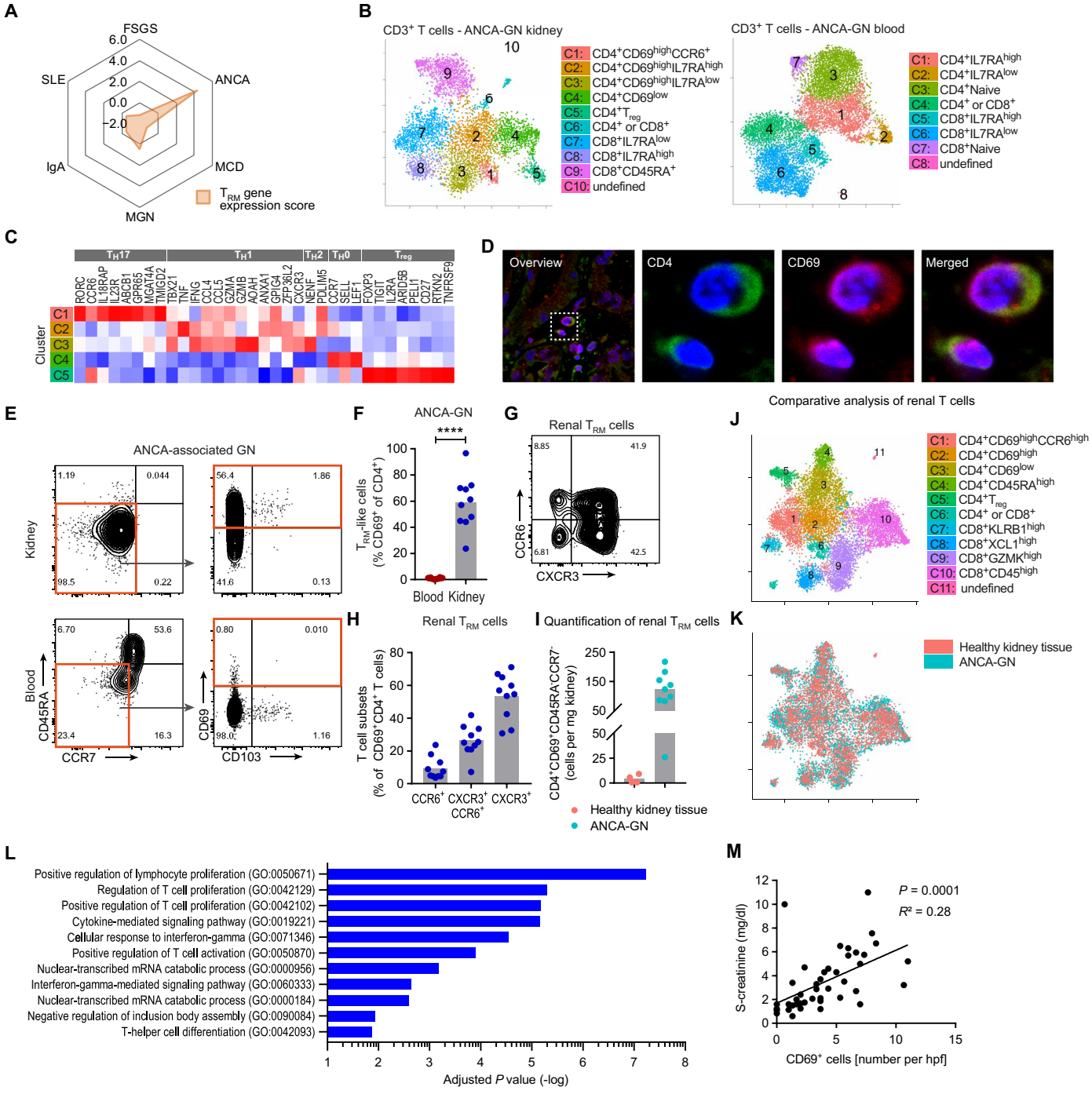

Fig. 2. High-dimensional single-cell analysis of tissue-resident memory T cells in patients with ANCA-GN. (**A**) Changes of mean of scores of the T_{RM} signature gene sets in the glomerular compartment of renal biopsies from patients with different nephritis entities relative to controls. MCD, minimal change disease; SLE, systemic lupus erythematosus; IgA, IgA nephritis; FSGS, focal segmental glomerulosclerosis; MGN, membranous ANCA-GN, antineutrophil cytoplasmic antibody-associated GN. (**B**) t-SNE plots of unsupervised clustering of FACS-sorted $CD3^+$ T cells from kidney biopsies and peripheral blood from patients with ANCA-GN. (**C**) Clusters C1 to C5 from patients with ANCA-GN were analyzed for T cell expression profiles. (**D**) Immunofluorescence reveals presence of intrarenal $CD4^+CD69^+$ T cells in patients with ANCA-associated GN. (**E**) T_{RM} cell phenotype and (**F**) frequencies of $CD69^+CD4^+CD45RA^-CCR7^-$ T cells from blood and kidney samples. $^{****}P < 0.0001$ (unpaired *t* test). (**G** and **H**) Chemokine receptor expression of renal $CD69^+CD4^+$ T_{RM} cells. (**I**) FACS-based quantification of $CD4^+CD69^+$ T cells from healthy kidney tissue and ANCA-GN kidneys. (**J**) t-SNE presentation and unsupervised clustering of combined scRNA-seq data of renal T cells from healthy human tissue (Fig. 1) and ANCA-GN (B). (**K**) Distribution of T cells from both groups of samples. (**L**) Pathway analysis of differentially expressed genes of $CD4^+CD69^+$ T cells [$CD4^+CD69^{high}$ (orange) and $CD4^+CD69^{high}CCR6^{high}$ (red) clusters] from healthy human tissue versus ANCA-GN using the Enrichr analysis tool. (**M**) Correlation of renal $CD69^+$ cells in biopsies of patients with ANCA-GN with serum-creatinine level at time of biopsy ($n = 46$).

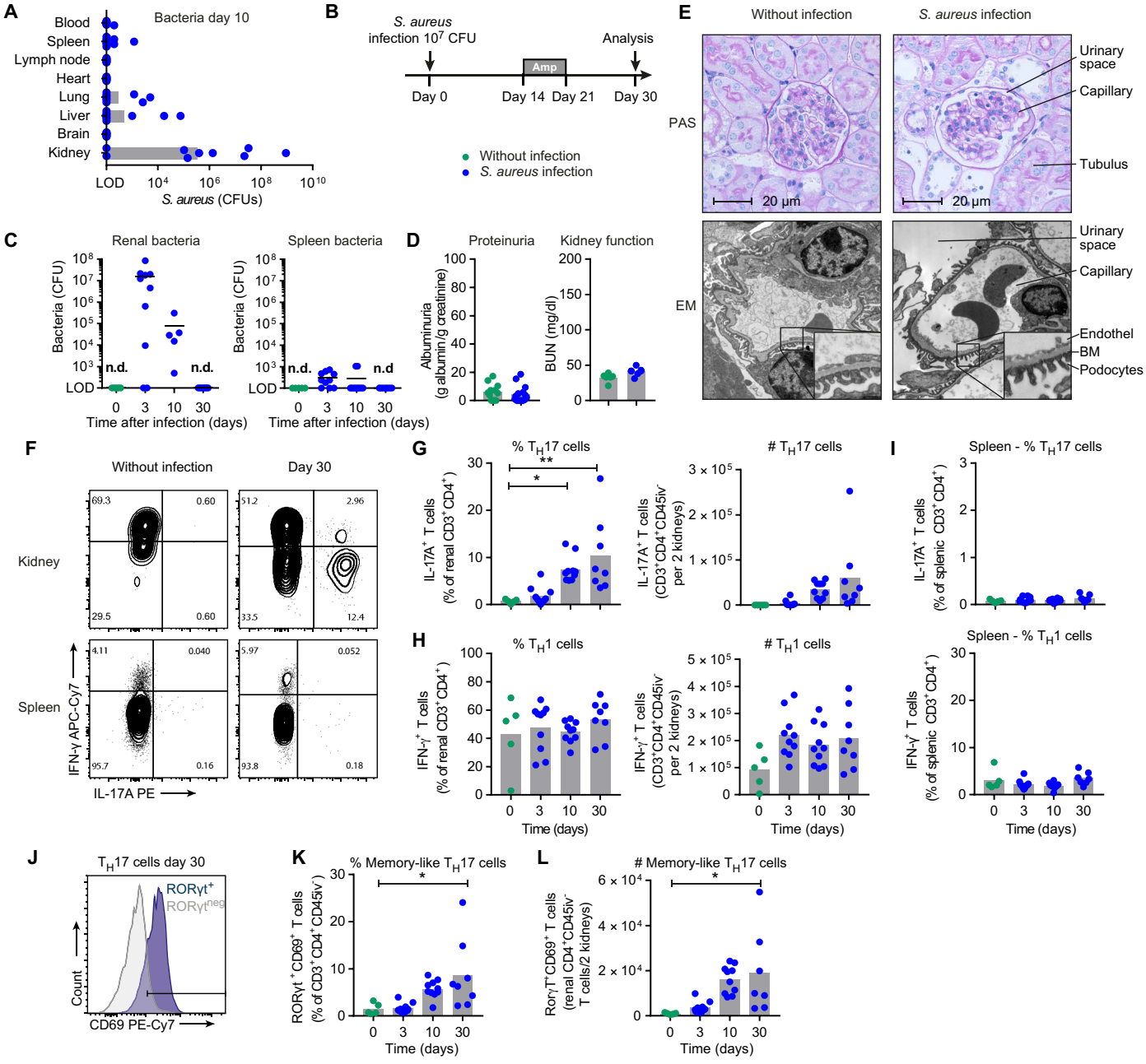

Fig. 3. Systemic *S. aureus* infection results in high bacterial titers in the kidney and induces renal CD4$^+$ T$_H$17 cells. (**A**) C57BL/6 mice were infected with *S. aureus*. To analyze the organ involvement of the infection, different lymphoid and nonlymphoid organs as indicated were analyzed for *S. aureus* [limit of detection (LOD)]. Data are representative of three independent experiments. (**B** to **E**) Bacteria were cleared with oral ampicillin and kidneys were analyzed at the indicated time points. (C) Bacterial titers in kidney and spleen. (D) At day 30, renal function with normal urinary protein and regular blood urea nitrogen (BUN) concentrations were detected. (E) Regular glomerular morphology (PAS staining) and absence of electron-dense deposits along the glomerular capillaries [electron microscopy (EM)] at day 30. (**F**) Flow cytometry of stimulated renal and splenic leukocytes at indicated time points after infection. CD45iv$^-$ CD3$^+$ CD4$^+$ T cells were assessed for IFN-γ and IL-17A production. Quantification of relative and absolute IL-17A– and IFN-γ–producing CD45iv$^-$ CD4$^+$ T cells in the kidney (**G** and **H**) and spleen (**I**). (**J** to **L**) CD69 and RORγt expression of renal CD45iv$^-$ T cells. Data are representative of three to four independent experiments. *$P < 0.05$ and **$P < 0.01$ [one-way analysis of variance (ANOVA) with multiple comparison test].

cells. *S. aureus* infection caused increased proportions of IL-17A$^+$ CD4$^+$ T cells as well as total numbers of these cells in the kidney, which remained elevated after clearance of *S. aureus* (Fig. 3, F and G, and fig. S5, A and B). In contrast, increase of frequencies and numbers of renal IFN-γ$^+$ CD4$^+$ T$_H$1 cells was less evident and infection resulted in only marginal changes of splenic T$_H$1 and T$_H$17 cells (Fig. 3, H and I). Ex vivo staining for RORγt confirmed the accumulation of T$_H$17 cells in kidneys from infected mice (fig. S5C). At day 30, the majority of renal RORγt$^+$ CD4$^+$ T cells had a CD69$^+$ phenotype of T$_{RM}$ cells (Fig. 3, J to L, and fig. S5C). Together, these findings indicate that infection with *S. aureus* induces renal T$_H$17 cells with the phenotype resembling T$_{RM}$ cells.

S. aureus–induced T_H17 cells persist in the kidney

T_{RM} cells are defined by their exclusion from recirculation. To test whether *S. aureus*–induced RORγt$^+$CD69$^+$CD4$^+$ T cells persisted in the kidney, we performed parabiosis of CD45.1 mice after *S. aureus* infection with CD45.2 control mice (Fig. 4A). After 4 weeks of parabiosis, splenic CD4$^+$ T cells showed equilibrium between host- and donor-derived cells. In contrast, CD69$^+$RORγt$^+$ CD4$^+$ T cells in the kidney of the previously infected mice were still host-derived (Fig. 4, B to E). We also used *Kaede* transgenic mice that ubiquitously express the *Kaede* protein, which is permanently converted from green to red fluorescence by light exposure (Fig. 4F) (*11*). *Kaede* transgenic mice were infected with *S. aureus*, and after clearance of infection, one kidney was exposed to light to induce photoconversion (*Kaede* red$^+$) of renal CD4$^+$ T cells (Fig. 4, G to K). Because of the limited depth of light penetration, only a fraction of renal cells can be photoconverted. Four weeks later, the light-exposed kidney still contained 10% *Kaede* red$^+$ CD4$^+$ T cells, indicating that these cells had persisted in the tissue. Converted CD4$^+$ T cells contained an enlarged fraction of up to 60% RORγt$^+$ cells, and in contrast to *Kaede* green$^+$ RORγt$^+$ cells, the vast majority of *Kaede* red$^+$ RORγt$^+$ cells were CD69$^+$. Thus, CD69$^+$ T_H17 cells represented a major population of persisting cells. Photoconverted cells were not detected in the spleen or the contralateral kidney. These experiments indicate that *S. aureus* infection induces bona fide T_{RM} cells with a T_H17 signature in the kidney, referred to as $T_{RM}17$ cells.

S. aureus aggravates crescentic GN and increases the renal T_H17 cell response

To investigate whether infection-induced $T_{RM}17$ cells can amplify renal autoimmune disease, we used an established model of crescentic GN (*6*). After passive transfer, anti-GBM sheep Igs bind within the glomerulus and provoke a T cell response against the deposited antigen. This response results in T_H1 cell– and T_H17 cell–driven formation of glomerular crescents, tubulointerstitial damage, and loss of renal function, resembling multiple aspects of human crescentic GN (*6*, *8*, *11*). Ten days after sheep Ig application, control mice showed proteinuria and crescent formation in about 20% of glomeruli (Fig. 5, A to D). Treatment of mice with resolved *S. aureus* infection resulted in significantly enhanced numbers of glomerular crescents, demonstrating a more severe course of crescentic GN. Induction of crescentic GN was associated with increased frequencies of renal T_H1 and T_H17 cells (Fig. 5, E and F). Aggravated crescentic GN after *S. aureus* infection was accompanied by even greater increases in the frequency of renal T_H17 cells as determined by IL-17A staining after polyclonal stimulation and of CD69$^+$ RORγt$^+$ $T_{RM}17$ cells (Fig. 5G). In contrast, renal IFN-γ$^+$ T_H1 cells were not further increased in mice with prior *S. aureus* infection. The more pronounced T_H17 response but similar T_H1 response in mice with prior *S. aureus* infection was also evident when total numbers of renal T_H1 and T_H17 cells were calculated (Fig. 5F). mRNA analysis corroborated the enhanced T_H17 response (Fig. 5H) because we observed higher renal expression of the T_H17-associated cytokines *Il17a*, *Cxcl5*, *Il6*, and *Il23a* in mice after *S. aureus* infection and crescentic GN induction. Compared with mice that did not have a prior *S. aureus* infection, renal *Il10* levels were not reduced, indicating that aggravated crescent formation and enhanced T_H17 cell responses in mice with prior infection were not due to impaired IL-10 production. IL-17A fate reporter mice (*Il17a*^{Cre} × *R26*^{eYFP}) (*36*), in which T cells that had produced IL-17A, constitutively expressed enhanced yellow fluorescent protein (eYFP), allowed for the localization of T_H17 cells in the kidney (Fig. 5I). eYFP$^+$ cells could not be detected in kidneys of control mice but were frequently found after recovery of *S. aureus* infection with or without subsequent induction of crescentic GN. In these mice, eYFP$^+$ cells were mainly found in the tubulointerstitium surrounding the glomeruli. In conclusion, *S. aureus* infection before crescentic GN induction results in aggravated disease, which is associated with an enhanced renal T_H17 cell response.

S. aureus–induced renal $T_{RM}17$ cells drive crescentic GN

Aggravated crescentic GN after *S. aureus* infection could be caused by persisting renal damage not detected in our extensive analysis or by alterations in the local composition and differentiation status of innate immune cells. To exclude T cell–independent mechanisms, *Rag1*$^{-/-}$ mice were infected with *S. aureus*, and after 7 days, bacteria were cleared with ampicillin. After recovery, mice were reconstituted with purified CD4$^+$ T cells from naïve mice and crescentic GN was induced (Fig. 5J). Reconstituted mice with and without prior infection showed similar severity of crescentic GN (Fig. 5, K and L), indicating that aggravation of disease in immunocompetent mice was likely due to *S. aureus*–induced changes in the renal T cell composition.

To link the aggravated crescentic GN directly with the enhanced T_H17 cell response in mice with prior *S. aureus* infection, we used an IL-17A neutralization approach. One group of mice was infected with *S. aureus* and a control group remained uninfected, and all mice were subsequently treated with ampicillin. Two months after infection, crescentic GN was induced in the presence of anti–IL-17A mAb (Fig. 6A). Blockade of IL-17A was associated with low levels of glomerular crescent formation, as described previously (*37*). Neutralization of IL-17A abolished the difference in crescent formation between mice without and with prior *S. aureus* infection (Fig. 6, B and C). After IL-17A neutralization, kidneys of *S. aureus*–infected mice still contained enhanced frequencies of renal IL-17A$^+$ and RORγt$^+$ T_H17 cells, whereas frequencies of IL-17A$^+$ or RORγt$^+$ T_H17 cells in the spleen were not affected (Fig. 6, D and E). Both groups of mice showed similar renal mRNA expression of the IL-17A target gene *Cxcl5* (Fig. 6F).

In an alternative approach, we used mice with a diphtheria toxin receptor (DTR) constitutively induced by *Il17a* expression (*Il17a*^{Cre} × *R26*^{DTR} × *R26*^{eYFP}) (*36*, *38*). In these mice, injection of DT resulted in effective depletion of T_H17 cells (Fig. 6G). Mice with resolved *S. aureus* infection and control mice were treated three times with DT to remove T_H17 cells, including those induced by *S. aureus*. Two weeks later, crescentic GN was induced (Fig. 6, H to K), and both groups showed similarly low levels of crescent formation, which indicates that DT treatment abolished the exacerbated kidney damage acquired by prior *S. aureus* infection. We also observed similar frequencies of renal IL-17A$^+$ T_H17 cells in both groups of mice, suggesting that DT had effectively removed preexisting T_H17 cells including those induced by *S. aureus* infection.

In addition to IL-17A–producing $T_{RM}17$ cells, IFN-γ$^+$ CD4$^+$ T_{RM} cells could be detected in the kidney of mice with prior *S. aureus* infection (Fig. 5, E and F). We neutralized IFN-γ to directly test whether IFN-γ–producing T_{RM} cells could amplify crescentic GN. In contrast to IL-17A blockade, IFN-γ neutralization did not prevent more pronounced crescent formation in mice with prior *S. aureus* infection (Fig. 6, L to O). Together, these results indicate that *S. aureus*–induced renal $T_{RM}17$ cells play a role and significantly contributed to the aggravated crescentic GN.

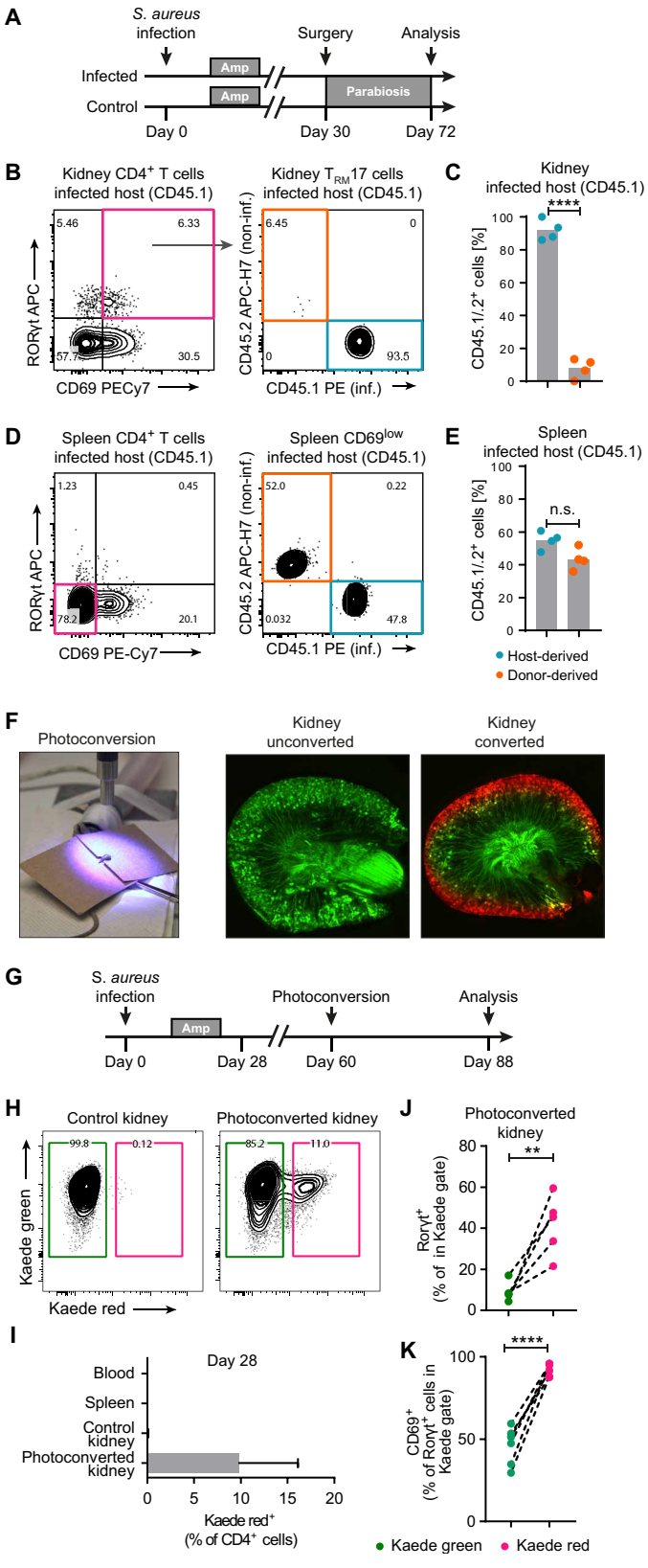

Fig. 4. *S. aureus* induces tissue-resident memory T_H17 cells in the kidney. (**A** to **E**) Parabiosis of *S. aureus*–infected and noninfected mice. (A) CD45.1$^+$ mice were infected with *S. aureus*. Two weeks later, infected and CD45.2$^+$ control mice were treated for 1 week with ampicillin in the drinking water. Thirty days after infection, the circulation of CD45.1$^+$ and CD45.2$^+$ mice was surgically connected. Spleen and kidney T cells were analyzed 28 days later by flow cytometry. (B) Identification of donor-derived (CD45.2$^+$) and host-derived (CD45.1$^+$) CD4$^+$ RORγt$^+$ CD69$^+$ T_{RM} cells in kidneys of previously infected host. (C) Frequencies of CD4$^+$ RORγt$^+$CD69$^+$ T_{RM} cells in kidneys of infected hosts. (D) Representative staining and (E) quantification of CD4$^+$ RORγt$^-$CD69$^-$ of spleen cells from previously infected CD45.1$^+$ mice. Data are combined from two independent experiments. ****$P < 0.0001$ (unpaired *t* test). n.s., not significant. (**F** to **I**) Use of photoconvertible *Kaede* transgenic mice to investigate tissue residency of renal T cells. (F) Photoconversion and fluorescence microscopy before and after renal photoconversion. (G) Experimental setup. Sixty days after *S. aureus* infection, one kidney was photoconverted and analyzed 4 weeks later. (H) *Kaede* red$^+$ T cells 4 weeks after photoconversion. (I) Frequency of photoconverted *Kaede* red$^+$ CD4$^+$ T cells in blood, spleen, and kidneys of *Kaede* mice 28 days after photoconversion (mean ± SD, $n = 3$). (**J**) Quantification of RORγt$^+$ cells among *Kaede* red$^+$ and *Kaede* green$^+$ CD4$^+$ T cells 4 weeks after photoconversion. (**K**) CD69 expression of *Kaede* red$^+$ and *Kaede* green$^+$ CD4$^+$ RORγt$^+$ T cells. Data are representative of three independent experiments. **$P < 0.01$ and ****$P < 0.0001$ (unpaired *t* test).

To investigate whether *S. aureus*–induced T cells residing in the kidney become reactivated and contribute to the T_H17 cell response during subsequent crescentic GN, we made use of *Kaede* transgenic mice. Mice were infected with *S. aureus*, and crescentic GN was induced after recovery from infection. Four days after crescentic GN induction, which is before the main wave of T_H17 cell recruitment to the kidney occurs (*39*), cells in one kidney were converted (*Kaede* red$^+$) (Fig. 6P). Three days later, a large population of *Kaede* red$^+$ CD4$^+$ T cells that expressed IL-17A was detected in the kidney of mice with preceding infection. In contrast, the vast majority of IL-17A–expressing CD4$^+$ T cells in kidneys of control mice were *Kaede* green$^+$ (Fig. 6, Q and P). This indicates that the majority of T_H17 cells in control mice were recruited to the kidney, but mice with prior infection had a large fraction of these cells derived from *S. aureus*–induced renal-resident T_H17 cells.

We tested the consequence of *S. aureus* infection–induced renal $T_{RM}17$ cells in a second model of crescentic GN, experimental autoimmune GN (EAG), a mouse model of Goodpasture syndrome (*40*). In this model, repeated immunization of DBA/1 mice with a fragment of type IV collagen induces antibodies as well as T_H1 and T_H17 cells specific to the collagen matrix of the GBM. Over a period of several weeks, mice develop severe crescentic GN and eventually succumb to renal failure (*14*). DBA/1 mice were infected with *S. aureus* and EAG was induced after recovery from infection (Fig. 7, A to D). In contrast to immunized control mice, a substantial number of previously infected mice succumbed to renal disease. After 10 weeks, surviving mice showed more severe crescentic GN in terms of enhanced crescent formation compared with mice that were not infected. Mice had increased frequencies of renal IL-17A$^+$ T_H17 cells, but similar frequencies of IFN-γ$^+$ T_H1 cells. Overall, results from the EAG model were consistent with the results observed after transfer of anti-GBM sheep Ig.

Listeria monocytogenes transiently infects the kidney and induces strong CD4$^+$ T_H1 cell responses (*41*). After induction of crescentic GN, mice with and without preceding *L. monocytogenes* infection showed similar levels of kidney injury and equal frequencies of renal RORγt$^+$ T_H17 cells (fig. S6). Thus, in contrast to *S. aureus* infection, which caused aggravation of renal damage in different models, *L. monocytogenes* infection did not change renal disease. These results indicate that the course and severity of renal autoimmunity

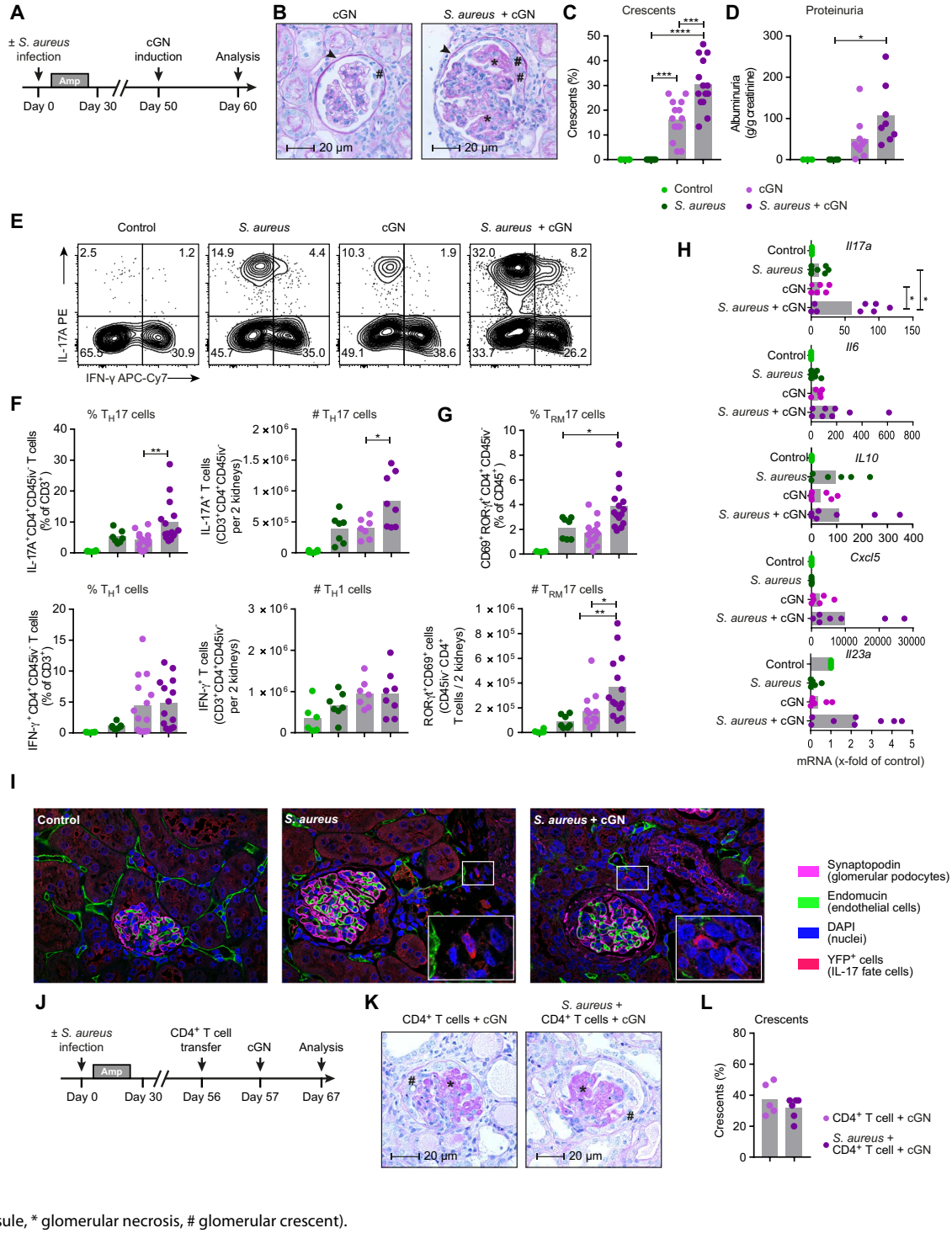

Fig. 5. *S. aureus* aggravates crescentic GN and exacerbates the renal T_H17 immune response. (**A**) Mice were infected as described in Fig. 3. After 8 weeks, experimental crescentic GN (cGN) was induced, and kidneys were analyzed 10 days later. (**B**) Preceding *S. aureus* infection causes enhanced glomerular crescent formation. PAS staining of renal tissue sections 10 days after cGN induction. (**C**) Quantification of glomerular crescents and (**D**) proteinuria. (**E**) Flow cytometry of renal CD45iv$^-$ CD4$^+$ T cells after intracellular staining for IL-17A and IFN-γ. (**F**) Quantification of renal CD45iv$^-$ T_H1 and T_H17 cells by their cytokine production as well as (**G**) renal tissue-resident T_H17 cells by RORγt and CD69 expression. (**H**) RT-PCR analysis of cytokines in the renal cortex at day 10 of cGN as indicated. Data are representative of four independent experiments. *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001 (one-way ANOVA with multiple comparison test). (**I**) Localization of $T_{RM}17$ cells analyzed by renal immunofluorescence microscopy. Data are representative of two independent experiments (n = 3 to 4 mice per group). (**J** to **L**) Analysis of the contribution of the innate immune response to *S. aureus*–induced aggravation of cGN. (J) *Rag1*$^{-/-}$ mice were infected with *S. aureus*. After bacterial clearing, CD4$^+$ T cells were transferred and cGN was induced subsequently. (K) Renal histology and (L) quantification of glomerular crescents of mice with and without preceding *S. aureus* infection. Data are representative of two independent experiments (unpaired t test) (► Bowman's capsule, * glomerular necrosis, # glomerular crescent).

depend on the type of prior infection and the renal T cell composition elicited during the antipathogen response and suggest a unique pathogenic role of $T_{RM}17$ cells in this context.

Infection-induced $T_{RM}17$ cells did not promote autoimmunity at distant sites

After systemic infection, *S. aureus* disseminated into peripheral tissues, particularly into the kidney, which was associated with renal expansion of the $T_{RM}17$ cell population (Fig. 3, A, K, and L). To test whether infection at distant sites also resulted in the generation of renal $T_{RM}17$ cells, mice received intradermal infection with *S. aureus* (fig. S7A) (*42*). Analysis after antibiotic treatment and recovery revealed that local skin infection with *S. aureus* did not induce $T_{RM}17$ cells in the kidney (fig. S7, B and C), suggesting that the presence of pathogens in tissue is a prerequisite for local $T_{RM}17$ generation.

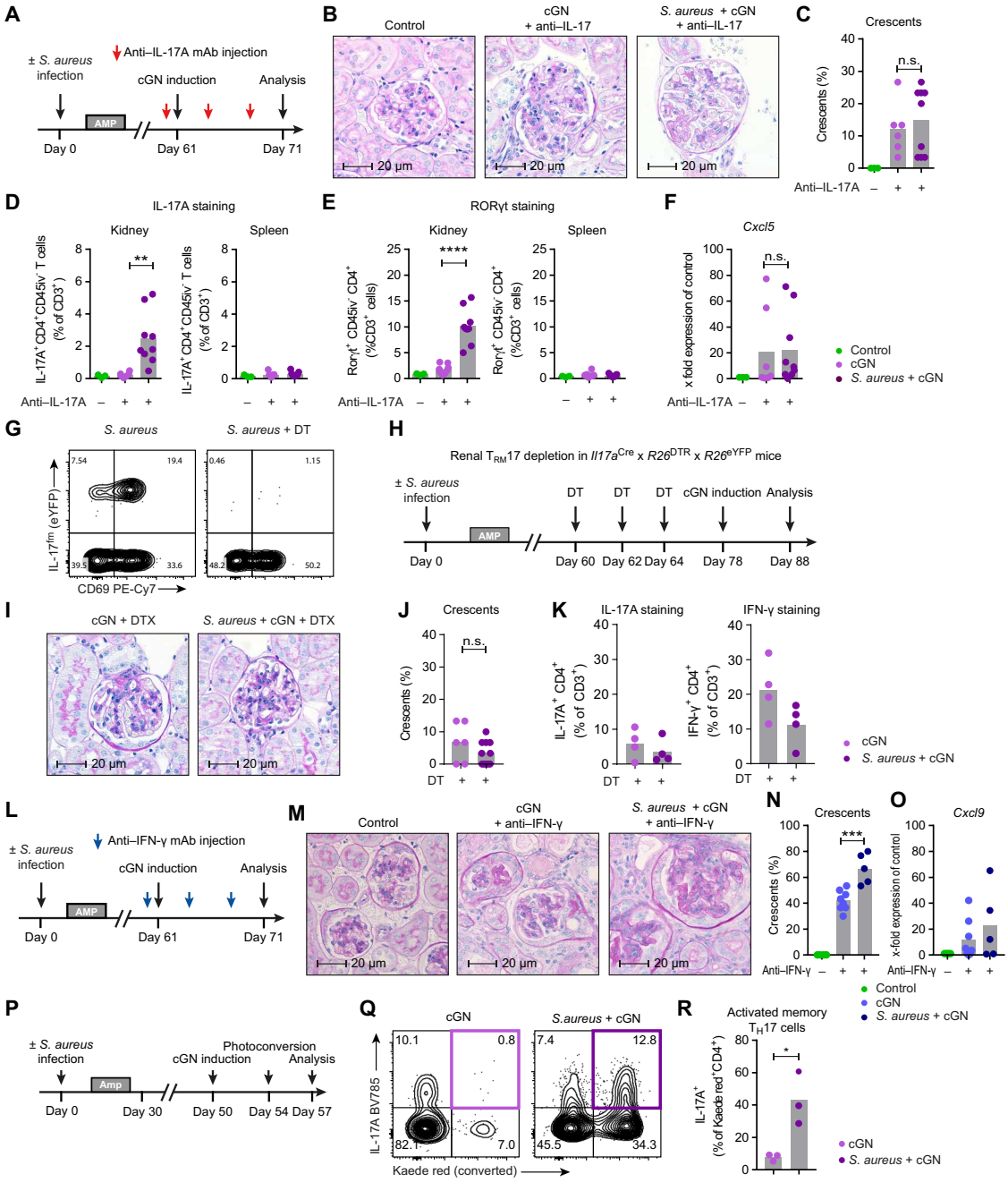

Fig. 6. *S. aureus*–induced renal tissue-resident memory T_H17 cells drive crescentic GN. (**A** to **F**) Inhibition of IL-17 signaling by monoclonal antibodies (mAb). (A) cGN was induced in *S. aureus*–infected and control mice, and at days −1, 3, and 6 of cGN induction, IL-17A was neutralized with mAb. (B) Representative PAS staining of renal tissue section of the respective groups. (C) Quantification of glomerular crescents, (D) IL-17A production, and (E) frequencies of RORγt$^+$ cells in renal and splenic CD45iv$^-$ CD4$^+$ T cells from anti–IL-17A–treated mice were assessed. (F) *Cxcl5* expression by quantitative polymerase chain reaction (qPCR). Data are representative of two independent experiments. $^{**}P < 0.01$ and $^{****}P < 0.0001$ (unpaired *t* test). (**G** to **K**) Depletion of *S. aureus*–induced $T_{RM}17$ cells before induction of experimental GN. (G) Depletion of IL-17A–producing cells in *Il17a*^{Cre} × *R26*^{DTR} × *R26*^{eYFP} mice with diphtheria toxin (DT). (H) Experimental setup of depletion of IL-17A fate cells in *Il17a*^{Cre} × *R26*^{DTR} × *R26*^{eYFP} mice with DT and induction of cGN. (I) Representative PAS staining and (J) quantification of glomerular crescents at day 10 of cGN in mice with T_H17 cell depletion before cGN induction. (K) After DT treatment, analysis of renal CD4$^+$ T cells from mice with and without prior *S. aureus* infection revealed similar frequencies of IL-17A– or INF-γ–expressing CD4$^+$ T cells in cGN. Data are representative of three independent experiments (unpaired *t* test). (**L** to **O**) Inhibition of IFN-γ signaling. (L) cGN was induced in *S. aureus*–infected and control mice, and at days −1, 3, and 6 of cGN induction, IFN-γ was neutralized with mAb. (M) Representative PAS staining of renal tissue section of the respective groups. (N) Quantification of glomerular crescents. (O) Renal *Cxcl9* expression by qPCR. Data are representative of two independent experiments. $^{***}P < 0.001$ (unpaired *t* test). (**P** to **R**) IL-17 expression is rapidly induced by cGN induction in CD4$^+$ T cells residing in the kidney. (P) *Kaede* transgenic mice were treated as in Fig. 4. At day 50 after infection, cGN was induced. Four days after cGN, cells in one kidney were photoconverted and analyzed at day 7 of cGN. (**Q** and **R**) IL-17A production by renal CD4$^+$ T cells in *Kaede* transgenic mice with cGN or *S. aureus* plus cGN was assessed by flow cytometry. Data are representative of three independent experiments. $^{*}P < 0.05$ (unpaired *t* test).

We also tested whether systemic *S. aureus* promoted autoimmunity in the skin using an imiquimod-induced dermal inflammation, which is a well-established mouse model of IL-17–driven psoriasis (*43*). Mice were intravenously infected with *S. aureus* and imiquimod was applied to one ear of each mouse after recovery from infection. Ears were scored for thickness and inflammation (fig. S7, D to G). In contrast to the GN models, preceding *S. aureus* infection did not aggravate dermal inflammation. These results suggest that *S. aureus* infection does not cause a generally increased susceptibility to autoimmune diseases. Aggravation of disease may depend on local generation of $T_{RM}17$ cells that become reactivated during an autoimmune response at the site.

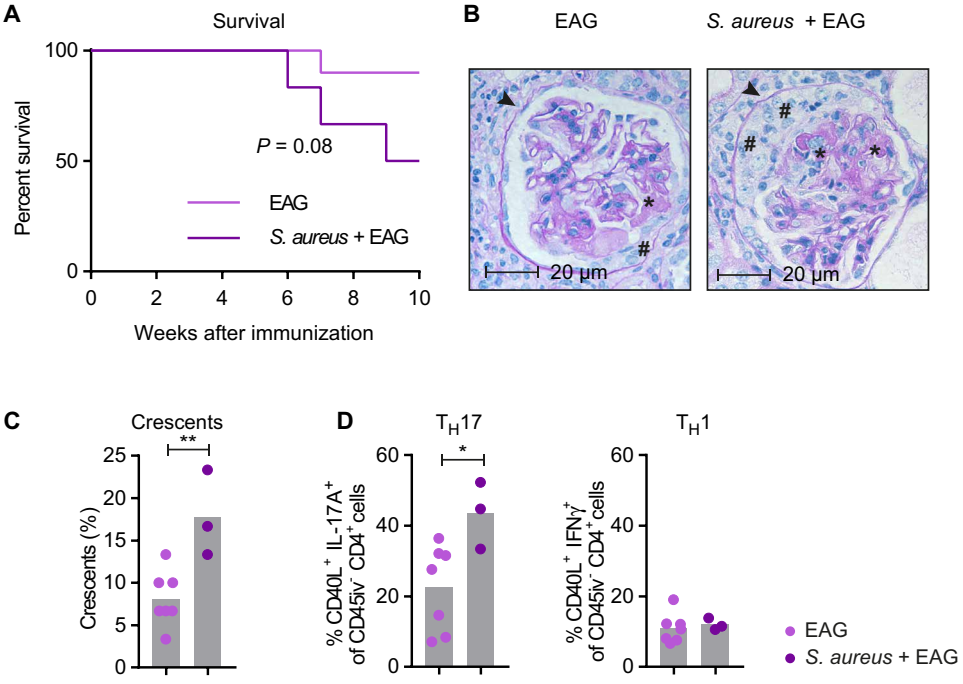

Fig. 7. Impact of *S. aureus* infection on experimental crescentic GN. (**A**) EAG was induced in DBA/1 mice. Survival of mice during the course of EAG. (**B**) PAS staining of glomeruli in EAG mice with and without preceding bacterial infection. (**C**) Quantification of renal damage (crescents) and (**D**) renal T_H1 and T_H17 cells by their cytokine production. Data are representative of two independent experiments $^*P < 0.05$ and $^{**}P < 0.01$ (unpaired *t* test) (► Bowman's capsule, * glomerular necrosis, # glomerular crescent).

Rapid IL-17 response of renal $T_{RM}17$ cells is cytokine-driven

To directly assess reactivation of renal $T_{RM}17$ cells, *Kaede* mice that were also transgenic for the fluorescent protein Katushka under the control of the *Il17a* promotor [*Kaede* × *Il17a*^{Kat} mice (*11*, *44*)] were infected with *S. aureus*. After recovery, one kidney was photoconverted and crescentic GN was induced 1 day later, and CD4$^+$ T cells from both kidneys were analyzed 2 days later (Fig. 8, A and B). In the nonexposed kidneys, about 1% of CD4$^+$ T cells were Kat$^+$, indicating that cells actively produced IL-17A. In the light-exposed kidney, Kat$^+$ cells were mainly found in the photoconverted CD4$^+$ T cell population when compared with nonconverted CD4$^+$ T cells. This result demonstrates that IL-17A production in the early phase of crescentic GN was mainly due to the activation of kidney-resident T cells and not to rapid recruitment of cells to the kidney. Because photoconversion of renal cells is incomplete, a fraction of IL-17A$^+$ cells in the nonconverted *Kaede* green$^+$ CD4$^+$ T cell population was most likely also derived from kidney-resident cells, which suggests that IL-17A production was almost exclusively due to renal-resident CD4$^+$ T cells.

It is unlikely that *S. aureus*–specific $T_{RM}17$ cells generated during infection recognize and become activated by unrelated antigens in two distinct models of crescentic GN. The rapid IL-17A response of *S. aureus*–induced renal T cells after crescentic GN induction suggests that activation is antigen independent. IL-17A fate reporter mice were infected to identify activation pathways of renal $T_{RM}17$ cells. After recovery from infection, crescentic GN was induced in one group of mice. At day 2 of crescentic GN, which is a very early time point preceding renal T cell recruitment and substantial tissue injury, scRNA-seq of renal IL-17A fate$^+$ CD4$^+$ T cells was performed. Unsupervised clustering of renal IL-17A fate$^+$ CD4$^+$ T cells from mice with and without crescentic GN defined eight distinct populations. The T cell composition from both groups of mice was relatively similar, and T cells from both groups were found in all clusters. This excluded extensive recruitment of T_H17 cells from extrarenal sites at this time point (fig. S8, A to C). In the most prominent clusters, C0 to C2, cells from mice with crescentic GN displayed up-regulation of a number of genes, including *Il7a*, as compared with cells from control animals (Fig. 8C and fig. S8C). To uncover activation pathways that were triggered in these T cells by induction of crescentic GN, we made use of the TRRUSTv2 database, which was generated to study transcriptional regulation based on gene expression profiles (*45*). The TRRUSTv2 analysis tool identified several transcription factors that might be active in these cells with signal transducer and activator of transcription 3 (STAT3) and nuclear factor κB (NFκB) (p50/Nfkb1) as top candidates (table S4). On the basis of these analyses and Kyoto Encyclopedia of Genes and Genomes reference pathways for STAT3 and NFκB (*46*), we proposed a model of $T_{RM}17$ cell activation by inflammatory cytokines such as IL-1β, IL-6, and IL-23 (Fig. 8D), which is also consistent with the observation that T_H17 cell can be activated by inflammatory cytokines (*47*).

Analyses of renal tissues revealed increased expression of *Il1b*, *Il6*, and *Il23a* mRNA at 1 day after crescentic GN induction (Fig. 8E). To test whether these cytokines induce IL-17A, CD4$^+$ T cells were sorted from the spleen and kidney of mice previously infected with *S. aureus* and were stimulated with a cocktail of IL-1β, IL-6, and IL-23, or with anti-CD3 and anti-CD28 mAb as a control (Fig. 8F), and cytokines in supernatants were determined. Anti-CD3/CD28 stimulation induced IL-17A and IFN-γ in T cells from the spleen and kidney. Renal CD4$^+$ T cells also responded to inflammatory cytokines in the absence of T cell receptor (TCR) stimulation by producing IL-17A, indicating that inflammatory cytokines can activate renal $T_{RM}17$ cells in the early phase of crescentic GN. On the basis of these results, mice were infected with *S. aureus*, and after recovery, crescentic GN was induced in the presence of neutralizing mAb against IL-1β, IL-6, and IL-23p40 (Fig. 8, G to I). Anti-cytokine mAb–treated mice demonstrated attenuated crescent formation when compared with mice treated with control IgG (isotype) (Fig. 8, G and H). We also observed reduced frequencies of renal RORγt$^+$ T_H17 cells, most likely as a consequence of reduced renal inflammation (Fig. 8I). Collectively, these results demonstrate that resident renal T_H17 cells can be activated by inflammatory cytokines and thereby cause aggravated crescentic GN.

DISCUSSION

In contrast to the role of T_{RM} cells in the defense against pathogens at interfaces with the environment, such as skin, gut, and airways,

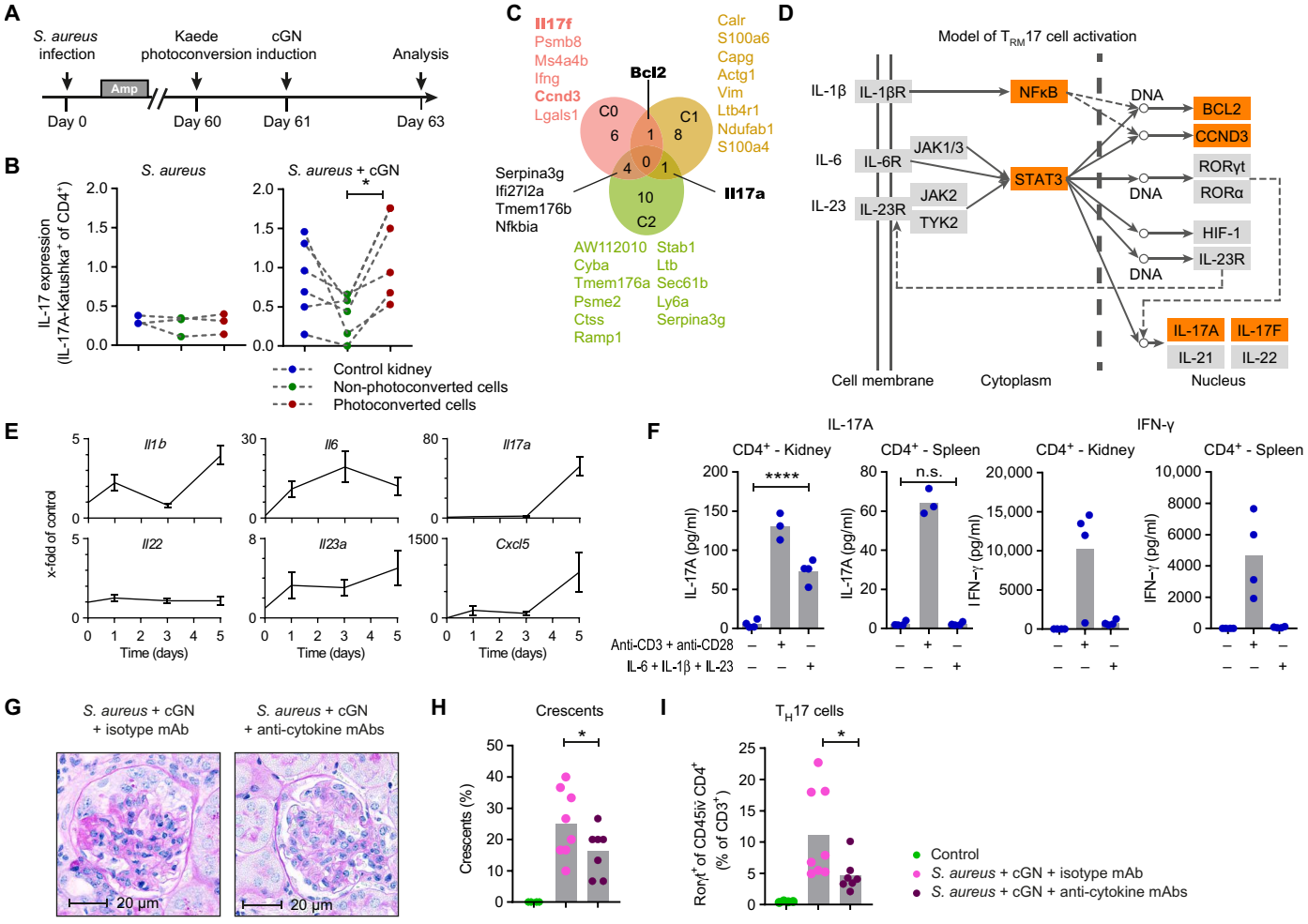

Fig. 8. Rapid IL-17 response in tissue-resident memory T cells is cytokine-driven. (**A** and **B**) cGN drives rapid IL-17 expression in $T_{RM}17$ cells in the kidney. (A) *Kaede* × *Il17a*^{Kat} mice were infected with *S. aureus*. After bacterial clearance, one kidney was photoconverted and cGN was induced. Two days after cGN, Katushka expression (indicating IL-17A production) was determined in renal CD45iv$^-$ CD4$^+$ T cells by flow cytometry. (B) Quantification of in vivo IL-17A production in kidneys at day 2 of cGN in *Kaede* × *Il17a*^{Kat} mice. Data are representative of two independent experiments. *$P < 0.05$ (one-way ANOVA with multiple comparison test). (**C** and **D**) Gene expression profiles induced by cGN in $T_{RM}17$ cells. (C) IL-17A fate reporter mice with prior *S. aureus* infection were subjected to cGN and control treatment. After 2 days, sorted renal CD4$^+$ eYFP$^+$ $T_{RM}17$ cells were analyzed by scRNA-seq. In infected mice, three clusters with up-regulation of *Il17a* or *Il17f* were identified (C0 to C2). Genes up-regulated after cGN in cells of the respective clusters are shown. (D) Proposed model of the potential regulatory network of $T_{RM}17$ cell activation based on scRNA profiles, TRRUSTv2 analysis of differentially expressed genes of cells from mice with and without cGN (clusters C0 to C2) and KEGG reference pathways (orange: proteins and transcription factors identified by scRNA-seq and TRRUSTv2). (**E** and **F**) $T_{RM}17$ cells can be activated by cytokines in the absence of TCR signaling. (E) Proinflammatory cytokines in the kidney were analyzed during the early phase of cGN by qPCR (mean ± SEM, $n = 5$ to 11). (F) FACS-sorted CD4$^+$ T cells from spleen and kidney were stimulated with proinflammatory cytokines for 72 hours. Cytokines were measured in the supernatants. Data are representative of three independent experiments. ****$P < 0.0001$ (unpaired *t* test). (**G** to **I**) After prior *S. aureus* infection, cGN was induced in mice. Cytokines (IL-1β, IL-6, and IL-23p40) were neutralized using mAb at days 0, 3, and 6 of cGN. (G and H) Glomerular crescents and (I) frequencies of T_H17 cells were determined at day 10 of cGN. Data are representative of two independent experiments. *$P < 0.05$ (one-tailed *t* test).

their function in autoimmune diseases is currently not well understood (*18*–*20*). The long-term persistence of T_{RM} cells in tissues often affected by chronic relapsing and remitting autoimmune diseases such as skin (psoriasis) (*48*), gut (IBD) (*49*, *50*), and brain (multiple sclerosis) (*51*) suggests a potential contribution of T_{RM} cells to these diseases. T_{RM} cells are detectable in active skin lesions of patients with psoriasis and persist in these areas after successful treatment (*48*), which might explain the recurrence of treated and resolved psoriasis lesions at the same site. The accumulation of CD4$^+$CD69$^+$ CD103$^+$ T cells in the mucosa of patients with IBD was predictive for the development of disease flares, and in experimental IBD, depletion of T_{RM} cells attenuated colitis activity, suggesting a critical role of T_{RM} cells in the pathogenesis of IBD (*50*). Steinbach *et al.* (*52*) recently reported that a transient viral infection of the mouse brain in early life induces brain T_{RM} cells that precipitate CNS autoimmune disease.

We detected T cells with a CD4$^+$ CD45RA^{low} CCR7^{low} CD69$^+$ phenotype and the signature transcriptional profile of CD4$^+$ T_{RM} cells (*21*) in the human kidney. When compared with healthy renal tissue from tumor nephrectomies, tissue from patients with ANCA-GN had substantially increased numbers of CD4$^+$ T_{RM} cells. In addition, in biopsies of a larger cohort of patients with active

ANCA-associated GN, high numbers of renal $CD69^+$ cells are correlated with impaired kidney function. In terms of mRNA expression profile and chemokine receptor surface expression, major subsets of renal $CD4^+CD69^+$ T_{RM} cells showed features of T_H1 or T_H17 cells and therefore were termed $T_{RM}1$ and $T_{RM}17$ cells, respectively. Direct comparison of $CD4^+$ T_{RM} cells derived from renal tissue of patients with ANCA-GN with $CD4^+$ T_{RM} cells from healthy renal tissue from patients with tumor nephrectomy revealed up-regulation of genes involved in activation, proliferation, and cytokine signaling. This expression profile likely reflects activation of T_{RM} cells in the context of an acute renal immune response in the patients with ANCA-GN.

S. aureus induces T_H17 cell responses, and *S. aureus*–specific T_H17 cells are relatively abundant in human peripheral blood (*34*, *53*). On the basis of our experimental data from mouse models, we hypothesized that $T_{RM}17$ cells specific for *S. aureus* could amplify an autoimmune reaction in the kidney. Patients with ANCA-associated vasculitis that carry *S. aureus* on their nasal mucosa show higher relapse rates and antibiotic treatment with trimethoprim and sulfamethoxazole (cotrimoxazole) prevents relapses (*35*, *54*). *S. aureus*–specific $T_{RM}17$ cells could be a link between *S. aureus* and GN in ANCA-vasculitis. Overt or subclinical systemic infections (*55*) could boost renal $T_{RM}17$ cells, which subsequently amplify a smoldering autoimmune reaction. Other pathogens that infect the human kidney are uropathogenic *Escherichia coli* strains and *Candida albicans*. We infected mice with uropathogenic *E. coli*, which induces a transient form of acute pyelonephritis (*56*), and with *C. albicans*, a well-known trigger of T_H17 cell response (*57*). Analysis after clearance of the renal infections revealed that both pathogens induced $T_{RM}17$ cell formation in the kidneys of mice (fig. S9). These results suggest that in addition to *S. aureus*, *C. albicans* and uropathogenic *E. coli* but also other so far unknown pathogens can induce T_{RM} cells in human kidneys.

It is currently unknown to which extent pathogen-specific T cells and particularly T cells specific for *S. aureus*, *E. coli*, *or C. albicans* contribute to $CD4^+$ $T_{RM}17$ cell populations in the human kidney. Because all three pathogens are inducers of T_H17 cells in humans (*34*, *58*), it is likely that a fraction of human renal $T_{RM}17$ cells is directed against these pathogens. $CD4^+$ T cells specific for these three pathogens are found in the blood of healthy individuals in the range of 1 in 10^3 to 10^4 $CD4^+$ T cells (*53*, *59*). We therefore estimate that at least 10^6 purified $CD4^+$ T cells would be required for the reliable detection of these pathogen-specific $CD4^+$ T cells. Using currently available approaches, it is not possible to obtain these quantities of $CD4^+$ T cells from renal biopsies or from tissue of tumor nephrectomies.

Our infection studies in mice support the concept that renal $T_{RM}17$ cells are generated in response to kidney infection because we found high numbers of $T_{RM}17$ cells that persisted in the kidney after resolution of infections. In the *S. aureus* infection model, parabiosis proved that the tissue residency of infection induced $T_{RM}17$ cells. Photoconversion of kidneys in *Kaede* transgenic mice confirmed this result and might provide a tool to circumvent ethically controversial parabiosis experiments in the future. It is therefore possible that $T_{RM}17$ cells identified in the healthy human kidney represent the memory of preceding infections.

Resolved *S. aureus* infection caused aggravation of renal disease in two different crescentic GN models. We carefully excluded persisting glomerular damage as cause for aggravated crescentic GN. *Rag1*$^{-/-}$ mice reconstituted with $CD4^+$ T cells after *S. aureus* infection and reconstituted controls showed similar crescentic GN development. Comparable severity of disease was also observed after depletion of $T_{RM}17$ cells or IL-17A neutralization in mice with and without prior infection. In contrast, neutralization of IFN-γ did not prevent the more pronounced crescent formation in mice with prior *S. aureus* infection. These experiments define the enlarged T_H17 cell population as a mediator of worsened disease, which is consistent with the detrimental function of T_H17 cells in both crescentic GN models (*11*, *14*). Labeling of cells in *Kaede* mice before crescentic GN revealed that renal $T_{RM}17$ cells become locally activated in the kidney. Renal T_H17 cells produced IL-17A after stimulation with IL-1β, IL-6, and IL-23, and transcriptional analyses indicated a response to inflammatory cytokines in renal T_H17 cells in the early stage of crescentic GN (*60*). Together, these results suggest that $T_{RM}17$ cells can respond to the local inflammation by production of IL-17A and most likely further cytokines that promote renal damage. Antigen-independent activation by inflammatory cytokines, including IL-1 family members, IL-6, IL-12, and IL-23, has been described not only for innate T cells, such as natural killer T cells (*61*), but also for conventional $CD4^+$ and $CD8^+$ effector T cells (*62*–*66*). The bystander activation of conventional T cells could support control of infection but could also contribute to tissue damage in autoimmune responses. In the model of experimental autoimmune encephalomyelitis, Lee *et al.* (*47*) could recently show that accumulation of T_H17 cells with irrelevant specificity in the inflamed central nervous system and bystander activation of these cells by IL-1β and IL-23 contribute to local injury. Our data indicate that $T_{RM}17$ cells induced by previous unrelated infections could function in a similar manner and promote tissue damage after activation by inflammatory cytokines.

Whether the generation and persistence of $CD4^+$ $T_{RM}17$ cells in nonlymphoid tissues in response to T_H17 cell–inducing microbes, e.g., *S. aureus*, *C. albicans*, or *E. coli*, are a common amplifier for T_H17 cell–driven autoimmune diseases in humans remain to be elucidated. In addition to $T_{RM}17$ cells, we detected T_{RM} cells of the T_H1 or T_H2 phenotype as well as $CD69^+$ $CD8^+$ T cells in human kidneys. It would be of interest to investigate whether these T cells can act in a similar way in amplifying immune-mediated disorders. In the murine crescentic GN model, infection with *L. monocytogenes* that induces a local $CD4^+$ T_H1 cell response (*67*) did not affect the course of renal disease. Moreover, IFN-γ neutralization did not prevent the more pronounced crescent formation in mice with prior *S. aureus* infection. However, these results do not exclude the idea that $T_{RM}1$ cells interfere with responses in other autoimmune diseases.

Together, we provide a concept for the predisposing role of microbial infections for T_H17-driven autoimmune diseases and identify $T_{RM}17$ cells as a potential therapeutic target (fig. S10), indicating that $T_{RM}17$ cell depletion rather than transient cytokine (IL-17) neutralization might be a causal treatment strategy in chronic relapsing and remitting autoimmune diseases.

MATERIALS AND METHODS

Study design

The aims of the study were to characterize T cell subsets in the kidney and to determine their potential role in renal autoimmunity. T cells from human kidney biopsies from controls and from individuals with ANCA-associated GN were analyzed by scRNA-seq, CITE-seq, flow cytometry, and immune histology. These assays revealed renal $CD4^+$

T_{RM} cells with phenotype and mRNA expression profile of T_H17 cells. For the functional characterization of renal $CD4^+$ T_{RM} cells, we combined mouse infection models (*S. aureus*, *L. monocytogenes*, *E. coli*, and *C. albicans*) with models of crescentic GN. Transgenic cytokine reporter mice and *Kaede* mice that allowed identification and tracking of cells in vivo were included in these experiments. Mice were analyzed using standard assays for renal function, as well as scRNA-seq, quantitative polymerase chain reaction, flow cytometry, and histology. Detailed methods are given below and in the Supplementary Materials.

Human studies

Human biopsies for flow cytometry and scRNA-seq were obtained either from the healthy part of kidneys after tumor nephrectomy or from renal biopsies of patients with ANCA-associated GN. Human kidney sections and clinical parameters were analyzed from patients included in the Hamburg GN Registry. For some of the analyses, matched blood samples from the same patient were used. These studies were approved by the Ethik-Kommission der Ärztekammer Hamburg, local ethics committee of the chamber of physicians in Hamburg (Registration numbers PV 5026 and PV 5822), and were conducted in accordance with the ethical principles stated by the Declaration of Helsinki. Informed consent was obtained from all participating patients. Information on the patient cohorts is provided in tables S1 to S3. Details on the analysis of human renal biopsies and of human T cells are given in the Supplementary Materials.

Mice

Mouse experiments were carried out in accordance with the national guidelines. The protocols were approved by local ethics committees. Mice were housed under specific pathogen–free conditions in individually ventilated cages with standard food and water ad libitum. During experiments, mice were monitored daily and mice with signs of severe disease were euthanized with an O_2/CO_2 mixture to minimize suffering. All experiments were conducted with age-matched male mice on C57BL/6 background, with the exception of EAG that was induced in male DBA/1 mice. The following transgenic mouse strains were used: *Kaede* mice (*11*), $Il17a^{Kat}$ mice (*44*), $Il17a^{cre} \times R26^{eYFP}$ mice (*36*, *39*), $Il17a^{Cre} \times R26^{DTR} \times R26^{eYFP}$ mice (*36*, *38*), CD45.1 congenic mice (B6.SJL-$Ptprc^a$ $Pepc^b$/*BoyJ*), and $Rag1^{-/-}$ mice (B6.129S7-$Rag1^{tm1Mom}$/J). Details on mouse experiments are given in the Supplementary Materials.

Statistics

Statistical analysis was performed using GraphPad Prism (La Jolla, CA). The results are shown as mean ± SEM or mean ± SD when presented as a bar graph or as single data points with the mean in a scatter dot plot. Differences between two individual groups were compared using a two-tailed *t* test. In the case of three or more groups, a one-way analysis of variance (ANOVA) with Bonferroni's multiple comparisons test was used.

SUPPLEMENTARY MATERIALS

immunology.sciencemag.org/cgi/content/full/5/50/eaba4163/DC1

Material and Methods

Fig. S1. scRNA-seq analysis of T cells from human blood and healthy kidney tissue from tumor nephrectomies.

Fig. S2. Profiling of T cells from human blood and healthy kidney tissue from tumor nephrectomies.

Fig. S3. scRNA-seq analysis of T cells from blood and kidney of patients with ANCA-GN.

Fig. S4. Profiling of T cells from kidney and blood of patients with ANCA-GN.

Fig. S5. Flow cytometry gating strategy for murine T cell analysis.

Fig. S6. Prior *L. monocytogenes* infection does not affect crescentic GN.

Fig. S7. Effect of local *S. aureus* skin infection on renal $T_{RM}17$ cell generation and effect of systemic *S. aureus* infection on imiquimod-induced skin inflammation.

Fig. S8. Gene expression analysis of renal $CD4^+$ T cells from nephritic mice previously infected with *S. aureus*.

Fig. S9. The T_H17-associated pathogens *C. albicans* and *E. coli* cause renal $T_{RM}17$ cell formation.

Fig. S10. Conceptual link of pathogen-induced tissue resident memory T cells with autoimmunity.

Table S1. Baseline characteristics of controls at time of biopsy.

Table S2. Baseline characteristics of patients with ANCA-GN at the time of biopsy.

Table S3. Clinical characteristics and ANCA-serotype of patients from the Hamburg GN Registry.

Table S4. List of transcription factors identified by TRRUSTv2 analysis.

Data file S1.

References (*68*–*79*)

View/request a protocol for this paper from *Bio-protocol*.

REFERENCES AND NOTES

1. K. W. Wucherpfennig, Mechanisms for the induction of autoimmunity by infectious agents. *J. Clin. Invest.* **108**, 1097–1104 (2001).
2. K. N. Konstantinov, C. J. Ulff-Møller, A. H. Tzamaloukas, Infections and antineutrophil cytoplasmic antibodies: Triggering mechanisms. *Autoimmun. Rev.* **14**, 201–203 (2015).
3. A. J. Steelman, Infection as an environmental trigger of multiple sclerosis disease exacerbation. *Front. Immunol.* **6**, 520 (2015).
4. E. A. Mann, S. A. Saeed, Gastrointestinal infection as a trigger for inflammatory bowel disease. *Curr. Opin. Gastroenterol.* **28**, 24–29 (2012).
5. W. G. Couser, R. J. Johnson, The etiology of glomerulonephritis: Roles of infection and autoimmunity. *Kidney Int.*. **86**, 905–914 (2014).
6. C. Kurts, U. Panzer, H. J. Anders, A. J. Rees, The immune system and kidney disease: Basic concepts and clinical implications. *Nat. Rev. Immunol.* **13**, 738–753 (2013).
7. W. G. Couser, Basic and translational concepts of immune-mediated glomerular diseases. *J. Am. Soc. Nephrol.* **23**, 381–399 (2012).
8. C. F. Krebs, T. Schmidt, J. H. Riedel, U. Panzer, T helper type 17 cells in immune-mediated glomerular disease. *Nat. Rev. Nephrol.* **13**, 647–659 (2017).
9. B. R. Lúdvíksson, M. C. Sneller, K. S. Chua, C. Talar-Williams, C. A. Langford, R. O. Ehrhardt, A. S. Fauci, W. Strober, Active Wegener's granulomatosis is associated with HLA-DR^+ $CD4^+$ T cells exhibiting an unbalanced Th1-Type T cell cytokine pattern: Reversal with IL-10. *J. Immunol.* **160**, 3602–3609 (1998).
10. J. D. Ooi, J. Petersen, Y. H. Tan, M. Huynh, Z. J. Willett, S. H. Ramarathinam, P. J. Eggenhuizen, K. L. Loh, K. A. Watson, P. Y. Gan, M. A. Alikhan, N. L. Dudek, A. Handel, B. G. Hudson, L. Fugger, D. A. Power, S. G. Holt, P. T. Coates, J. W. Gregersen, A. W. Purcell, S. R. Holdsworth, N. L. La Gruta, H. H. Reid, J. Rossjohn, A. R. Kitching, Dominant protection from HLA-linked autoimmunity by antigen-specific regulatory T cells. *Nature* **545**, 243–247 (2017).
11. C. F. Krebs, H.-J. Paust, S. Krohn, T. Koyro, S. R. Brix, J.-H. Riedel, P. Bartsch, T. Wiech, C. Meyer-Schwesinger, J. Huang, N. Fischer, P. Busch, H.-W. Mittrücker, U. Steinhoff, B. Stockinger, L. Garcia Perez, U. O. Wenzel, M. Janneck, O. M. Steinmetz, N. Gagliani, R. A. K. Stahl, S. Huber, J.-E. Turner, U. Panzer, Autoimmune renal disease is exacerbated by S1P-receptor-1-dependent intestinal Th17 cell migration to the kidney. *Immunity* **45**, 1078–1092 (2016).
12. H.-J. Paust, J.-E. Turner, O. M. Steinmetz, A. Peters, F. Heymann, C. Hölscher, G. Wolf, C. Kurts, H.-W. Mittrücker, R. A. K. Stahl, U. Panzer, The IL-23/Th17 axis contributes to renal injury in experimental glomerulonephritis. *J. Am. Soc. Nephrol.* **20**, 969–979 (2009).
13. S. A. Summers, O. M. Steinmetz, M. Li, J. Y. Kausman, T. Semple, K. L. Edgtton, D. B. Borza, H. Braley, S. R. Holdsworth, A. R. Kitching, Th1 and Th17 cells induce proliferative glomerulonephritis. *J. Am. Soc. Nephrol.* **20**, 2518–2524 (2009).
14. S. Hünemörder, J. Treder, S. Ahrens, V. Schumacher, H. J. Paust, T. Menter, P. Matthys, T. Kamradt, C. Meyer-Schwesinger, U. Panzer, H. Hopfer, H.-W. Mittrücker, T_{H1} and T_{H17} cells promote crescent formation in experimental autoimmune glomerulonephritis. *J. Pathol.* **237**, 62–71 (2015).
15. A. Suarez-Fueyo, S. J. Bradley, D. Klatzmann, G. C. Tsokos, T cells and autoimmune kidney disease. *Nat. Rev. Nephrol.* **13**, 329–343 (2017).
16. E. M. Disteldorf, C. F. Krebs, H. J. Paust, J. E. Turner, G. Nouailles, A. Tittel, C. Meyer-Schwesinger, G. Stege, S. Brix, J. Velden, T. Wiech, U. Helmchen, O. M. Steinmetz, A. Peters, S. B. Bennstein, A. Kaffke, C. Llanto, S. A. Lira, H. W. Mittrücker, R. A. K. Stahl, C. Kurts, S. H. E. Kaufmann, U. Panzer, CXCL5 drives neutrophil recruitment in T_H17-mediated GN. *J. Am. Soc. Nephrol.* **26**, 55–66 (2015).
17. S. N. Mueller, L. K. Mackay, Tissue-resident memory T cells: Local specialists in immune defence. *Nat. Rev. Immunol.* **16**, 79–89 (2016).

18. O. Gaide, R. O. Emerson, X. Jiang, N. Gulati, S. Nizza, C. Desmarais, H. Robins, J. G. Krueger, R. A. Clark, T. S. Kupper, Common clonal origin of central and resident memory T cells following skin immunization. *Nat. Med.* **21**, 647–653 (2015).
19. P. A. Szabo, M. Miron, D. L. Farber, Location, location, location: Tissue resident memory T cells in mice and humans. *Sci. Immunol.* **4**, eaas9673 (2019).
20. M. C. Amezcua Vesely, P. Pallis, P. Bielecki, J. S. Low, J. Zhao, C. C. D. Harman, L. Kroehling, R. Jackson, W. Bailis, P. Licona-Limón, H. Xu, N. Iijima, P. S. Pillai, D. H. Kaplan, C. T. Weaver, Y. Kluger, M. S. Kowalczyk, A. Iwasaki, J. P. Pereira, E. Esplugues, N. Gagliani, R. A. Flavell, Effector T_H17 cells give rise to long-lived T_{RM} cells that are essential for an immediate response against bacterial infection. *Cell* **178**, 1176–1188.e15 (2019).
21. B. V. Kumar, W. Ma, M. Miron, T. Granot, R. S. Guyer, D. J. Carpenter, T. Senda, X. Sun, S. H. Ho, H. Lerner, A. L. Friedman, Y. Shen, D. L. Farber, Human tissue-resident memory T cells are defined by core transcriptional and functional signatures in lymphoid and mucosal sites. *Cell Rep.* **20**, 2921–2934 (2017).
22. P. Hombrink, C. Helbig, R. A. Backer, B. Piet, A. E. Oja, R. Stark, G. Brasser, A. Jongejan, R. E. Jonkers, B. Nota, O. Basak, H. C. Clevers, P. D. Moerland, D. Amsen, R. A. W. van Lier, Programs for the persistence, vigilance and control of human $CD8^+$ lung-resident memory T cells. *Nat. Immunol.* **17**, 1467–1478 (2016).
23. S. C. Jameson, D. Masopust, Understanding subset diversity in T cell memory. *Immunity* **48**, 214–226 (2018).
24. J. M. Schenkel, D. Masopust, Tissue-resident memory T cells. *Immunity* **41**, 886–897 (2014).
25. J.-E. Turner, M. Becker, H.-W. Mittrücker, U. Panzer, Tissue-resident lymphocytes in the kidney. *J. Am. Soc. Nephrol.* **29**, 389–399 (2018).
26. E. Z. Macosko, A. Basu, R. Satija, J. Nemesh, K. Shekhar, M. Goldman, I. Tirosh, A. R. Bialas, N. Kamitaki, E. M. Martersteck, J. J. Trombetta, D. A. Weitz, J. R. Sanes, A. K. Shalek, A. Regev, S. A. McCarroll, Highly parallel genome-wide expression profiling of individual cells using nanoliter droplets. *Cell* **161**, 1202–1214 (2015).
27. M. Stoeckius, C. Hafemeister, W. Stephenson, B. Houck-Loomis, P. K. Chattopadhyay, H. Swerdlow, R. Satija, P. Smibert, Simultaneous epitope and transcriptome measurement in single cells. *Nat. Methods* **14**, 865–868 (2017).
28. E. P. Mimitou, A. Cheng, A. Montalbano, S. Hao, M. Stoeckius, M. Legut, T. Roush, A. Herrera, E. Papalexi, Z. Ouyang, R. Satija, N. E. Sanjana, S. B. Koralov, P. Smibert, Multiplexed detection of proteins, transcriptomes, clonotypes and CRISPR perturbations in single cells. *Nat. Methods* **16**, 409–412 (2019).
29. F. A. Vieira Braga, G. Kar, M. Berg, O. A. Carpaij, K. Polanski, L. M. Simon, S. Brouwer, T. Gomes, L. Hesse, J. Jiang, E. S. Fasouli, M. Efremova, R. Vento-Tormo, C. Talavera-López, M. R. Jonker, K. Affleck, S. Palit, P. M. Strzelecka, H. V. Firth, K. T. Mahbubani, A. Cvejic, K. B. Meyer, K. Saeb-Parsy, M. Luinge, C. A. Brandsma, W. Timens, I. Angelidis, M. Strunz, G. H. Koppelman, A. J. van Oosterhout, H. B. Schiller, F. J. Theis, M. van den Berge, M. C. Nawijn, S. A. Teichmann, A cellular census of human lungs identifies novel cell states in health and in asthma. *Nat. Med.* **25**, 1153–1163 (2019).
30. C. D. Cohen, K. Frach, D. Schlondorff, M. Kretzler, Quantitative gene expression analysis in renal biopsies: A novel protocol for a high-throughput multicenter application. *Kidney Int.*. **61**, 133–140 (2002).
31. L. S. Cauley, L. Lefrançois, Guarding the perimeter: Protection of the mucosa by tissue-resident memory T cells. *Mucosal Immunol.* **6**, 14–23 (2013).
32. F. Sallusto, Heterogeneity of human $CD4^+$ T cells against microbes. *Annu. Rev. Immunol.* **34**, 317–334 (2016).
33. E. Y. Chen, C. M. Tan, Y. Kou, Q. Duan, Z. Wang, G. Vaz Meirelles, N. R. Clark, A. Ma'ayan, Enrichr: Interactive and collaborative HTML5 gene list enrichment analysis tool. *BMC Bioinformatics* **14**, 128 (2013).
34. C. E. Zielinski, F. Mele, D. Aschenbrenner, D. Jarrossay, F. Ronchi, M. Gattorno, S. Monticelli, A. Lanzavecchia, F. Sallusto, Pathogen-induced human T_H17 cells produce IFN-γ or IL-10 and are regulated by IL-1β. *Nature* **484**, 514–518 (2012).
35. C. A. Stegeman, J. W. Tervaert, P. E. de Jong, C. G. Kallenberg; Dutch Co-trimoxazole Wegener Study Group, Trimethoprim-sulfamethoxazole (co-trimoxazole) for the prevention of relapses of Wegener's granulomatosis. *N. Engl. J. Med.* **335**, 16–20 (1996).
36. K. Hirota, J. H. Duarte, M. Veldhoen, E. Hornsby, Y. Li, D. J. Cua, H. Ahlfors, C. Wilhelm, M. Tolaini, U. Menzel, A. Garefalaki, A. J. Potocnik, B. Stockinger, Fate mapping of IL-17-producing T cells in inflammatory responses. *Nat. Immunol.* **12**, 255–263 (2011).
37. H.-J. Paust, J.-E. Turner, J.-H. Riedel, E. Disteldorf, A. Peters, T. Schmidt, C. Krebs, J. Velden, H. W. Mittrücker, O. M. Steinmetz, R. A. K. Stahl, U. Panzer, Chemokines play a critical role in the cross-regulation of Th1 and Th17 immune responses in murine crescentic glomerulonephritis. *Kidney Int.*. **82**, 72–83 (2012).
38. T. Buch, F. L. Heppner, C. Tertilt, T. J. A. J. Heinen, M. Kremer, F. T. Wunderlich, S. Jung, A. Waisman, A Cre-inducible diphtheria toxin receptor mediates cell lineage ablation after toxin administration. *Nat. Methods* **2**, 419–426 (2005).
39. C. F. Krebs, J.-E. Turner, H.-J. Paust, S. Kapffer, T. Koyro, S. Krohn, F. Ufer, M. A. Friese, R. A. Flavell, B. Stockinger, O. M. Steinmetz, R. A. K. Stahl, S. Huber, U. Panzer, Plasticity of Th17 cells in autoimmune kidney diseases. *J. Immunol.* **197**, 449–457 (2016).
40. H. Hopfer, J. Holzer, S. Hünemörder, H. J. Paust, M. Sachs, C. Meyer-Schwesinger, J. E. Turner, U. Panzer, H.-W. Mittrücker, Characterization of the renal $CD4^+$ T-cell response in experimental autoimmune glomerulonephritis. *Kidney Int.*. **82**, 60–71 (2012).
41. M. Kursar, K. Bonhagen, A. Köhler, T. Kamradt, S. H. E. Kaufmann, H.-W. Mittrücker, Organ-specific $CD4^+$ T cell response during *Listeria monocytogenes* infection. *J. Immunol.* **168**, 6382–6387 (2002).
42. J. Tintelnot, F. Ufer, J. B. Engler, H. Winkler, K. Lücke, H. W. Mittrücker, M. A. Friese, Arc/Arg3.1 defines dendritic cells and Langerhans cells with superior migratory ability independent of phenotype and ontogeny in mice. *Eur. J. Immunol.* **49**, 724–736 (2019).
43. L. van der Fits, S. Mourits, J. S. A. Voerman, M. Kant, L. Boon, J. D. Laman, F. Cornelissen, A.-M. Mus, E. Florencia, E. P. Prens, E. Lubberts, Imiquimod-induced psoriasis-like skin inflammation in mice is mediated via the IL-23/IL-17 axis. *J. Immunol.* **182**, 5836–5845 (2009).
44. N. Gagliani, M. C. Amezcua Vesely, A. Iseppon, L. Brockmann, H. Xu, N. W. Palm, M. R. de Zoete, P. Licona-Limón, R. S. Paiva, T. Ching, C. Weaver, X. Zi, X. Pan, R. Fan, L. X. Garmire, M. J. Cotton, Y. Drier, B. Bernstein, J. Geginat, B. Stockinger, E. Esplugues, S. Huber, R. A. Flavell, Th17 cells transdifferentiate into regulatory T cells during resolution of inflammation. *Nature* **523**, 221–225 (2015).
45. H. Han, J.-W. Cho, S. Lee, A. Yun, H. Kim, D. Bae, S. Yang, C. Y. Kim, M. Lee, E. Kim, S. Lee, B. Kang, D. Jeong, Y. Kim, H. N. Jeon, H. Jung, S. Nam, M. Chung, J. H. Kim, I. Lee, TRRUST v2: An expanded reference database of human and mouse transcriptional regulatory interactions. *Nucleic Acids Res.* **46**, D380–D386 (2018).
46. M. Kanehisa, S. Goto, KEGG: Kyoto encyclopedia of genes and genomes. *Nucleic Acids Res.* **28**, 27–30 (2000).
47. H.-G. Lee, J.-U. Lee, D.-H. Kim, S. Lim, I. Kang, J.-M. Choi, Pathogenic function of bystander-activated memory-like $CD4^+$ T cells in autoimmune encephalomyelitis. *Nat. Commun.* **10**, 709 (2019).
48. I. Gallais Serezal, C. Classon, S. Cheuk, M. Barrientos-Somarribas, E. Wadman, E. Martini, D. Chang, N. X. Landén, M. Ehrström, S. Nylén, L. Eidsmo, Resident T cells in resolved psoriasis steer tissue responses that stratify clinical outcome. *J. Invest. Dermatol.* **138**, 1754–1763 (2018).
49. S. Omenetti, C. Bussi, A. Metidji, A. Iseppon, S. Lee, M. Tolaini, Y. Li, G. Kelly, P. Chakravarty, S. Shoaie, M. G. Gutierrez, B. Stockinger, The intestine harbors functionally distinct homeostatic tissue-resident and inflammatory Th17 cells. *Immunity* **51**, 77–89.e6 (2019).
50. S. Zundler, E. Becker, M. Spocinska, M. Slawik, L. Parga-Vidal, R. Stark, M. Wiendl, R. Atreya, T. Rath, M. Leppkes, K. Hildner, R. López-Posadas, S. Lukassen, A. B. Ekici, C. Neufert, I. Atreya, K. P. J. M. van Gisbergen, M. F. Neurath, Hobit- and Blimp-1-driven $CD4^+$ tissue-resident memory T cells control chronic intestinal inflammation. *Nat. Immunol.* **20**, 288–300 (2019).
51. K. Steinbach, I. Vincenti, M. Kreutzfeldt, N. Page, A. Muschaweckh, I. Wagner, I. Drexler, D. Pinschewer, T. Korn, D. Merkler, Brain-resident memory T cells represent an autonomous cytotoxic barrier to viral infection. *J. Exp. Med.* **213**, 1571–1587 (2016).
52. K. Steinbach, I. Vincenti, K. Egervari, M. Kreutzfeldt, F. van der Meer, N. Page, B. Klimek, I. Rossitto-Borlat, G. Di Liberto, A. Muschaweckh, I. Wagner, K. Hammad, C. Stadelmann, T. Korn, O. Hartley, D. D. Pinschewer, D. Merkler, Brain-resident memory T cells generated early in life predispose to autoimmune disease in mice. *Sci. Transl. Med.* **11**, eaav5519 (2019).
53. P. Bacher, F. Heinrich, U. Stervbo, M. Nienen, M. Vahldieck, C. Iwer, K. Vogt, J. Kollet, N. Babel, B. Sawitzki, C. Schwarz, S. Bereswill, M. M. Heimesaat, G. Heine, G. Gadermaier, C. Asam, M. Assenmacher, O. Kniemeyer, A. A. Brakhage, F. Ferreira, M. Wallner, M. Worm, A. Scheffold, Regulatory T cell specificity directs tolerance versus allergy against aeroantigens in humans. *Cell* **167**, 1067–1078.e16 (2016).
54. C. A. Stegeman, J. W. Tervaert, W. J. Sluiter, W. L. Manson, P. E. de Jong, C. G. Kallenberg, Association of chronic nasal carriage of *Staphylococcus aureus* and higher relapse rates in Wegener granulomatosis. *Ann. Intern. Med.* **120**, 12–17 (1994).
55. N. B. Parahitiyawa, L. J. Jin, W. K. Leung, W. C. Yam, L. P. Samaranayake, Microbiology of odontogenic bacteremia: Beyond endocarditis. *Clin. Microbiol. Rev.* **22**, 46–64 (2009).
56. M. Schiwon, C. Weisheit, L. Franken, S. Gutweiler, A. Dixit, C. Meyer-Schwesinger, J. M. Pohl, N. J. Maurice, S. Thiebes, K. Lorenz, T. Quast, M. Fuhrmann, G. Baumgarten, M. J. Lohse, G. Opdenakker, J. Bernhagen, R. Bucala, U. Panzer, W. Kolanus, H. J. Gröne, N. Garbi, W. Kastenmüller, P. A. Knolle, C. Kurts, D. R. Engel, Crosstalk between sentinel and helper macrophages permits neutrophil migration into infected uroepithelium. *Cell* **156**, 456–468 (2014).
57. A. Vrablikova, L. Czernekova, R. Cahlikova, Z. Novy, M. Petrik, S. Imran, Z. Novak, M. Krupka, V. Cerovsky, J. Turanek, M. Raska, Lasioglossins LLIII affect the morphogenesis of *Candida albicans* and reduces the duration of experimental vaginal candidiasis in mice. *Microbiol. Immunol.* **61**, 474–481 (2017).
58. K. Atarashi, T. Tanoue, M. Ando, N. Kamada, Y. Nagano, S. Narushima, W. Suda, A. Imaoka, H. Setoyama, T. Nagamori, E. Ishikawa, T. Shima, T. Hara, S. Kado, T. Jinnohara, H. Ohno, T. Kondo, K. Toyooka, E. Watanabe, S. I. Yokoyama, S. Tokoro, H. Mori, Y. Noguchi, H. Morita, I. I. Ivanov, T. Sugiyama, G. Nuñez, J. G. Camp, M. Hattori, Y. Umesaki, K. Honda,

Th17 cell induction by adhesion of microbes to intestinal epithelial cells. *Cell* **163**, 367–380 (2015).
59. P. Bacher, T. Hohnstein, E. Beerbaum, M. Röcker, M. G. Blango, S. Kaufmann, J. Röhmel, P. Eschenhagen, C. Grehn, K. Seidel, V. Rickerts, L. Lozza, U. Stervbo, M. Nienen, N. Babel, J. Milleck, M. Assenmacher, O. A. Cornely, M. Ziegler, H. Wisplinghoff, G. Heine, M. Worm, B. Siegmund, J. Maul, P. Creutz, C. Tabeling, C. Ruwwe-Glösenkamp, L. E. Sander, C. Knosalla, S. Brunke, B. Hube, O. Kniemeyer, A. A. Brakhage, C. Schwarz, A. Scheffold, Human anti-fungal Th17 immunity and pathology rely on cross-reactivity against *Candida albicans*. *Cell* **176**, 1340–1355.e15 (2019).
60. M. J. McGeachy, D. J. Cua, S. L. Gaffen, The IL-17 family of cytokines in health and disease. *Immunity* **50**, 892–906 (2019).
61. P. J. Brennan, M. Brigl, M. B. Brenner, Invariant natural killer T cells: An innate activation scheme linked to diverse effector functions. *Nat. Rev. Immunol.* **13**, 101–117 (2013).
62. R. E. Berg, E. Crossley, S. Murray, J. Forman, Memory $CD8^+$ T cells provide innate immune protection against *Listeria monocytogenes* in the absence of cognate antigen. *J. Exp. Med.* **198**, 1583–1593 (2003).
63. L. Guo, G. Wei, J. Zhu, W. Liao, W. J. Leonard, K. Zhao, W. Paul, IL-1 family members and STAT activators induce cytokine production by Th2, Th17, and Th1 cells. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 13463–13468 (2009).
64. J. E. Kohlmeier, T. Cookenham, A. D. Roberts, S. C. Miller, D. L. Woodland, Type I interferons regulate cytolytic activity of memory $CD8^+$ T cells in the lung airways during respiratory virus challenge. *Immunity* **33**, 96–105 (2010).
65. B. Becher, S. Spath, J. Goverman, Cytokine networks in neuroinflammation. *Nat. Rev. Immunol.* **17**, 49–59 (2017).
66. T. Chu, A. J. Tyznik, S. Roepke, A. M. Berkley, A. Woodward-Davis, L. Pattacini, M. J. Bevan, D. Zehn, M. Prlic, Bystander-activated memory CD8 T cells control early pathogen load in an innate-like, NKG2D-dependent manner. *Cell Rep.* **3**, 701–708 (2013).
67. M. Kursar, K. Bonhagen, A. Köhler, T. Kamradt, S. H. E. Kaufmann, H. W. Mittrücker, Antigen-specific $CD8^+$ T cell responses in intestinal tissues during murine listeriosis. *Microbes Infect.* **6**, 8–16 (2004).
68. M. J. Horsburgh, J. L. Aish, I. J. White, L. Shaw, J. K. Lithgow, S. J. Foster, σB modulates virulence determinant expression and stress resistance: Characterization of a functional *rsbU* strain derived from *Staphylococcus aureus* 8325-4. *J. Bacteriol.* **184**, 5457–5467 (2002).
69. K. E. Foulds, L. A. Zenewicz, D. J. Shedlock, J. Jiang, A. E. Troy, H. Shen, Cutting edge: CD4 and CD8 T cells are intrinsically different in their proliferative responses. *J. Immunol.* **168**, 1528–1532 (2002).
70. J. D. Ooi, J.-H. Jiang, P. J. Eggenhuizen, L. L. Chua, M. van Timmeren, K. L. Loh, K. M. O'Sullivan, P. Y. Gan, Y. Zhong, K. Tsyganov, L. R. Shochet, J. Ryan, C. A. Stegeman, L. Fugger, H. H. Reid, J. Rossjohn, P. Heeringa, S. R. Holdsworth, A. Y. Peleg, A. R. Kitching, A plasmid-encoded peptide from *Staphylococcus aureus* induces anti-myeloperoxidase nephritogenic autoimmunity. *Nat. Commun.* **10**, 3392 (2019).
71. K. T. Bæk, D. Frees, A. Renzoni, C. Barras, N. Rodriguez, C. Manzano, W. L. Kelley, Genetic variation in the *Staphylococcus aureus* 8325 strain lineage revealed by whole-genome sequencing. *PLOS ONE* **8**, e77122 (2013).
72. A. Louie, G. L. Drusano, P. Banerjee, Q. F. Liu, W. Liu, P. Kaw, M. Shayegani, H. Taber, M. H. Miller, Pharmacodynamics of fluconazole in a murine model of systemic candidiasis. *Antimicrob Agents Chemother.* **42**, 1105–1109 (1998).
73. P. Kamran, K. I. Sereti, P. Zhao, S. R. Ali, I. L. Weissman, R. Ardehali, Parabiosis in mice: A detailed protocol. *J. Vis. Exp.* **80**, 50556 (2013).
74. K. G. Anderson, K. Mayer-Barber, H. Sung, L. Beura, B. R. James, J. J. Taylor, L. Qunaj, T. S. Griffith, V. Vezys, D. L. Barber, D. Masopust, Intravascular staining for discrimination of vascular and tissue leukocytes. *Nat. Protoc.* **9**, 209–222 (2014).
75. B. Rissiek, M. Lukowiak, F. Raczkowski, T. Magnus, H. W. Mittrücker, F. Koch-Nolte, In vivo blockade of murine ARTC2.2 during cell preparation preserves the vitality and function of liver tissue-resident memory T cells. *Front. Immunol.* **9**, 1580 (2018).
76. M. Marouf, P. Machart, V. Bansal, C. Kilian, D. S. Magruder, C. F. Krebs, S. Bonn, Realistic in silico generation and augmentation of single-cell RNA-seq data using generative adversarial networks. *Nat. Commun.* **11**, 166 (2020).
77. T. Stuart, A. Butler, P. Hoffman, C. Hafemeister, E. Papalexi, W. M. Mauck III, Y. Hao, M. Stoeckius, P. Smibert, R. Satija, Comprehensive integration of single-cell data. *Cell* **177**, 1888–1902.e21 (2019).
78. V. G. Tusher, R. Tibshirani, G. Chu, Significance analysis of microarrays applied to the ionizing radiation response. *Proc. Natl. Acad. Sci. U.S.A.* **98**, 5116–5121 (2001).
79. J. Tao, L. Mariani, S. Eddy, H. Maecker, N. Kambham, K. Mehta, J. Hartman, W. Wang, M. Kretzler, R. A. Lafayette, JAK-STAT signaling is activated in the kidney and peripheral blood cells of patients with focal segmental glomerulosclerosis. *Kidney Int.* **94**, 795–808 (2018).

Acknowledgments: FACS sorting was performed at the UKE FACS sorting core facility. Next-generation sequencing was performed at the Deutsche Forschungsgemeinschaft (DFG)–funded "Competence Centre for Genome Analysis Kiel" at the University of Kiel. **Funding:** This study was supported by grants from the DFG (SFB1192 to C.F.K., F.K.-N., J.-E.T., S.H., N.G., V.G.P., E.H., C.M.-S., T.W., C.Ku., T.H., U.P., and H.-W.M.; SFB1286 to S.B.; SFBTR57 to C.Ku.), grants from the Deutsche Nierenstiftung and Deutsche Gesellschaft für Nephrologie to C.F.K., a grant from the Werner Otto Stiftung to L.U.B.E., a grant from the eMed Consortia "Fibromap" from the Federal Ministry of Education and Research to V.G.P., and grants Conicyt Proyecto AFB 170004 and Conicyt/FONDEQUIP/EQM140016 to M.R.B. The ERCB was supported by the Else Kröner-Fresenius Foundation. **Author contributions:** C.F.K., D.R., U.P., and H.-W.M. wrote the manuscript and designed figures. C.F.K., D.R., H.-J.P., H.R., C.Ku., F.K.-N., M.R.B., U.P., and H.-W.M. designed experiments. C.F.K., D.R., H.-J.P., P.B., S.N., M.V.R., M.H., A.B., L.U.B.E., M.Z., M.B., J.S., M.R., S.F., T.W., S.K., M.N.W., V.G.P., C.S., T.B., N.S., C.M.-S., and E.H. conducted experiments and acquired data. C.F.K., D.R., Y.Z., H.-J.P., P.B., C.Ki., C.D.C., M.T.L., and M.H. analyzed data. C.F.K., D.R., Y.Z., T.B.H., S.B., U.P. and H.-W.M. interpreted data. C.F.K. and H.-W.M. did statistical analysis. J.-E.T., J.-H.R., S.H., and N.G. edited the manuscript. **Competing interests:** The authors declare that they have no competing interests. **Data and materials availability:** The datasets generated and analyzed during the current study are available from the corresponding authors on reasonable request. All datasets used for figures are provided as Supplementary Materials. The single-cell gene expression count and metadata tables containing clustering and quality control metrics for each cell are available at FigShare: https://figshare.com/s/7912de1afc7fd5bbefd4.

Submitted 2 December 2019
Resubmitted 11 May 2020
Accepted 16 July 2020
Published 7 August 2020
10.1126/sciimmunol.aba4163

Citation: C. F. Krebs, D. Reimers, Y. Zhao, H.-J. Paust, P. Bartsch, S. Nuñez, M. V. Rosemblatt, M. Hellmig, C. Kilian, A. Borchers, L. U. B. Enk, M. Zinke, M. Becker, J. Schmid, S. Klinge, M. N. Wong, V. G. Puelles, C. Schmidt, T. Bertram, N. Stumpf, E. Hoxha, C. Meyer-Schwesinger, M. T. Lindenmeyer, C. D. Cohen, M. Rink, C. Kurts, S. Franzenburg, F. Koch-Nolte, J.-E. Turner, J.-H. Riedel, S. Huber, N. Gagliani, T. B. Huber, T. Wiech, H. Rohde, M. R. Bono, S. Bonn, U. Panzer, H.-W. Mittrücker, Pathogen-induced tissue-resident memory T_H17 ($T_{RM}17$) cells amplify autoimmune kidney disease. *Sci. Immunol.* **5**, eaba4163 (2020).

Science Immunology

Pathogen-induced tissue-resident memory T_H17 ($T_{RM}17$) cells amplify autoimmune kidney disease

Christian F. Krebs, Daniel Reimers, Yu Zhao, Hans-Joachim Paust, Patricia Bartsch, Sarah Nuñez, Mariana V. Rosemblatt, Malte Hellmig, Christoph Kilian, Alina Borchers, Leon U. B. Enk, Michael Zinke, Martina Becker, Joanna Schmid, Stefanie Klinge, Milagros N. Wong, Victor G. Puelles, Constantin Schmidt, Tabea Bertram, Natascha Stumpf, Elion Hoxha, Catherine Meyer-Schwesinger, Maja T. Lindenmeyer, Clemens D. Cohen, Michael Rink, Christian Kurts, Sören Franzenburg, Friedrich Koch-Nolte, Jan-Eric Turner, Jan-Hendrik Riedel, Samuel Huber, Nicola Gagliani, Tobias B. Huber, Thorsten Wiech, Holger Rohde, Maria Rosa Bono, Stefan Bonn, Ulf Panzer and Hans-Willi Mittrücker

Sci. Immunol. **5**, eaba4163.
DOI: 10.1126/sciimmunol.aba4163

Autoimmunity promoter

Tissue-resident memory T (T_{RM}) cells are involved in peripheral immunity against reinfection, but their role in autoimmunity is unclear. Krebs *et al.* examine the contribution of T_{RM} cells in patients with antineutrophil cytoplasmic antibody (ANCA)–dependent glomerulonephritis (GN). They identified multiple T cell subsets in healthy kidney tissue biopsies, but a marked increase in $CD4^+$ T_{RM} cells was seen in kidney biopsies from patients with ANCA-GN. They infected mice with *Staphylococcus aureus*, which induced renal T_H17 cells that had a T_{RM} cell phenotype and persisted in kidney tissue. In a mouse model of crescentic GN, *S. aureus* infection aggravated kidney pathology and appeared to drive localized renal autoimmune responses. These findings provide critical insight into the role of $CD4^+$ T_{RM} cells in contributing to autoimmune disease.

Science Immunology (ISSN 2470-9468) is published by the American Association for the Advancement of Science, 1200 New York Avenue NW, Washington, DC 20005. The title *Science Immunology* is a registered trademark of AAAS.