IL-17C/IL-17 Receptor E Signaling in CD4\(^+\) T Cells Promotes T\(_{H17}\) Cell-Driven Glomerular Inflammation


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ABSTRACT

The IL-17 cytokine family and the cognate receptors thereof have a unique role in organ-specific autoimmunity. Most studies have focused on the founding member of the IL-17 family, IL-17A, as the central mediator of diseases. Indeed, although pathogenic functions have been ascribed to IL-17A and IL-17F in the context of immune-mediated glomerular diseases, the specific functions of the other IL-17 family members in immunity and inflammatory kidney diseases is largely unknown. Here, we report that compared with healthy controls, patients with acute Anti-neutrophil cytoplasmatic antibody (ANCA)-associated crescentic glomerulonephritis (GN) had significantly elevated serum levels of IL-17C (but not IL-17A, F, or E). In mouse models of crescentic GN (nephrotoxic nephritis) and pristane-induced lupus nephritis, deficiency in IL-17C significantly ameliorated the course of GN in terms of renal tissue injury and kidney function. Deficiency of the unique IL-17C receptor IL-17 receptor E (IL-17RE) provided similar protection against crescentic GN. These protective effects associated with a reduced T\(_{H17}\) response. Bone marrow transplantation experiments revealed that IL-17C is produced by tissue-resident cells, but not by lymphocytes. Finally, IL-17RE was highly expressed by CD4\(^+\) T\(_{H17}\) cells, and loss of this expression prevented the T\(_{H17}\) responses and subsequent tissue injury in crescentic GN. Our findings indicate that IL-17C promotes T\(_{H17}\) cell responses and immune-mediated kidney disease via IL-17RE expressed on CD4\(^+\) T\(_{H17}\) cells. Targeting the IL-17C/IL-17RE pathway may present an intriguing therapeutic strategy for T\(_{H17}\)-induced autoimmune disorders.


Significance Statement

Recent studies have established the crucial role of the T\(_{H17}/IL-17A\) pathway in the pathogenesis of autoimmune diseases, such as human and experimental crescentic GN. So far, the focus of most studies has been on the IL-17 founding member IL-17A as the central mediator of diseases. Here, we report that serum IL-17C levels were significantly elevated in patients with ANCA-associated crescentic GN. Moreover, using a murine model of crescentic GN, we provide direct evidence that IL-17C promotes T\(_{H17}\) cell responses and kidney injury via IL-17 receptor E, expressed on CD4\(^+\) T\(_{H17}\) cells. These findings have significant implications for the understanding of how the IL-17 cytokine family contributes to organ-specific T\(_{H17}\) autoimmunity and potentially for the development of novel treatment strategies.
transcription factor RORγt3 and the expression of CCR6,4,5 play a pivotal role in crescentic GN by producing proinflammatory cytokines.6–9 In particular, IL-17A and IL-17F may mediate tissue injury by the induction of renal CXCL1 and CXCL5 expression.10–12 These chemokines, in turn, recruit neutrophils into the kidney, which ultimately contributes to the organ damage.13

The IL-17 cytokine family consists of six members (IL-17A–F), of which IL-17A and F are the most closely related and best characterized ones.14 Their biologic effects are mediated by binding to heterodimeric receptors of the IL-17 receptor family (IL-17RA–RE). Except for the receptor for IL-17B (IL-17RB), all receptor complexes contain the ubiquitously expressed subunit IL-17RA and a second, ligand-specific receptor. So far, only the roles of IL-17A and IL-17F have been analyzed in the pathogenesis of immune-mediated glomerular diseases.10,11,15–17 The potential effect of the other IL-17 family members in renal autoimmunity and inflammation is unknown.

First, we assessed the serum levels of the IL-17 family members in patients with ANCA-associated crescentic GN and analyzed the systemic and renal expression patterns of these cytokines and their receptors during the time course of an experimental model of crescentic GN (nephrotic nephritis [NTN]).18 Unexpectedly, these analyses demonstrated an up-regulation of IL-17C and its specific receptor IL-17RE, suggesting a functional role for the IL-17C/IL-17RE axis in immune-mediated kidney diseases, and represented the rationale for our study.

Pioneer studies indicated that IL-17C might share similar biologic features with IL-17A, as it stimulates inflammation by upregulating cytokines and chemokines that are characteristic of a T helper 17 response in epithelial cells.19 Moreover, the involvement of IL-17C in the immunopathogenesis of murine models for multiple sclerosis and arthritis,20,21 potentially by direct effects on the T helper 17 response, has been reported.20

The goal of this study was to elucidate the role of the IL-17C/IL-17RE axis in immune-mediated glomerular diseases. We therefore induced an experimental model of crescentic GN (NTN) to (1) define the effect of IL-17C and its receptor IL-17RE on the clinical outcome of the GN, (2) determine the significance of this ligand receptor pair for the T-cell immune response, and (3) identify the mechanisms by which the IL-17C/IL-17RE pathway might drive renal tissue injury in experimental crescentic GN.

RESULTS

Serum IL-17C Levels Are Elevated in Patients with ANCA-Associated Crescentic GN

Using an ultrasensitive electrochemiluminescence immunoassay, we analyzed the serum levels of IL-17A, C, E, and F in 70 patients with biopsy-confirmed, acute ANCA-associated crescentic GN and in 20 healthy controls. Unexpectedly, we observed a marked elevation (P<0.001) in IL-17C protein levels in the ANCA GN patient group compared with healthy controls (Figure 1). No significant differences were detected in the levels of the remaining IL-17 family members (Figure 1). We also analyzed the levels of TNF-α and IL-6 as general markers for inflammation, which were significantly higher in the patient population compared with the control group (Figure 1). The clinical and demographic baseline characteristics of the patient group are shown in Supplemental Figure 1.

IL-17C and IL-17RE Expression Is Upregulated in Experimental Crescentic GN

Next, we aimed at investigating the expression pattern of the IL-17 family members and their receptors during the time course of NTN. Renal quantitative PCR (qPCR) analyses during different periods of time (day 0 to day 30) of nephritic mice revealed the regulation of Il-17a mRNA expression, which is in agreement with our previous data.22 In addition, Il-17c expression was upregulated 12 hours after NTN induction (Figure 2A). A similar mRNA expression pattern of Il-17a and Il-17c was detected in the spleen (Figure 2B). We also
Figure 2. Expression of IL-17C and IL-17RE is upregulated in experimental crescentic GN. Renal expression of the IL-17 family and their receptors in crescentic GN. (A) qPCR analysis of Il-17a–f mRNA expression levels in the kidney, (B) the spleen, and (C) renal expression levels of Il-17ra, Il-17rc, and Il-17re at indicated time points after NTN induction. Controls (Con; n=5) and nephritic wild-type mice (n=4–7). mRNA levels are expressed as x-fold of controls. Symbols represent individual data points with the mean as a horizontal line.
observed a regulation of the mRNA expression of IL-17rc and IL-17re, which are the specific receptor subunits of IL-17A and IL-17C, respectively (Figure 2C). The receptor chain IL-17RA, which serves as a common receptor chain for all IL-17 ligands (except for IL-17B), was highly expressed throughout the course of crescentic GN, but did not show any changes in its expression pattern (Figure 2C).

IL-17C Promotes Renal Tissue Injury in Crescentic GN

On the basis of the expression pattern of Il-17c in human and experimental GN described above, we examined whether IL-17C signaling is involved in the pathogenesis of crescentic GN. Therefore, we analyzed Il-17c−/− mice using the NTN mouse model. The histologic and functional parameters were analyzed to compare the clinical outcome in wild-type and Il-17c−/− mice upon NTN induction (Figure 3). Quantification of the renal tissue damage, in terms of glomerular crescent formation and tubulointerstitial injury 10 days after NTN induction, revealed a reduction in Il-17c−/− mice (Figure 3, A and B). In line with these results, we also found a significant reduction in BUN levels, and to a lesser degree in serum creatinine levels, whereas we did not find any changes in the urinary albumin-to-creatinine ratio (ACR) (Figure 3C).

Assessment of renal leukocyte recruitment by immunohistochemical staining revealed that the tubulointerstitial infiltration of F4/80+ macrophages and CD3+ T cells was significantly

![Figure 3](https://www.jasn.org/)

**Figure 3.** IL-17C promotes renal tissue injury in crescentic GN. (A) Representative photographs of PAS-stained kidney sections from control (Con), nephritic wild-type (WT), and nephritic Il-17c−/− mice at day 10 of NTN (original magnification, ×400). (B) Quantification of glomerular crescent formation and tubulointerstitial damage. A combination of results from two independent experiments is shown. Controls (n=5), nephritic wild-type (n=11), and nephritic Il-17c−/− mice (n=15). (C) BUN, serum creatinine, and ACR determined in the aforementioned groups. (D) Quantification of tubulointerstitial F4/80+, glomerular MAC-2+ and CD3+, and tubulointerstitial CD3+ cells in the aforementioned groups. (E) Representative photographs of GR-1–stained kidney sections from nephritic wild-type and nephritic Il-17c−/− mice. Original magnification, ×400. (F) Quantification of tubulointerstitial GR-1+ cells in the aforementioned groups. Symbols represent individual data points with the mean as a horizontal line (*P<0.05; **P<0.01; ***P<0.001).
Figure 4. IL-17C-driven tissue injury in crescentic GN is IL-17A-dependent. (A) Representative FACS plots of intracellular cytokine staining for IL-17A and IFN-γ in renal T cells (pre-gated on singlets, live, CD45+, CD3+, and CD4+ cells). (B) Quantification of intracellular cytokine FACS analysis for IL-17A, IL-17F, and IFN-γ in renal CD4+ T cells. Data are representative of two independent experiments.
revealed a predominant downregulation of TH17/IL-17A response in nephritic Il-17c−/− mice (Figure 3D). Staining of tubulointerstitial GR-1+ neutrophils showed a significant reduction in these cells in nephritic Il-17c−/− mice (Figure 3, E and F).

### Amelioration of the GN Disease Course in Il-17C−/− Mice Is a Consequence of a Reduced Th17/IL-17A Response

To elucidate the immunologic mechanisms that lead to a less severe course of GN in Il-17C−/− mice, we investigated the renal and systemic immune responses in these animals in more detail. Flow cytometry studies revealed a significant reduction in renal T17 cells, whereas the T17 cell response in the kidney was unaffected (Figure 4, A and B). To determine whether IL-17C might also modulate systemic immunity in the NTN model, we measured the serum concentration of several cytokines, including IL-17C. The concentration of IL-17C in the serum of nephritic mice was increased at day 10 and, as expected, was not detectable in nephritic Il-17C−/− mice (Figure 4C). The analysis of serum IL-17A levels revealed a significant reduction in nephritic Il-17c−/− mice compared with nephritic wild-type mice. Other effector cytokines related to Th17 immune responses did not show significant differences. The IFN-γ serum level was slightly, but significantly, increased in Il-17c−/− mice.

IL-17C did not have major effects on the humoral immune response because nephritic wild-type and nephritic Il-17c−/− mice had similar serum titers of anti-sheet total IgG, IgG1, IgG2a/c, IgG2b, and IgG3 antibodies directed against the nephritogenic antigen (Supplemental Figure 2, A and B).

To further investigate the influence of IL-17C on the local immune/inflammatory response in the kidney, expression analyses of the renal cortex using a cytokine and chemokine pathway-focused PCR array (RT2 Profiler) were performed. These analyses revealed a predominant downregulation of T17/IL-17A–specific target genes in nephritic Il-17c−/− mice (Figure 4D). Accordingly, qPCR analysis confirmed significantly reduced Cxcl2 and Cxcl5 mRNA expression in nephritic Il-17c−/− mice. Likewise, we found a reduction in the neutrophil-attracting chemokine Cxcl1 as well as Ccl20, which interacts with the chemokine receptor CCR6 expressed on T17 cells (Figure 4E). Moreover, IL-17C deficiency resulted in a reduction in the renal expression of IL-1β and IL-6, whereas IL-23 expression was not affected (Figure 4E). This suggests that the downregulated T17/IL-17A immune response in nephritic Il-17c−/− mice might be responsible for the less severe crescentic GN. To test this hypothesis, we treated nephritic wild-type and nephritic Il-17c−/− mice with a neutralizing anti-IL-17A antibody. IL-17A neutralization diminished the differences in kidney damage between the wild-type and knockout groups (Figure 4F). In line with these results, we did not observe a difference in the functional parameters of BUN, serum creatinine, or ACR (Figure 4G). Taken together, these results strongly indicate that the ameliorated GN disease course in Il-17C−/− mice is a consequence of a reduced T17/IL-17A response.

### IL-17C Is Produced by Resident Tissue Cells

To study whether IL-17C in the kidney is mainly produced by resident renal tissue cells or by hematopoietic cells, we performed bone marrow (BM) transplantation studies using wild-type and Il-17C−/− mice. Twenty-four hours after induction of nephritis (the time point of highest renal Il-17C mRNA expression), we obtained high Il-17C expression levels in animals with wild-type renal tissue cells. By contrast, Il-17C mRNA expression in Il-17c−/− mice receiving wild-type BM or Il-17c−/− BM was not elevated (Supplemental Figure 3). These data indicate that IL-17C is mainly expressed by resident kidney cells.

### IL-17C Does Not Induce Chemokine Expression in Resident Kidney Cells

In order to investigate whether IL-17C has direct effects on resident kidney cells, murine proximal tubular and murine mesangial cells were cultured and stimulated with IL-17C (1–100 ng/ml), or TNF-α and IL-17A as positive control. RT-PCR analysis revealed the mRNA expression of IL-17RA and IL-17RE in both cell lines (data not shown). Stimulation with IL-17C alone had no effect on Cxcl1 and Cxcl5 mRNA expression and protein formation (Supplemental Figure 4, A–D), but a moderate, synergistic effect was observed after stimulation together with a IL-22 or TNF-α (data not shown). Taken together, these results argue against major effects of IL-17C on resident kidney cells, which is in contrast to the pathophysiologic concept of IL-17A and IL-17F.

### IL-17RE Deficiency Ameliorates Renal Injury in Crescentic GN

To analyze whether deficiency in IL-17RE, the specific receptor of IL-17C, also protects against immune-mediated glomerular
disease, we compared the clinical course of experimental GN between wild-type mice and Il-17re+/− mice. The evaluation of kidney sections stained with periodic acid–Schiff (PAS) for the presence of crescents and tubulointerstitial injury revealed a significant reduction in the destruction of regular tissue structures in nephritic Il-17re−/− mice (Figure 5, A and B). BUN and serum creatinine levels of nephritic Il-17re−/− mice were significantly reduced as compared with nephritic wild-type mice (Figure 5C), but no effect on ACR was detectable (Figure 5C).

The migration of F4/80+ macrophages and CD3+ T cells into the kidney revealed no difference among nephritic wild-type and nephritic Il-17re−/− mice (Figure 5D), whereas the number of glomerular Mac-2+ mononuclear phagocytes was increased in nephritic Il-17re−/− mice (Figure 5D). Most importantly, the tubulointerstitial GR-1+ cell infiltration was significantly reduced in nephritic Il-17re−/− mice (Figure 5, E and F).

**IL-17RE Is Required for the Promotion of Renal T\(_{H17}\) Cell Responses**

In line with the observed immunologic phenotype in Il-17c−/− mice, IL-17RE deficiency resulted in a significant reduction in the renal T\(_{H17}\) cell response in GN, whereas no effect was seen on the T\(_{H11}\) cell population (Figure 6, A and B). Analyses of the mRNA expression profiles of whole renal cortices revealed that the absence of IL-17RE had a predominant effect on T\(_{H17}\)/IL-17A target genes because the neutrophil-attracting chemokines Cxcl1 and Cxcl5 showed a decreased upregulation in nephritic Il-17re−/− mice compared with their nephritic wild-type counterparts (Figure 6C). In contrast, T\(_{H11}\) target

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**Figure 5.** IL-17RE–mediated signaling aggravates crescentic GN. (A) Representative photographs of PAS-stained kidney sections from control (Con), nephritic wild-type (WT), and nephritic Il-17re−/− mice at day 9 of NTN (original magnification, ×400). (B) Quantification of glomerular crescent formation and tubulointerstitial damage. Data are representative of two independent experiments. Controls (n=2), nephritic wild-type (n=6), and nephritic Il-17re−/− mice (n=5). (C) BUN, serum creatinine, and ACR were determined in the aforementioned groups. (D) Quantification of tubulointerstitial F4/80+, glomerular Mac-2+, glomerular CD3+, and tubulointerstitial CD3+ cells in the aforementioned groups. (E) Representative photographs of GR-1–stained kidney sections from nephritic wild-type and nephritic Il-17re−/− mice. Original magnification, ×400. (F) Quantification of tubulointerstitial GR-1+ cells in the aforementioned groups. Symbols represent individual data points with the mean as a horizontal line (*P<0.05; ***P<0.001).
genes such as Ccl5 and Cxcl9 were not affected in nephritic Il-17re−/− mice. The serum titers of anti-sheep total IgG, IgG1, IgG2a/c, IgG2b, and IgG3 antibodies of nephritic wild-type and nephritic Il-17re−/− mice showed no effect of IL-17RE–mediated signaling on the humoral immune response (Supplemental Figure 2, C and D).

**IL-17RE Is Expressed by CD4+ T\(_{h17}\) Cells and Is Instrumental for T\(_{h17}\) Responses and Subsequent Tissue Injury in Crescentic GN**

To investigate the cellular expression pattern of IL-17RE, NK1.1+ NK cells, CD11b+CD11c+MHC2+ monocytes/macrophages, CD19+ B cells, and CD3+/CD4+ T cells from the kidneys of control and nephritic wild-type and nephritic Il-17re−/− mice were sorted by FACS. Subsequent RT-PCR analysis revealed that Il-17re expression is primarily upregulated by CD4+ T cells (Figure 7A). Using IL-17A YFP+ fate reporter mice,\(^ {23}\) we isolated CD4+ T\(_{h17}\) cells from the kidney and, by qPCR, compared their Il-17re mRNA expression with CD4+ T\(_{h17}\)− cells. As shown in Figure 7B, Il-17re expression was increased in the T\(_{h17}\) cell population. Finally, we polarized CD4+ T cells in vitro in T\(_{h17}\), T\(_{h17}\), regulatory T cells, and T\(_{h0}\) cells and assessed the Il-17re expression. In line with the other experiments, T\(_{h17}\) cells demonstrated the highest expression level of the receptor (Figure 7C). Because of the lack of suitable IL-17RE antibodies, it is currently not feasible to confirm these data by flow cytometry.

To study the potential function of IL-17RE in the T\(_{h17}\) response, we isolated CD4+ T cells from the spleen of CD45.1 wild-type and CD45.2 Il-17re−/− mice and transferred them into Rag1−/− mice (competitive adoptive transfer, 1:1 ratio),

![Figure 6](https://www.jasn.org/BASIC_RESEARCH/1217.png)

**Figure 6.** IL-17RE promotes renal T\(_{h17}\) cell responses in crescentic GN. (A) Representative FACS plots of intracellular cytokine staining for IL-17A and IFN-γ in renal T cells (gated on singlets, live, CD45+, CD3+, and CD4+ cells). (B) Quantification of intracellular cytokine FACS analysis for IL-17A, IL-17F, and IFN-γ in renal CD4+ T cells. Nephritic wild-type (WT; n=6) and nephritic Il-17re−/− mice (n=5). (C) Renal qPCR mRNA expression analysis of Cxcl1, Cxcl2, Cxcl5, and Cxcl9, as well as Ccl5 and Ccl20. mRNA levels are expressed as x-fold of controls (Con). Data are representative of two independent experiments with controls (n=2), nephritic wild-type (n=6), and nephritic Il-17re−/− mice (n=5). Data are presented as individual data points with the mean as a horizontal line (*P<0.05; **P<0.01).
Figure 7. IL-17RE is highly expressed by CD4+ T cells and promotes renal tissue injury in crescentic GN. (A) Regulation of Il-17re mRNA expression on selected FACS sorted renal leukocyte subsets under nephritic conditions (n=4). (B) Il-17re mRNA expression profile of IL-17A-, YFP-, and IL-17A+, YFP+ sorted renal CD4+ T cells of nephritic IL-17A YFP+ fate reporter mice. (C) Il-17re mRNA expression profile of cells isolated from naïve mice and cultured under TH0-, TH1-, TH17-, or Treg-polarizing conditions for 65 hours (n=5). mRNA levels are expressed as x-fold of controls. (D) Representative FACS plots and quantification of IL-17A+ cells from nephritic Rag1−/− mice repopulated with CD45.1 wild-type (WT) and CD45.2 Il-17re−/− CD4+ T cells. (E) Representative photographs of PAS-stained kidney sections of Rag1−/− controls (Con; n=6), nephritic Rag1−/− mice repopulated with 1.5×10^6 wild-type CD4+ T cells (n=6), and nephritic Rag1−/− mice repopulated with 1.5×10^6 Il-17re−/− CD4+ T cells (n=8) at day 10 of NTN (original magnification, ×400). (F) Quantification of glomerular crescent formation and tubulointerstitial damage of the aforementioned groups.
which are deficient in T cells and B cells. Subsequently, we induced NTN and analyzed the “origin” of renal T117 cells at day 10, using the congeneric markers CD45.1 and CD45.2. As shown in Figure 7D, the deficiency in IL-17RE on CD4+ T cells led to a significantly reduced renal T117 cell response compared with the wild-type group.

In addition, we performed adoptive transfer experiments to investigate the effect of IL-17RE–expressing CD4+ T cells on the clinical course of crescentic GN. Therefore, CD4+ T cells from the spleen of wild-type and II-17re−/− mice were isolated and separately transferred into Rag1−/− mice. Six days after the adoptive cell transfer, NTN was induced. Rag1−/− mice reconstituted with II-17re−/− CD4+ T cells developed a less severe course of the GN with respect to glomerular crescent formation and tubulointerstitial injury (Figure 5, B and C). A comparison of pristane-treated wild-type and Rag1−/− mice revealed that the percentage of abnormal glomeruli was significantly reduced in II-17re−/− mice (Figure 7G), as was the expression of Cxcl5 (Figure 7H).

**IL-17C Plays a Role in a Chronic Model of Lupus Nephritis**

We then assessed the effect of IL-17C deficiency in the chronic model of pristane-induced lupus nephritis. The application of pristane resulted in early mortality because of the development of severe pulmonary vasculitis, which was comparable between wild-type and II-17c−/− mice (Supplemental Figure 5A). Twelve months after pristane injection (the point of time when lupus nephritis was established), renal analysis was performed. PAS staining of renal tissue sections showed glomerular alterations, including hypercellularity, segmental proliferation, hyalinosis, and capillary wall thickening, as well as an increase of glomerular area as a sign of immune-mediated kidney damage (Supplemental Figure 5A). During the past decade, the interaction of IL-17C and IL-17 receptor (IL-17R) has been identified as one pathway to upregulate IL-17C in these disease entities. IL-17C then drives inflammatory effects by stimulating innate immune responses in epithelial cells to fight off the infection. Yet little is known about the involvement of the IL-17C/IL-17R axis in the pathogenesis of autoimmune diseases. Recently, it was suggested that the axis plays a role in autoimmune hepatitis by induction of IL-2 in CD4+ T cells which, in turn, stimulate NK cells to cause hepatic damage. Also, IL-17C had proinflammatory effects in an imiquimod-induced mouse model of autoimmune skin inflammation.

**Discussion**

Here, we demonstrate that the IL-17C/IL-17RE axis significantly contributes to renal tissue injury in a murine model of crescentic GN. Our findings indicate that IL-17C promotes T117 cell responses via IL-17RE, expressed on CD4+ T117 cells. This results in increased renal expression of IL-17 target genes, such as the chemokines CXCL1 and CXCL5, and further recruitment of neutrophils, ultimately leading to tissue injury.

In recent years IL-17A and, to lesser degree, IL-17F and IL-17RA have been identified as important proinflammatory regulators and potential therapeutic targets in autoimmune and inflammatory kidney diseases, including human and experimental crescentic GN. However, the potential function of other IL-17 cytokines and IL-17 receptors is still unclear. We measured serum protein levels of the IL-17 cytokine family members in 70 patients with ANCA-associated GN, the most common form of crescentic GN. Surprisingly, we found no significant differences in IL-17A, E, and F levels in the ANCA GN patient group compared with healthy controls, but a highly significant increased level of IL-17C. An upregulated expression of IL-17C was also detectable in a model of crescentic GN (NTN), suggesting a potential role of this cytokine in crescentic GN. We were not able to detect significant serum levels of IL-17B and D.

The biologic role of IL-17C is not well characterized and its function in renal autoimmune and inflammatory kidney diseases is unknown. IL-17C was cloned in 2000 by large-scale screen of expressed sequence tag databases for proteins homologous to IL-17A. Six years later, the specific receptor subunit IL-17RE was identified by a sequence-based homology search. The observation that IL-17C binds to IL-17RE was made in 2011. During the past decade, the interaction of IL-17C and IL-17RE has been reported to have proinflammatory effects by stimulating innate immune responses on epithelial cells in response to bacterial or sterile inflammatory stimuli. Toll-like receptor signaling has been identified as one pathway to upregulate IL-17C in these disease entities. IL-17C then drives innate immune responses in an autocrine manner in epithelial cells to fight off the infection. Yet little is known about the involvement of the IL-17C/IL-17RE axis in the pathogenesis of autoimmune diseases. Recently, it was suggested that the axis plays a role in autoimmune hepatitis by induction of IL-2 in CD4+ T cells which, in turn, stimulate NK cells to cause hepatic damage. Also, IL-17C had proinflammatory effects in an imiquimod-induced mouse model of autoimmune skin inflammation.
of cultured T\textsubscript{H}17 cells overexpressing the receptor subunit.\textsuperscript{20} They also report that IL-17C is implicated in the pathogenesis of experimental autoimmune encephalomyelitis. Moreover, IL-17C serum levels were increased in patients with active inflammatory bowel diseases.\textsuperscript{34}

We studied the function of IL-17C in a well established model of crescentic GN (NTN). Compared with wild-type mice, IL-17C-deficient mice developed less severe disease, with significantly better renal function, and a reduced frequency of glomerular crescent formation and tubulointerstitial tissue injury. In line with this, the lack of IL-17RE (the unique receptor for IL-17C) also resulted in an ameliorated course of the GN in terms of renal function and tissue damage. Additionally, studies in the chronic model of pristane-induced lupus nephritis showed that IL-17C-deficient lupus mice had a moderately improved course of the disease. In summary, these results demonstrated for the first time the pathogenic role of the IL-17C/IL-17RE axis in immune-mediated glomerular disease.

Mechanistically, we provide evidence that the IL-17C/IL-17RE axis directly stimulates the nephritogenic T\textsubscript{H}17 response and thereby promotes renal tissue injury in crescentic GN. This hypothesis is on the basis of the finding that (1) renal IL-17A–producing T\textsubscript{H}17 cells, the expression of IL-17 target genes, and the infiltration of neutrophils were selectively reduced in nephritic IL-17C–/– and IL-17RE–/– mice; (2) application of a neutralizing IL-17A antibody diminished the observed differences in kidney damage between nephritic wild-type and IL-17C–/– animals; (3) IL-17RE is highly expressed on renal CD4\textsuperscript{+} T\textsubscript{H}17 cells; and (4) the transfer of IL-17RE–deficient CD4\textsuperscript{+} T cell into Rag1–/– mice resulted in a reduced renal T\textsubscript{H}17 response and subsequent tissue injury in crescentic GN.

Our attempts to study the IL-17C/IL-17RE–induced signaling pathways in T\textsubscript{H}17 cells that potentiate the T\textsubscript{H}17 cell response were hampered by the lack of suitable FACS antibodies for IL-17RE and the lack of available IL-17RE reporter mice, and remains to be fully elucidated. First experiments indicated that IL-17C/IL-17RE did not influence the polarization of naive T cells toward T\textsubscript{H}17 cells \textit{in vitro} (data not shown), suggesting that other pathways such as T\textsubscript{H}17 cell maintenance, expansion, or stabilization are involved in the process.

Our study highlighted major differences in the biologic function of IL-17C compared with IL-17A and IL-17F. IL-17C is predominantly expressed by resident cells in the kidney, whereas in the spleen different cell populations, including mononuclear phagocytes, expressed IL-17C mRNA. Further studies are clearly needed to characterize in detail the cellular source of IL-17C under inflammatory and homeostatic conditions. So far, we have not been able to establish reliable IL-17C immunohistochemical staining in the kidney or spleen. In contrast, IL-17A and F are derived from renal T\textsubscript{H}17 cells or γδ T cells in GN. Moreover, IL-17C has only weak effects on resident kidney cells, in terms of proinflammatory chemokine induction, but directly promotes systemic T\textsubscript{H}17 response via IL-17RE signaling. This is different compared with IL-17A and F, which are supposed to act locally on renal cells and might explain why systemic levels of IL-17C reflect the local T\textsubscript{H}17 response in the kidney better than IL-17A or F. On the basis of our finding that IL-17C stimulates the T\textsubscript{H}17 cell response upstream of IL-17A, targeting of the IL-17C/IL-17RE pathway may present an intriguing therapeutic strategy for T\textsubscript{H}17-driven autoimmune and inflammatory diseases, especially because neutralization of the T\textsubscript{H}17/IL-17A axis has already been confirmed to be an effective concept of treating autoimmune diseases such as psoriasis.\textsuperscript{35,36}

**CONCISE METHODS**

**Human Study Cohort**
A total of 70 patients were recruited in this study (Hamburg Glomerulonephritis Registry). The inclusion criterion was biopsy-proven, ANCA-associated GN. The trial was performed in accordance with the declaration of Helsinki. After informed consent was obtained, patient data and renal biopsy samples were collected according to the guidelines of the respective local ethics committees.

**Animals**
IL-17C–/– mice were obtained from the Mutant Mouse Regional Resource Centers (University of California, Davis, CA). IL-17RE–/– mice were kindly provided by Bristol-Myers Squibb Company (Princeton, NJ). Rag1–/– mice were obtained from the Jackson Laboratory. All mice were on the C57BL/6J background. Age-matched C57BL/6J wild-type controls were bred in our facility. All mice were raised under specific pathogen-free conditions. All animal experiments were performed according to national and institutional animal care and ethical guidelines and were approved by the local authorities.

**Induction of Experimental GN and Functional Studies**
NTN was induced by intraperitoneal injection of 2.5 mg of nephrotoxic sheep serum per gram of body weight into 8–12-week-old male mice.\textsuperscript{10} Lupus nephritis was induced by intraperitoneal injection of 0.5 ml pristane into 8–10-week-old male mice.\textsuperscript{7,38} Urine samples were collected by housing the mice in metabolic cages for 3–5 hours. Urinary albumin excretion was determined using standard ELISA technology (Mice-Albumin Kit; Bethyl), and urinary creatinine, BUN, and serum creatinine levels were measured using standard laboratory methods.

**qPCR Analyses**
Total RNA of the renal cortex and cell subsets were prepared according to standard laboratory methods. qPCR analyses were performed for 40 cycles on a StepOnePlus Real-Time PCR system (Applied Biosystems, Foster City, CA). The samples were run in duplicate and normalized to 18S rRNA. The cytokine and chemokine expression profiles were determined using the Mouse cytokines & chemokines RT2 Profiler PCR Array (Qiagen, Hilden, Germany). The samples were normalized to the housekeeping gene β-actin (Actb). Data were analyzed using the StepOne Software v2.0.
Electrochemiluminescence Immunoassay
Mouse cytokine serum levels as well as serum samples from patient visits to our clinic, thawed on ice, were analyzed using a customized Meso Scale Discovery immunoassay (U-Plex Th17 Combo 2). This technology includes biotinylated and conjugated SULFO-TAG detection antibodies to simultaneously measure different analytes in a one microwell well. Unprocessed serum from each patient (50 μl) was thawed and analyzed for the cytokines according to the manufacturer’s protocol (Meso Scale Discovery, Rockville, MD). Electrochemiluminescence was detected using the QuickPlex SQ120 instrument. Analyses were performed with the MSD Discovery Workbench software v4.0.

Morphologic Analyses
Immunohistochemistry was performed using routine laboratory methods. Glomerular crescent formation and tubulointerstitial injury were assessed in PAS-stained paraffin sections in a blinded fashion, as described.11

Isolation of Leukocytes from Murine Kidney
The leukocytes from murine kidneys were isolated as previously described.39 Briefly, kidneys were digested for 45 minutes at 37°C by adding 0.4 mg/ml collagenase D (Roche, Mannheim, Germany) and 0.01 mg/ml DNase I (Roche) to RPMI 1640 (Life Technologies, Karlsruhe, Germany) medium supplemented with 10% heat-inactivated FCS (Gibco, Eggenstein, Germany). Subsequently, kidneys were finely minced using the gentle MACS Dissociator (Miltenyi Biotec, Teterow, Germany). Single-cell suspensions were separated using Percoll density gradient centrifugation.

Culturing and Stimulation of Mouse Kidney Tubular and Mesangial Cells
Mouse kidney tubular cells40 and mouse kidney mesangial cells41 were cultured in DMEM medium (Life Technologies) containing 3%–10% FCS (Gibco), 100 U/ml penicillin, and 100 μg/ml streptomycin (Life Technologies) at 37°C with 5% CO2. The cells were incubated in serum-free DMEM medium 24 hours before stimulation. Cells were stimulated with IL-17C (10 ng/ml), TNF-α (10 ng/ml), and IL-22 (100 ng/ml), or with a combination. Cells were harvested after 4 hours of stimulation and stored at −80°C for mRNA analyses. The protein levels in the supernatant were determined after 24 hours of incubation, using a specific ELISA according to the manufacturer’s protocol (R&D Systems, Wiesbaden, Germany).

CD4+ T Cell Polarization
For polarization of naïve CD4+ cells, splenocytes were isolated from naïve wild-type mice using the MACS CD4+ T Cell Isolation Kit, mouse (Miltenyi Biotec). A total of 2×10^6 cells/well was plated in anti-CD3 antibody-coated 96-well plates (eBioscience, San Diego, CA) and incubated for 65 hours with soluble anti-CD28. The following cytokines and neutralizing antibodies were used: for Th0 conditions, anti–IFN-γ and IL-2; for Th1-polarizing conditions, anti–IL-4 and recombinant IL-12; for Th17-polarizing conditions, anti–IL-4, anti–IFN-γ, IL-6, TGF-β, IL-1β, and IL-23; and for Treg-polarizing conditions, anti–IFN-γ, IL-2, and TGF-β (all BioLegend).

Assessment of the Humoral and Cellular Nephritogenic Immune Responses
Mouse anti-sheep IgG antibody titers were determined in sera by ELISA as previously described.42 Anti–dsDNA-IgG and anti–U1-snRNP-IgG antibodies were analyzed by ELISA as previously described.11

Flow Cytometry
Cells were stained with fluorochrome-labeled antibodies directed against CD45, CD3, CD4, CD8, γδTCR, NK1.1, IL-17A, IL-17F, IL-22, IFN-γ, CD11b, and Ly6G (BioLegend, BD Biosciences, eBio-science, or R&D Systems) as previously described.39 Flow cytometry measurements were performed using the BD FACS LSR II. Data were analyzed by using the FlowJo software (Tree Star).

Neutralization Experiments
The animals were treated with 400 μg of a neutralizing mouse anti-murine IL-17A antibody (clone MM17F3; BioCell). The antibody was injected intraperitoneally on days 2, 4, and 8 after induction of NTN, as described.22

BM Transplantation
BM transplantation was performed as previously described.12 Briefly, age-matched IL-17C−/− mice and C57BL/6J wild-type mice received 9.5 Gy total body irradiation. Each recipient mouse (IL-17C−/− mice or wild-type) obtained 3×10^6 BM cells (IL-17C−/− or wild-type cells) by intravenous injection. NTN was induced 2 weeks after irradiation and BM transplantation.

Cell Transfer in Rag1−/− Mice
CD4+ cells were isolated by magnetic-activated cell sorting with the Mouse CD4+ Cell Isolation Kit II (Miltenyi Biotec). We intravenously injected 1.5×10^6 cells into Rag1−/− mice. NTN was induced 6 days after cell transfer.

Statistical Analyses
The results are shown as the mean±SEM when presented as a bar graph or as single data points with the mean in a scatter dot plot. Differences between two individual experimental groups were compared using a two-tailed t test. For survival analysis the Kaplan–Meier plot with a log-rank test was used. P<0.05 was considered to be statistically significant.

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DISCLOSURES
None.

REFERENCES


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