Funktion von immunregulatorischen Zytokinen in glomerulären Nierenerkrankungen

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1 ZUSAMMENFASSUNG

Neben der Diabetischen Nephropathie und der Arteriellen Hypertonie sind die Glomerulonephritiden (GN) eine der Hauptursachen von chronischen Nierenerkrankungen. Durch die Infiltration von verschiedenen Leukozyten-Subtypen kommt es zu einer irreversiblen Gewebeschädigung in zentralen Kompartimenten der Niere, die schließlich zum Funktionsverlust des Organs führt.

In einer der hier vorliegenden Arbeiten wurde die Funktion des immunregulatorischen und gewebsregenerierenden Zytokins Interleukin (IL)-22 in Mausmodellen der Glomerulonephritis untersucht. Es konnte erstmals die Expression des IL-22 Rezeptors im Glomerulum nachgewiesen werden und im Weiteren belegen die Daten einen frühen Anstieg der Expression von IL-22 in der Niere nach Induktion der experimentellen GN. Die Studie veranschaulicht, dass γδ T-Zellen die Hauptproduzenten von IL-22 in der frühen Phase der Immunantwort darstellen, während IL-17-produzierende $CD4^+$ T-Helferzellen zum späteren Zeitpunkt der Erkrankung die IL-22 Produktion übernehmen. Funktionelle Untersuchungen in IL-22-defizienten Mäusen und nach antikörpervermittelter IL-22 Blockade wiesen jedoch keine spezifische Funktion des endogenen IL-22 in den zwei untersuchten Mausmodellen der Glomerulonephritis nach. Im Hinblick auf eine mögliche Anwendung von IL-22-Therapiestrategien in anderen Erkrankungen beim Menschen wie z. B. Atopischer Dermatitis, können anhand dieser Daten Interferenzen oder Nebenwirkungen in der Niere ausgeschlossen werden.

In Autoimmunerkrankungen und chronischen Entzündungen können, neben den $CD4^+$ T-Helferzellen, Zellpopulationen des angeborenen Immunsystems eine entscheidende Rolle bei der Entstehung und der Regulation von Immunantworten spielen. Die neuentdeckte Population der Innate Lymphoid Cells (ILCs) stellt aufgrund ihrer immunregulatorischen Eigenschaften in diesem Zusammenhang eine interessante Zellpopulation dar. Durch die charakteristische Expression von Typ-2-Zytokinen besitzen insbesondere die Typ-2-ILCs (ILC2s) eine wichtige gewebsregenerierende Funktion.

In zwei weiteren hier vorgelegten Publikationen wurden die ILCs in der humanen und murinen Niere erstmalig charakterisiert und funktionell im Mausmodell der Glomerulonephritis untersucht. Es zeigte sich, dass ILC2s die größte Population der ILCs in der gesunden humanen und murinen Niere ausmachen. Nach Applikation des ILC2-aktiverenden Zytokins IL-33 konnte zum ersten Mal eine Verminderung des immunvermittelten Gewebeschadens im Mausmodell der rapid progressiven GN nachgewiesen werden. In der zweiten Studie wurde eine IFN-γ- und IL-27-abhängige Inhibition der ILC2s in der Niere im Mausmodell des Lupus erythematodes (SLE), einer systemischen Autoimmunerkrankung mit starker Beteiligung der Niere, beobachtet. Hier führte die Applikation von IL-33 zu einer erhöhten Produktion der von ILC2s gebildeten Effektorzytokine und zu einer verminderten Mortalität der erkrankten Tiere.

Beide Studien deuten auf eine entscheidende protektive Rolle von ILC2s bei der Entstehung und Progression des glomerulären Schadens sowie bei der Modulation der lokalen Immunantwort in der Niere hin. Zukünftige Untersuchungen werden zeigen, ob und wie das vielversprechende therapeutische Potential der ILC2s bei der Entstehung von Autoimmunerkrankungen und chronischen Entzündungen im Menschen als wirkungsvolle Therapie genutzt werden kann.

2 SUMMARY

In addition to diabetic nephropathy and arterial hypertension, glomerulonephritis (GN) is one of the major causes of chronic kidney diseases. The infiltration of various leukocyte subsets induces an irreversible tissue damage in pivotal compartments of the kidney which consequently leads to a functional loss of the organ.

The first study presented in this work, investigated the function of the immune-regulatory and tissue-regenerative cytokine IL-22 in mouse models of glomerulonephritis. For the first time it could be shown, that the IL-22 receptor is expressed in the glomerulum. In the course of experimental glomerulonephritis, the data show an early upregulation of IL-22 in the kidney. The study identifies γδ T cells as an early innate source of IL-22 whereas IL-17-producing $CD4^+$ T cells induce IL-22 expression at later time points of the disease. Functional analyses in IL-22-deficient mice and after antibody-mediated blockade of IL-22 no specific function of the endogenous IL-22 in two analyzed mouse models for glomerulonephritis was found. Concerning prospective IL-22 therapies in other human diseases such as atopic dermatitis, possible side effects in the kidney can be excluded.

In addition to $CD4^+$ T cells, other cell populations of the innate immune system play a key role in the development and regulation of immune responses in autoimmune diseases and chronic inflammation. In this context, the recently discovered cell population of Innate Lymphoid Cells (ILCs) represents due to their immune-regulatory function an interesting cell population. In particular type 2 ILCs (ILC2s) are of specific research interest, because their characteristic type 2 cytokine expression allows ILC2s to orchestrate tissue regeneration at barrier organs.
In two further publications presented in this work, the cell population of ILCs has been characterised for the first time in the kidney of mice and men and the function of ILCs in the mouse model of glomerulonephritis has been investigated. The results identified ILC2s as the major ILC population in the human and murine kidney. After administration of an ILC2-activating cytokine IL-33, a reduction of immune-mediated renal tissue damage in the mouse model for rapid progressive GN could be observed

for the first time. In the second study, IFN-γ- and IL-27-dependent inhibition of ILC2s in the kidney in the mouse model for systemic lupus erythematodes (SLE), a systemic autoimmune disease with manifestation in the kidney, was observed. Administration of IL-33 resulted in an increased production of ILC2-dependent effector cytokines and reduced mortality in diseased animals.

Both studies illustrate a pivotal protective role of ILC2s in the development and progression of glomerular injury and modulation of local immune responses in the kidney. Future investigations will show, whether the promising therapeutic potential of ILC2s in the establishment of autoimmune diseases and chronic inflammation in humans can be exploited to develop new effective therapies.

3 EINLEITUNG

3.1 Chronische Nierenerkrankungen

Chronische Nierenerkrankungen betreffen rund 10 % der in der westlichen, industrialisierten Welt lebenden Menschen. Im Gegensatz zu akuten Nierenerkrankungen, wird die Niere bei Patienten mit chronischen Nierenerkrankungen über Monate bis hin zu Jahren irreversibel geschädigt, was in vielen Fällen in einer terminalen Niereninsuffizienz mündet. Nierenersatztherapien wie z. B. Dialyse oder Nierentransplantationen sind dann zwingend erforderlich, gehen jedoch mit einer hohen Morbidität und Mortalität der betroffenen Patienten einher. Hinzu kommt, dass mit zunehmender Abnahme der Nierenfunktion das Risiko für kardiovaskuläre Erkrankungen drastisch ansteigt.[1,2]

3.1.1 Glomerulonephritiden

Neben Diabetes Mellitus und Arterieller Hypertonie, gehören die Glomerulonephritiden (GN) zu den häufigsten Ursachen von chronischen Nierenerkrankungen. Unter dem Begriff der Glomerulonephritiden sind eine Vielzahl von immunvermittelten Erkrankungen zusammengefasst, die zu einer abakteriellen Entzündung im Glomerulum, der Filtrationseinheit der Niere, und anderen Kompartimenten des selbigen Organs führen. Man unterscheidet zwischen einer primären, auf die Niere begrenzten Glomerulonephritis und einer sekundären, aus einer systemischen Grunderkrankung hervorgehenden Glomerulonephritis.[3]
Die verschiedenen Formen der Glomerulonephritis unterscheiden sich in erster Linie durch die Position des im Glomerulum induzierten Schadens. Hier können entweder die Endothelzellen, die Mesangialzellen oder die für die Filtrationsbarriere essentiellen Podozyten betroffen sein (Abbildung 1). Auch der klinische Verlauf der Glomerulonephritis variiert stark und kann von einer asymptomatischen Hämaturie (Blutausscheidungen im Urin) bis hin zur Entwicklung eines nephrotischen Syndroms, das durch eine hohe Proteinurie, Ödeme und eine Hyperlipoproteinämie (erhöhte

Ansammlung von Lipoproteinen und ihrer transportierten Lipide bspw. Cholesterin, Triglyzeride im Blut) charakterisiert ist, gekennzeichnet sein.
Unabhängig von dem zugrundeliegenden Pathomechanismus, weisen alle Formen der Glomerulonephritis eine gestörte Immunantwort auf, die zu einer lokalen Inflammation im Glomerulum führt und mit einer finalen Schädigung der Filtrationseinheit der Niere einhergeht.[3]

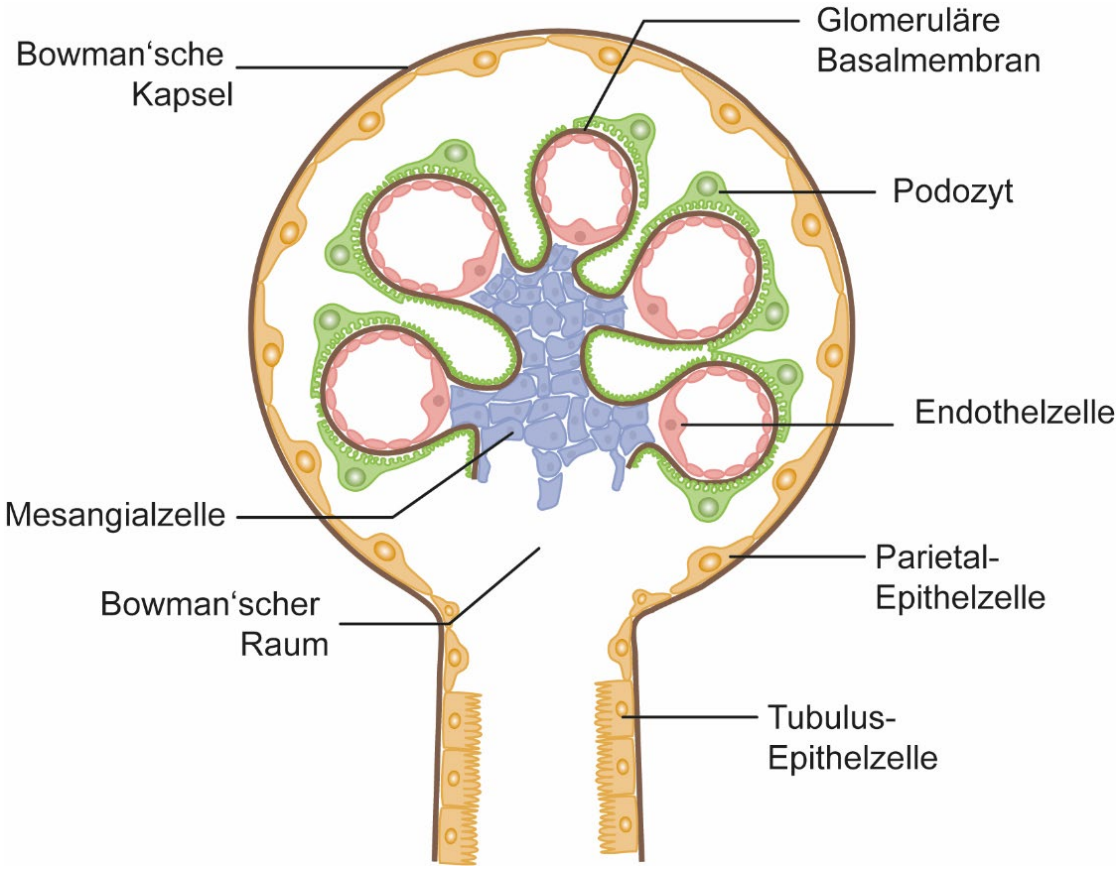

Abbildung 1: Das hier dargestellte Glomerulum ist die Filtrationseinheit der Niere und hat die Aufgabe das Blut zu filtrieren und den Primärharn zu produzieren. Die Glomeruli sind in der Nierenrinde (Cortex) lokalisiert und von der Bowman'schen Kapsel umschlossen, die von einer Schicht aus Parietal-Epithelzellen überzogen ist. Die glomeruläre Filtrationsbarriere besteht aus Endothelzellen, der glomerulären Basalmembran (GBM) und viszeralen Epithelzellen, auch Podozyten genannt. Mesangialzellen befinden sich zwischen den glomerulären Zellen und geben dem Kapillarbündel seine Struktur. Moleküle, die kleiner als 68 kDa sind können die GBM passieren und durchwandern anschließend das renale Tubulussystem.

Die rapid progressive Glomerulonephritis (RPGN), auch crescentic Glomerulonephritis genannt, ist die aggressivste und schlecht prognostizierteste Form der immunvermittelten Nierenerkrankungen und kann unbehandelt binnen Tagen bis Wochen zum terminalen Nierenversagen führen. Bei der RPGN verursachen eine Ablagerung von Antikörpern und/oder durch Immunzellen fehlgeleitete Entzündungsprozesse einen irreversiblen Schaden im Glomerulum.[4]
Die RPGN wird aufgrund unterschiedlicher histologischer Merkmale in drei Subklassen unterteilt: Bei der schwersten, aber seltensten Form der RPGN werden Auto-Antikörper gegen das Typ-IV-Kollagen gebildet, das Teil der glomerulären Basalmembran (GBM) ist. Diese Membran stellt die Filtrationsbarriere für das Blut dar und ist somit essentiell für die Funktion der Niere. Die zweite Subklasse der RPGN zeichnet sich durch Ablagerung von Immunkomplexen im Glomerulum aus und kommt häufig als Begleiterscheinung von systemischen Erkrankungen wie z. B. dem systemischen Lupus erythematodes (SLE) vor. Zu der dritten und häufigsten Subklasse gehört die pauci-immun fokal-segmental nekrotisierende Glomerulonephritis. Diese Subklasse tritt oft in Verbindung mit einer Anti-Neutrophilen zytoplasmatischen Antikörper (ANCA)-assoziierten Vaskulitis auf.[5] Für diese Erkrankung ist die Bildung von Auto-Antikörpern charakteristisch, die gegen zytoplasmatische Proteine in Neutrophilen und Monozyten gerichtet sind. Nach Präsentation dieser Antigene auf der Oberfläche der Zellen aktivieren ANCA die Neutrophile und induzieren die Freisetzung von Reaktiven Oxygen Spezies (ROS) und lytischen Enzymen, die dann die Endothelzellen schädigen.[4-6]

In allen Formen der RPGN kommt es zu einer Ruptur der glomerulären Basalmembran, die eine Infiltration von zirkulierenden Leukozyten in die Bowman'sche Kapsel zur Folge hat. Im weiteren Verlauf beginnen die Parietal-Epithelzellen stark zu proliferieren und es kommt zur Bildung einer fibrinösen Struktur, deren Form einem Halbmond ähnelt (engl. crescent) (Abbildung 2). Schlussendlich sorgt eine vollständige Sklerose der Glomeruli für einen Funktionsverlust der Niere.[7]

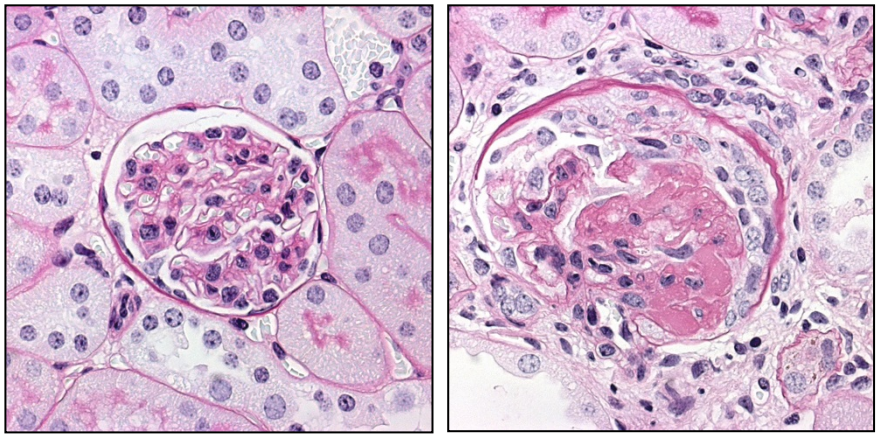

Abbildung 2: Die histologische Färbung (Periodsäure-Schiff'sche-Färbung) von in Paraffin eingebettetem Nierengewebe zeigt ein gesundes Glomerulum (links) und ein Glomerulum mit der typischen pathologischen Halbmond-Struktur (crescent) an Tag zehn nach Induktion der experimentellen GN in C57BL/6 Mäusen.

Die der rapid progressiven Glomerulonephritis zugrundeliegenden pathophysiologischen Mechanismen sind allerdings nur unzureichend verstanden. Die heutzutage angewendeten Therapien beruhen auf unspezifischen immunsuppressiven Medikamenten, die mit hohen Nebenwirkungen einhergehen und nur partiell effektiv sind. Aus diesem Grund ist es unbedingt notwendig durch ein wachsendes Verständnis der einzelnen Grunderkrankungen neue zielgerichtete und wirkungsvolle Medikamente zu entwickeln, mit denen die Nierenschädigung aufgehalten oder sogar geheilt werden kann.[4]

3.2 Lymphozyten und ihre Rolle in der RPGN

Die Infiltration von Effektorlymphozyten in die Niere ist entscheidend für die Entstehung von Glomerulonephritiden.[8]

Lymphozyten werden entsprechend ihrer Zugehörigkeit zur angeborenen bzw. adaptiven Immunantwort eingeteilt. Neben den bekannten Hauptakteuren des adaptiven Immunsystems, den $CD4^+$ T-Helferlymphozyten und zytotoxischen $CD8^+$ T-Lymphozyten, gibt es andere T-Zellpopulationen, die Eigenschaften von beiden Armen des Immunsystems vereinen. Zu dieser Gruppe von Zellen gehören die γδ T-Zellen, die Natürlichen Killer (NK) T-Zellen und die Mucosa-assoziierten invarianten T (MAIT)-Zellen, die unter dem Namen innate-like T-Lymphozyten zusammengefasst werden (Abbildung 3). Im Gegensatz zu den konventionellen T-Lymphozyten, die nach Erstkontakt mit einem Antigen und anschließender Reifung im Lymphknoten hochspezifisch auf nur ein Antigen reagieren können, besitzen die innate-like T-Zellen einen T-Zellrezeptor mit nur eingeschränkter Spezifität. Dies erlaubt ihnen mehrere molekulare Strukturen des gleichen Musters zu erkennen und eine schnelle Immunantwort zu initiieren. Die innate-like T-Zell-aktivierenden Strukturen sind entweder aus körpereigenen Stoffwechselprozessen oder durch Phagozytose und Abbau von körperfremden Mikroorganismen entstanden. Hierzu gehören phosphorylierte Stoffwechselprodukte (γδ T-Zellen), Lipide (NK T-Zellen) und der Vitamin B2-Metabolit Riboflavin (MAIT-Zellen).[9,10]

Neben den innate-like und adaptiven T-Lymphozyten, gibt es Lymphozyten des angeborenen Immunsystems, die sich in ihrer Wirkungsweise grundlegend von den anderen Zellgruppen unterscheiden. Diese Zellen zeichnen sich durch das Fehlen des T-Zellrezeptors aus. Zu dieser Zellgruppe gehören die konventionellen Natürlichen Killer (NK) Zellen und die Familie der erst kürzlich entdeckten sogenannten Innate Lymphoid Cells (ILCs) (Abbildung 3).[9]

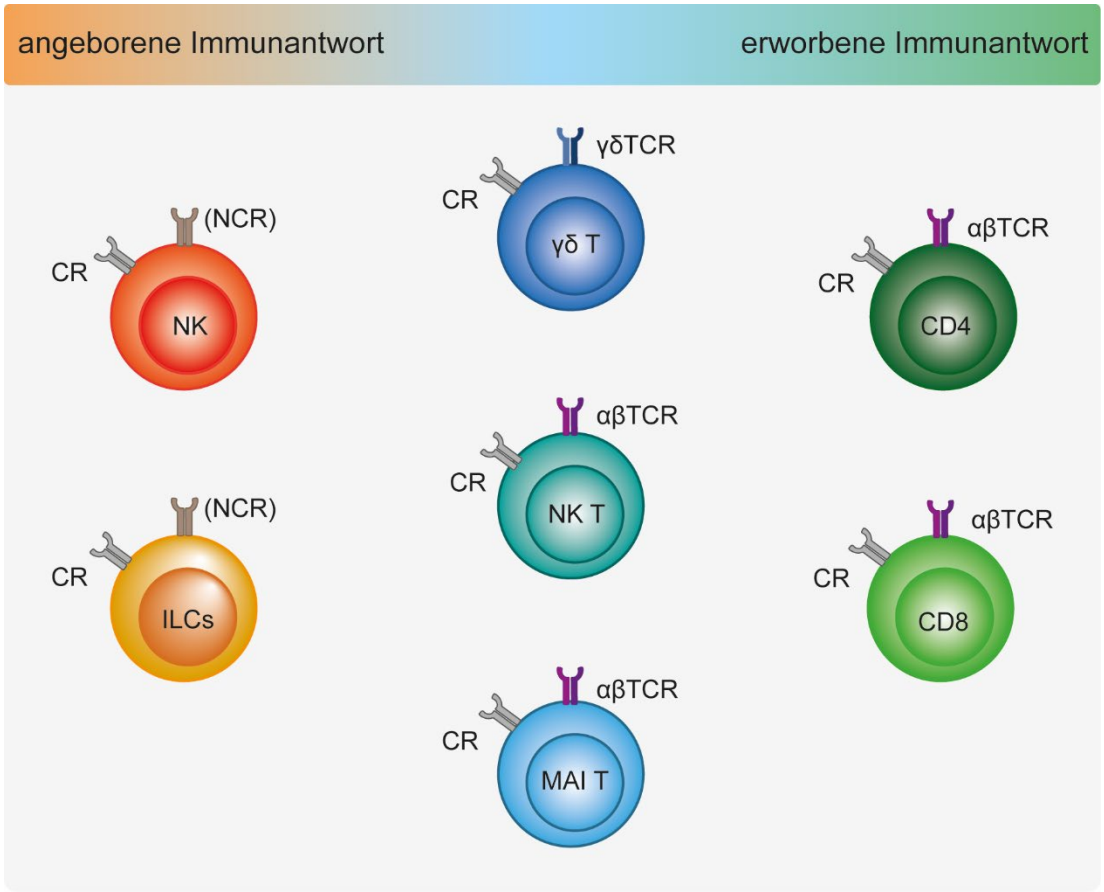

Abbildung 3: Übersicht über die adaptiven, innate-like und angeborenen Lymphozyten. Konventionelle CD4⁺ und CD8⁺ T-Lymphozyten werden durch die Bindung ihres T-Zellrezeptors mit dem beladenen MHC-Komplex auf antigenpräsentierenden Zellen und über co-stimulatorische Moleküle und Zytokine aktiviert. γδ T-Zellen, NK T-Zellen und MAIT-Zellen als innate-like T-Zellen werden entweder durch Zytokinsignale oder über ihren T-Zellrezeptor mit eingeschränkter Spezifität aktiviert. Lymphozyten des angeborenen Immunsystems agieren T-Zellrezeptor-unabhängig und werden meistens durch Zytokine aktiviert, seltener durch Natürliche Killer Rezeptoren (NCR). (CR = Zytokinrezeptor).

Lange Jahre herrschte das Paradigma, dass B-Lymphozyten und ihre sekretierten Antikörper den glomerulären Schaden in chronischen Nierenerkrankungen vermitteln.[11] Erst die Entwicklung von geeigneten Tiermodellen und die anschließende funktionelle Untersuchung von einzelnen Zellgruppen trug entscheidend zum Verständnis über die Entstehung und Progression der Glomerulonephritis bei.[8] Eines der am häufigsten verwendeten Mausmodelle für die humane crescentic Glomerulonephritis ist das sogenannte Nephrotoxische Nephritis (NTN)-Modell. Hier werden Mäusen aus dem Schaf oder dem Kaninchen stammende heterologe

Antikörper systemisch injiziert, die spezifisch an die glomeruläre Basalmembran binden und so eine Entzündung in der Niere auslösen.[12] Obwohl dieses Modell aufgrund des xenogenen anti-GBM-Antikörpers keine reale Autoimmunerkrankung darstellt, ähnelt die Pathologie dieses Modells der crescentic Glomerulonephritis im Menschen und ist somit ein wertvolles Hilfsmittel für die Untersuchungen der immunvermittelten Prozesse in der Niere.[4]
In einem anderen Mausmodell führt die Applikation von Adriamycin, einem Zytostatikum, zu einem spezifischen Podozytenschaden, wodurch es zu einem Verlust der Filtrationsbarriere und damit zu einer starken Proteinurie, einer progressiven Glomerulosklerose und einer tubulointerstitiellen Fibrose kommt. Diese Adriamycin-Nephropathie ist ein beliebtes Modell für die Untersuchung der humanen fokal-segmentalen Glomerulosklerose, einer hoch proteinurischen Form der Glomerulonephritis.[13]

Holdsworth und Kollegen lieferten den ersten Beweis über einen T-Lymphozyten-vermittelten Schaden in der GN. In der experimentellen RPGN konnten sie eindrücklich zeigen, dass antigenspezifische $CD4^+$ und $CD8^+$ T-Lymphozyten eine fehlgeleitete Immunantwort auslösen, die durch die Infiltration von Makrophagen und Neutrophilen potenziert wird und zu einem glomerulären Schaden führt.[8,14]
Mit der Entdeckung einer neuen $CD4^+$ T-Zellpopulation änderte sich die Sichtweise auf die der RPGN zugrundeliegenden Pathomechanismen maßgeblich. Die im Jahr 2005 neuentdeckten T_H17-Zellen werden durch das Zytokin Interleukin (IL)-23 aktiviert und sekretieren eine Reihe von immunregulatorischen Zytokinen wie z. B. IL-17A, IL-22 und Tumornekrose Faktor-α (TNF-α). Den ersten Nachweis über eine funktionelle Beteiligung von IL-17A an renalen Entzündungsmechanismen lieferte 2009 eine Studie von Paust und Turner *et al.*. Hier konnte belegt werden, dass Mäuse mit einer IL-17A- oder IL-23-Defizienz nach Induktion der experimentellen RPGN einen verminderten glomerulären Schaden und eine verbesserte Nierenfunktion im Vergleich zu den Wildtyp-Tieren aufwiesen. Die Tiere zeigten eine unveränderte T_H1-Immunantwort was darauf hindeutet, dass in Abwesenheit von IL-17 die T_H1-abhängige Entzündungsreaktion nicht ausreicht, um eine Gewebeschädigung zu initiieren.[15]
Im Folgenden konnte im gleichen Tiermodell mit Hilfe von durchflusszytometrischen Analysen anschaulich gezeigt werden, dass in der frühen Phase der experimentellen

Glomerulonephritis innate-like γδ T-Zellen die Hauptproduzenten von IL-17 sind und somit maßgeblich zur Initiation der pathogenen Immunreaktion beitragen.[16]

Inwieweit neben den adaptiven und innate-like T-Lymphozyten, Lymphozyten des angeborenen Immunsystems und die von ihnen produzierten immunregulatorischen Zytokine an der Pathogenese der immunvermittelten glomerulären Nierenerkrankungen beteiligt sind, ist Gegenstand der hier vorliegenden Untersuchungen.

3.2.1 IL-22 als immunregulatorisches Zytokin

Zytokine spielen bei der Entstehung und Aufrechterhaltung von immunvermittelten lokalen Entzündungsreaktionen eine essentielle Rolle. Das Zytokin IL-22 stellt aufgrund seiner immunregulatorischen Funktion einen interessanten und vielseitigen Immunmediator dar.[17,18]
IL-22 spielt sowohl in der angeborenen als auch in erworbenen Immunantwort eine entscheidende Rolle und wird von verschiedenen Leukozyten-Subtypen exprimiert. Kurz nach der Entdeckung wurde IL-22 häufig in IL-17-produzierenden $CD4^+$ T-Helferzellen nachgewiesen, weswegen das Zytokin initial zur Familie der T_H17-Zytokine gezählt wurde. Heute ist aber bekannt, dass IL-22 unabhängig von IL-17 von verschiedenen Zelltypen sekretiert werden kann.[17] Neben $CD4^+$ T-Helferzellen als Hauptproduzenten von IL-22, sekretieren γδ T-Zellen, NK T-Zellen und Typ-3-ILCs ebenso IL-22. Nur wenige Studien wiesen eine IL-22 Produktion in Dendritischen Zellen nach.[17]
IL-22 unterscheidet sich besonders in der Wirkungsweise von anderen klassischen Zytokinen. Der IL-22 Rezeptor ist nicht auf anderen Immunzellen,[19] sondern fast ausschließlich auf Epithelzellen der Haut, in Geweben des Verdauungstraktes und der Atemwege, in Zellen der Leber, der Niere und den Gelenken exprimiert.[17,18] Diese Tatsache erlaubt IL-22 eine direkte Wirkung auf nicht-hämatopoietische Zellen an Grenzflächen des Körpers und ist dort in erster Linie am Erhalt der Gewebeintegrität und Pathogenabwehr beteiligt.[18,20] Durch die Induktion von anti-mikrobiellen

Peptiden, Chemokinen sowie Mucus-bildenden Molekülen in Epithelzellen sorgt IL-22 für die Gewebeimmunität an natürlichen Barrieren des Körpers. IL-22 vermittelt diese schützenden Effekte in Synergie mit anderen Zytokinen wie zum Beispiel IL-17A, TNF-α oder IL-1β.[21] Zwei Studien konnten anschaulich zeigen, dass IL-22 essentiell für die Abwehr gegen extrazelluläre gram-negative Bakterien wie *Klebsiella pneumoniae* in der Lunge[22] und *Citrobacter rodentium* im Darm ist.[23,21]
Zusätzlich zur Pathogenabwehr besitzt IL-22 eine wichtige gewebsregenerative Funktion. Umfassende Studien belegen, dass IL-22 durch die Induktion von anti-apoptotischen Genen und Aktivierung von proliferativen Zellzyklusmechanismen die Epithelzellen der Haut, der Leber, des Darms, des Thymus sowie der Lunge regenerieren kann.[17,20]
Neben den erwähnten protektiven Funktionen, zeichnet sich IL-22 durch pathogene Eigenschaften aus (Abbildung 4).[24] Durch die Induktion von insbesondere pro-inflammatorischen Genen trägt IL-22 entscheidend zur Initiierung von lokalen oder systemischen Entzündungsreaktionen bei.[21] Dieser Anstieg der IL-22-IL-22R-Achse ist mit vielen humanen Erkrankungen assoziiert und in Mausmodellen für Autoimmunerkrankungen, Entzündungen und Krebs konnte für IL-22 eine pathogene Rolle nachgewiesen werden.[25]
Es scheint paradox, dass IL-22 sowohl eine protektive als auch eine inflammatorische Rolle besitzt.[17,21] Eingehenden Studien zu Folge hängt es von der molekularen Umgebung und dem Zytokinmilieu ab, ob IL-22 eine protektive oder pathogene Rolle im Gewebe übernimmt.[21]
IL-22 kann durch die Aktivierung von immunregulatorischen Prozessen schwerwiegende Veränderungen im Gewebe induzieren. Aus dem Gesichtspunkt erscheint es sinnvoll, dass ein natürlicher Inhibitor, das IL-22 Bindungsprotein (IL-22BP), in der Lage ist die IL-22-abhängigen Prozesse zu regulieren und falls nötig eine Hyperaktivierung von IL-22 zu limitieren. IL-22BP stellt die lösliche Form der IL-22 Rezeptor 1 Untereinheit dar und wird von Dendritischen Zellen und wie kürzlich nachgewiesen von $CD4^+$ T-Zellen sekretiert.[17,26] Diese endogene Regulation von IL-22 verleiht der IL-22-Signalkaskade ein zusätzliches Maß an Komplexität.

Zusammenfassend ist das immunregulatorische Zytokin IL-22 an einer Vielzahl von Prozessen beteiligt, die abhängig vom Zytokinmilieu entweder gewebsprotektiv oder pathogen auf Epithelzellen wirken. Besonders aufgrund der regenerativen

Mechanismen im Gewebe besitzt IL-22 ein großes therapeutisches Potential bei chronischen Entzündungsreaktionen und bei der Gewebereparatur. Um diese Therapiestrategien zu etablieren sind jedoch weitere Untersuchungen der IL-22-vermittelten molekularen Mechanismen und der Interaktion von IL-22 mit anderen Immunzellen und Zytokinen notwendig.[17,18,20]

Welche Rolle IL-22 im immunvermittelten glomerulären Schaden spielt und ob es bei chronischen Nierenentzündungen ein therapeutisches Potential besitzt, ist das zentrale Thema der vorliegenden Arbeit.

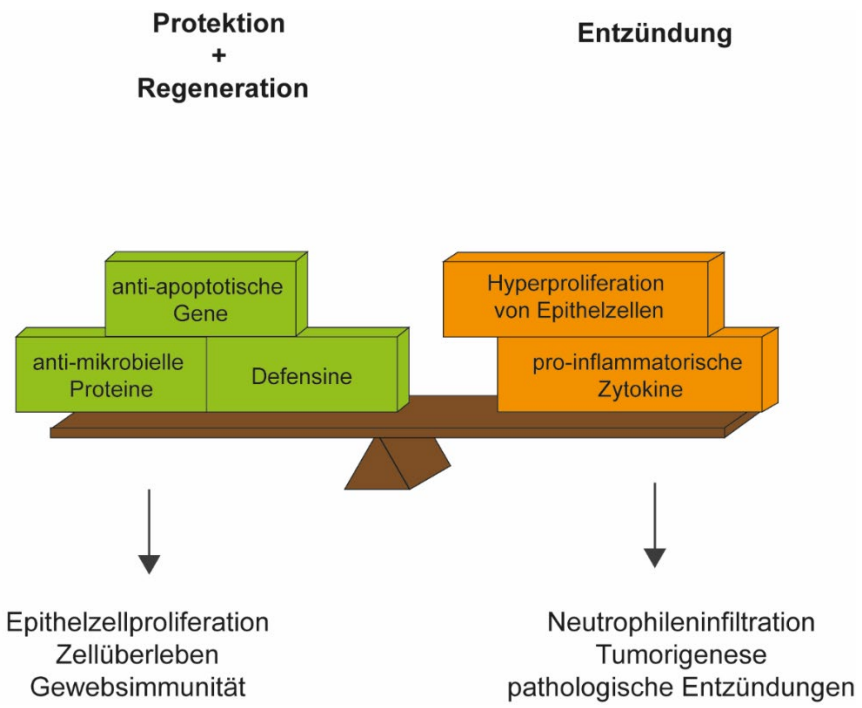

Abbildung 4: Die Rolle von IL-22 als zweischneidiges Schwert. IL-22 kann entweder durch die Induktion von anti-apoptotischen Genen, Mucus-bildenden Proteinen oder Defensinen die Abwehr gegen exogene Pathogene an Grenzflächen des Körpers vermitteln. Zusätzlich induziert IL-22 die Proliferation von Epithelzellen und sorgt so für den Erhalt der Gewebeintegrität. Dem gegenüber steht die pathogene Rolle von IL-22, denn in Synergie mit anderen pro-inflammatorischen Zytokinen kann IL-22 Entzündungsreaktionen induzieren. Welche Funktion IL-22 ausführt, hängt vom umliegenden Zytokinmilieu ab.

3.2.2 ILCs und ihre Funktion in Autoimmunerkrankungen

Der zunehmende Fokus der immunologischen Forschung auf die zellulären Mechanismen des angeborenen Immunsystems im letzten Jahrzehnt machte erst die Entdeckung einer neuen Zellpopulation möglich. Neben den schon lange bekannten NK-Zellen gehören die ILCs zu den Lymphozyten des angeborenen Immunsystems.[27,28] Entsprechend ihrer Zytokinproduktion und ihrer Transkriptionsfaktoren werden sie in drei helper-like ILC-Subtypen, die ILC1s, ILC2s und ILC3s, eingeteilt. Nach der aktuellsten Nomenklatur werden die NK-Zellen aufgrund ihrer zytotoxischen Eigenschaft ebenso zu den ILCs gezählt.[29]
Die Zytokinproduktion und Transkriptionsmuster der NK-Zellen und der helper-like ILCs sind den T-Lymphozyten des erworbenen Immunsystems sehr ähnlich und repräsentieren das Pendant des angeborenen Immunsystems.[30]
NK-Zellen stellen den Gegenpart der zytotoxischen $CD8^+$ T-Lymphozyten dar, während die ILC1s die T_H1-Zellen, die ILC2s die T_H2-Zellen und die ILC3s die T_H17-Zellen des adaptiven Immunsystems widerspiegeln.[27] In Abbildung 5 sind die bisherigen Erkenntnisse über die Aktivierung der ILC-Subtypen und ihre Effektorfunktionen schematisch dargestellt.

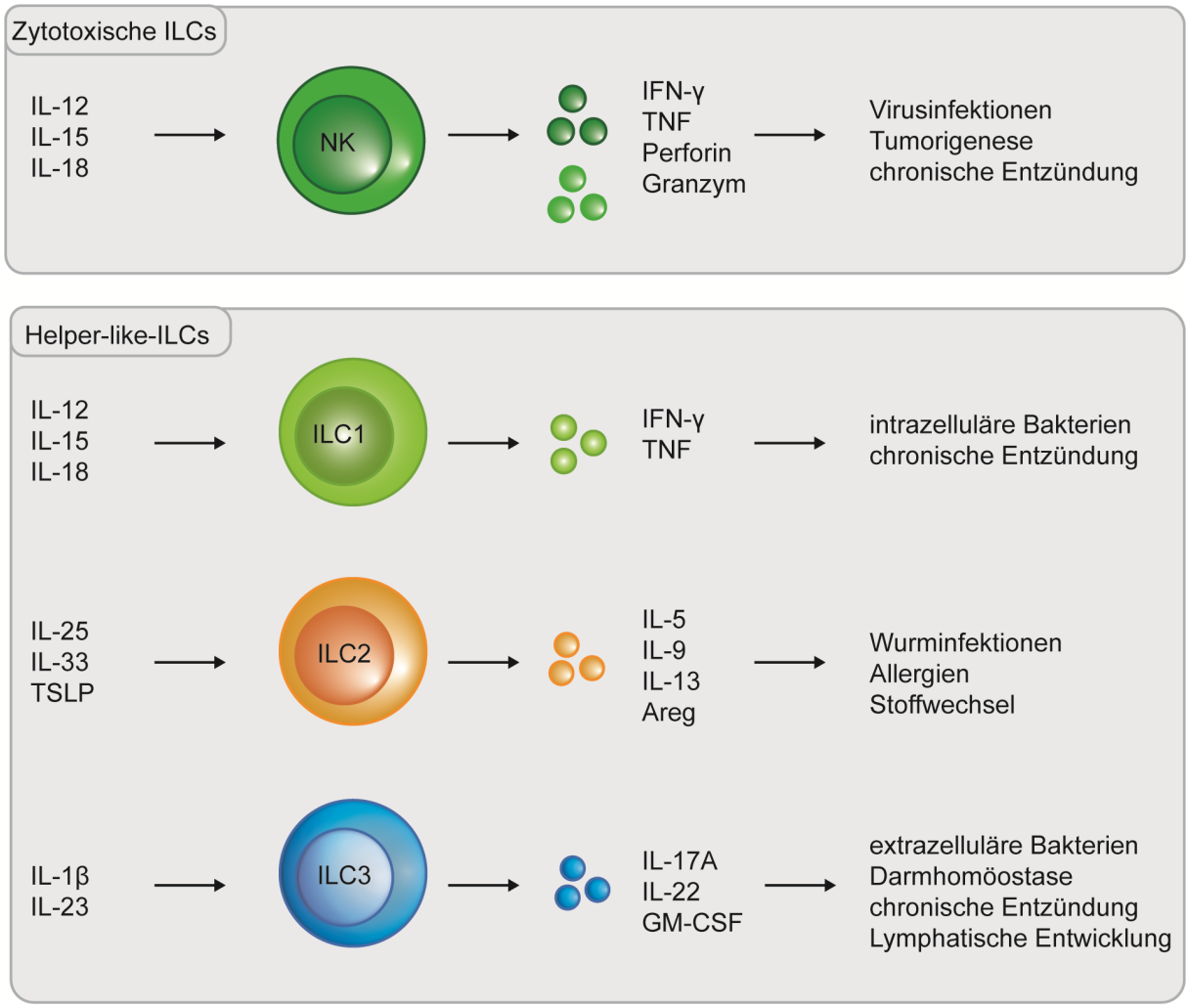

Abbildung 5: ILCs werden entsprechend ihrer Funktion in zytotoxische ILCs und helper-like-ILCs eingeteilt. NK-Zellen sekretieren neben IFN-γ und TNFs Perforin und Granzym und gehören daher zu den zytotoxischen ILCs. Helper-like Typ-1, 2 und 3 ILCs können durch eine Vielzahl von unterschiedlichen Alarminen und Zytokinen aktiviert und damit zur Zytokinproduktion angeregt werden und tragen so zur Pathogenabwehr und Homöostase in vielen Geweben bei. Fehlregulierte ILC-Immunantworten können aber auch zu chronischen Entzündungen und Krebs führen. (Areg = Amphiregulin; GM-CSF = Granulozyten-Makrophagen-Koloniestimulierender Faktor; NCR = Natürlicher Zytotoxizitätsrezeptor; TNF = Tumornekrose Faktor; TSLP = Thymisch-stromales Lymphopoietin).

ILCs haben sich evolutionär entwickelt, um eine schnelle Immunantwort auf Pathogene oder andere exogene Stimuli zu induzieren. Aufgrund der Tatsache, dass ILCs zum größten Teil gewebsständige Zellen sind, kann eine frühe Aktivierung der ILCs, die Zeit der antigenabhängigen Reifung von T-Lymphozyten und Wanderung zwischen Lymphknoten und Ort der Entzündung bzw. Infektion überbrücken.[31]

Besonders häufig sind ILCs an den natürlichen Barrieren des Körpers wie z. B. der Haut, der Lunge und dem Darm zu finden und werden dort antigenunabhängig über ihre Zytokinrezeptoren aktiviert.[32] Myeloide Zellen oder Epithelzellen stellen dabei die Quelle der ILC-aktivierenden Alarmine und Zytokine dar.[33]

ILCs vermitteln ihre immunregulatorische Funktion in erster Linie über die Sekretion von Zytokinen und sorgen so für die Initialisierung einer Immunantwort.[34] Neuen Erkenntnissen zufolge sind ILCs nicht nur in der Lage durch die frühe Aktivierung und Sekretion von Typ-1-, 2- und 3-Zytokinen Immunantworten zu initiieren, sondern ILCs sind interessanterweise auch an der Regulation von T-Lymphozyten und Dendritischen Zellen beteiligt.[35,36] Durch die Expression des major histocompatibility complex (MHC)-II präsentieren ILCs Antigene und induzieren so eine Aktivierung von T-Lymphozyten. Ebenso erlaubt die Expression von co-stimulatorischen Molekülen ILCs mit anderen Immunzellen zu interagieren.[33,37] Zu einem gewissen Teil erhalten sich ILCs durch diese interaktiven Prozesse selbst, da T-Zellen nach Bindung an den MHC II-Komplex das Zytokin IL-2 exprimieren, das wiederum ein essentieller Wachstumsfaktor für ILCs ist.[38]

Die Forschung der letzten Jahre führte zu der Erkenntnis, dass helper-like ILCs eine wichtige regulatorische Funktion in der Gewebehomöostase und der Pathogenabwehr übernehmen.[27] Besonders die Effektorzytokine IL-22 (ILC3s) und der Wachstumsfaktor Amphiregulin (ILC2s) induzieren in Epithelzellen anti-apoptotische Gene und proliferative Prozesse, um die Gewebeintegrität im Darm oder beispielsweise der Lunge nach Infektionen aufrechtzuerhalten.[38]
Demgegenüber steht die pathogene Rolle der ILCs, denn sie tragen durch ihre Aktivierung maßgeblich zur Entstehung von Autoimmunerkrankungen und chronischen Entzündungen bei. Ähnlich wie adaptive T-Lymphozyten, sind ILC1s und ILC3s durch die Expression von IFN-γ und IL-17A bei Erkrankungen wie der Multiplen Sklerose, bei entzündlichen Darmerkrankungen (IBD), bei der Psoriasis, der Spondyloarthritis, der rheumatoiden Arthritis und weiteren systemischen rheumatoiden Erkrankungen beteiligt. ILC2s hingegen wirken mehrheitlich protektiv, können jedoch abhängig vom zugrundeliegenden molekularen Umfeld auch pathogene Prozesse induzieren. Somit ist eine eindeutige Einteilung der ILCs in pro- und anti-inflammatorische Subtypen nicht möglich.[34]

ILCs stellen aufgrund ihrer entscheidenden Rolle bei der Gewebehomöostase, der Pathogenabwehr und der Entstehung von Autoimmunerkrankungen ein potentielles Ziel neuer Therapiestrategien dar. Einerseits kann die protektive Funktion der ILCs ausgenutzt werden, um beispielsweise die körpereigene Pathogenabwehr zu unterstützen oder die Regeneration von geschädigtem Gewebe zu verbessern. Andererseits können Strategien zur Eindämmung der pathogenen Funktionen von ILCs bspw. durch neutralisierende Antikörper, die ILC-aktivierenden Zytokine oder die Effektorzytokine der ILCs blockieren ein Therapieziel darstellen.[37]
Eine große Schwierigkeit aller Therapieansätze besteht in der Spezifität. Da ILCs als angeborene Gegenspieler der Th1-, Th2- und Th17-Zellen die gleichen Effektorzytokine wie ihre adaptiven Pendants sekretieren, muss ausgeschlossen werden, dass T-Zellantworten durch die Therapie verändert werden.

In der hier vorliegenden Arbeit wurde eingehend untersucht, ob ILCs an immunvermittelten Entzündungsprozessen in der Niere beteiligt sind.

4 FRAGESTELLUNG

Besitzt IL-22 eine immunregulatorische Funktion in immunvermittelten glomerulären Nierenerkrankungen?

Die Expression des IL-22 Rezeptors in der gesunden Niere sowie die zellulären Quellen und Aktivierungswege des Zytokins IL-22 wurden eingehend in der experimentellen RPGN analysiert und identifiziert. Mit Hilfe von gendefizienten Mäusen und einer antikörpervermittelten Blockade wurde die Funktion von IL-22 in Tiermodellen der Glomerulonephritis umfassend untersucht.

Wie hoch ist der residente Anteil an ILCs in der humanen Niere und welche Rolle spielen sie in der experimentellen Glomerulosklerose?

Neu etablierte durchflusszytometrische Methoden und die Weiterentwicklung der Leukozytenisolation aus gesundem Nierengewebe erlaubten eine Charakterisierung der drei ILC-Subklassen in der humanen Niere. Im Adriamycin-induzierten Tiermodell der Glomerulosklerose wurde die Rolle der ILCs im Hinblick auf die Typ-2-ILCs näher untersucht.

Welche Rolle spielen nierenresidente Typ-2-ILCs bei der Progression der autoimmunvermittelten Inflammation der Niere und wie sind sie reguliert?

Nachdem gezeigt wurde, dass Typ 2 ILCs eine regulatorische Funktion bei der Entstehung des glomerulären Schadens übernehmen können, wurde die Rolle von ebendiesen Zellen in der Progression der Nierenmanifestation beim systemischen Lupus erythematodes im Mausmodell eingehend studiert.

5 ERGEBNISSE

Die oben genannten Fragestellungen wurden ausführlich behandelt und die Ergebnisse wurden in drei wissenschaftlichen Publikationen veröffentlicht. Die im Folgenden aufgeführten Publikationen sind Grundlage der vorliegenden kumulativen Dissertationsschrift und befinden sich in voller Länge im Anhang.

Endogenous IL-22 is dispensable for experimental glomerulonephritis.
Gnirck AC, Wunderlich W, Becker M, Xiong T, Weinert E, Meyer-Schwesinger C, Dumoutier L, Renauld JC, Huber S, Panzer U, Turner JE. (2019) **Am J Physiol Renal Physiol** 316(4):F712-F722

T cell-derived IFN-γ downregulates protective group 2 innate lymphoid cells in murine lupus erythematosus.
Düster M, Becker M *, Gnirck AC, Wunderlich W, Panzer U, Turner JE.* (2018) **Eur J Immunol** 48(8):1364-1375 *contributed equally

IL-33-mediated expansion of type 2 innate lymphoid cells protects from progressive glomerulosclerosis.
Riedel JH, Becker M*, Kopp K, Düster M, Brix SR, Meyer-Schwesinger C, Kluth LA, Gnirck AC, Attar M, Krohn S, Fehse B, Stahl RAK, Panzer U, Turner JE.* (2017) **J Am Soc Nephrol** 28(7):2068-2080 *contributed equally

6 DISKUSSION

6.1 Die Funktion von IL-22 in immunvermittelten glomerulären Nierenerkrankungen

In den letzten Jahren ist das Interesse an dem Zytokin IL-22 und seiner Rolle als Immunregulator an Grenzflächen wie z. B. der Haut, des Darms und der Lunge stetig gestiegen.[17] Der Grund dafür liegt in der protektiven Eigenschaft von IL-22 im geschädigten Gewebe anti-apoptotische und anti-mikrobielle Prozesse ebenso wie die Proliferation der Epithelzellen zu induzieren und somit die Integrität der Grenzfläche zu erhalten oder diese zu regenerieren.[17,18] Eben jene Signalwege können jedoch auch zu einer Hyperproliferation der Epithelzellen und Produktion von pro-inflammatorischen Molekülen führen, die mit einer pathogenen Entzündungsreaktion oder einer Tumorgenese einhergehen. Aufgrund dieser ambivalenten Eigenschaften wird IL-22 oft mit einem zweischneidigen Schwert verglichen. Welche Seite des Schwertes in Aktion tritt, bestimmt das Zytokinmilieu und die molekulare Umgebung im Gewebe.[17,18]
In vielen Erkrankungen wie beispielsweise der Psoriasis, der entzündlichen Darmerkrankung (IBD) und der rheumatoiden Arthritis ist die Expression von IL-22 hochreguliert. Häufig bleibt die Frage jedoch ungeklärt, ob der Anstieg der Expression von IL-22 die Ursache für die Erkrankung ist oder in Folge der Entzündungskaskade induziert wird.[21]

IL-22 stellt aufgrund seiner pathogenen und regenerativen Eigenschaften ein potentielles Ziel neuer Therapiestrategien dar.[18] Zurzeit befindet sich ein IL-22-neutralisierender Antikörper für Patienten mit Atopischer Dermatitis in Phase II einer klinischen Studie. In anderen Strategien wird das regenerative Potential von IL-22 durch eine Induktion der IL-22 Expression ausgenutzt, um so eine Geweberegeneration herbeizuführen. Die Entwicklung von spezifischen IL-22 Aktivatoren steht jedoch noch am Anfang und detaillierte Studien sind notwendig, um riskante Nebenwirkungen im Menschen auszuschließen.[39]

Immunvermittelte Schäden sind häufig ein Grund für den Verlust der Filtrationsbarriere der Niere.[3] In diesem Zusammenhang ist die Untersuchung des immunregulatorischen Zytokins IL-22 von großem Interesse. In der Tat wurde eine, im Verhältnis zu anderen Organen, hohe Expression des IL-22 Rezeptors in der humanen gesunden Niere nachgewiesen.[19,40] Dies ist ein erster Hinweis auf eine mögliche regulatorische Rolle von IL-22 in der Niere. Ob IL-22 für einen Erhalt der Nierenfunktion sorgt oder die Schädigung der Niere begünstigt, ist zurzeit Gegenstand von verschiedenen Untersuchungen.

In Tiermodellen des akuten Nierenversagens, eine Erkrankung bei der mehrheitlich die Epithelzellen des tubulären Apparates geschädigt werden, konnten erste Studien zeigen, dass IL-22 in erster Linie auf Tubulusepithelzellen wirkt und dort durch Hemmung der Apoptose die Zellen des Tubulusapparates regeneriert.[41,42] Inwieweit diese Ergebnisse auf die glomerulären immunvermittelten Erkrankungen übertragbar sind, wurde bisher noch nicht näher beleuchtet.[43]

In der hier vorliegenden Publikation wurde die Funktion von IL-22 in der Pathogenese der glomerulären Schädigung in zwei Mausmodellen umfassend untersucht. Im Mausmodell der RPGN (NTN-Modell) wurde ein schneller Anstieg der IL-22 Expression in der Nierenrinde zwölf Stunden nach Induktion der Erkrankung beobachtet. Nach einem kurzzeitigen Abfall der IL-22 Expression stieg diese an Tag Sieben der crescentic GN wieder an. Diese Daten belegen eine sehr frühe Aktivierung von IL-22, die auf eine Beteiligung von IL-22 in der angeborenen Immunantwort hinweist. Ähnliche Beobachtungen wurden in Tiermodellen des akuten Nierenversagens veröffentlicht und bestätigen diese Erkenntnisse.[41,43]

Die zelluläre Quelle von IL-22 kann abhängig vom Organ und dem Kontext der Erkrankung stark variieren.[17] Die Untersuchungen der vorliegenden Studie zeigten, dass in der experimentellen crescentic GN γδ T-Zellen in der Niere schon nach zwölf Stunden eine hohe Menge an IL-22 sekretieren. An Tag Sieben übernehmen die $CD4^+$ T-Zellen die Produktion von IL-22 in der Niere.
Diese Beobachtung geht mit den Ergebnissen einer Studie von Turner *et al.* (2012) einher, die im Mausmodell der RPGN zeigen konnte, dass γδ T-Zellen früh in die Niere

infiltrieren und durch die Sekretion von IL-17A den glomerulären Schaden begünstigen.[16]
Die Ergebnisse der vorliegenden Studie zeigten ebenfalls, dass die zelluläre Quelle von IL-22 im Verlauf der Erkrankung variiert. Ähnliche Beobachtungen konnten schon in anderen immunologisch relevanten Organen gemacht werden. Im Darm sorgen ILC3s zum frühen Zeitpunkt einer *Citrobacter rodentium*-Infektion (ein enteropathogenes Bakterium) für die Produktion von IL-22, während $CD4^+$ T-Zellen zum späteren Zeitpunkt der Immunreaktion stark expandieren.[17] Im einem Mausmodell der Psoriasis, sind sowohl $CD4^+$- als auch γδ T-Zellen für die Produktion von IL-22 verantwortlich, unter Normalbedingungen obliegt die IL-22 Sekretion ausschließlich den γδ T-Zellen.

In der erworbenen Immunantwort stellen T_H17-Zellen die zelluläre Quelle von IL-22 dar.[17] Die der Arbeit vorliegenden Ergebnisse zeigen, dass an Tag Sieben der cGN $CD4^+$ T-Lymphozyten IL-22 und IL-17A co-exprimieren. Allerdings konnte keine IL-17A-unabhängige IL-22 Expression in $CD4^+$ T-Zellen beobachtet werden. Somit sprechen die hier gezeigten Ergebnisse gegen die Existenz einer auf die Produktion von IL-22 spezialisierten T_H22-Population in der Niere, die in anderen Zusammenhängen beschrieben wurde.[44]
Weitere Studien wiesen in der Lunge und im Colon nach, dass neben T-Lymphozyten ebenso nicht-lymphoide Zellen, wie z. B. Neutrophile oder Makrophagen, in der Lage sind IL-22 zu sekretieren.[45,46] Die durchflusszytometrischen Analysen in der vorliegenden Arbeit zeigten keine Hinweise für eine myeloide IL-22^+-Zellpopulation in der Niere.
Zum frühen Zeitpunkt einer Immunantwort werden in vielen Organen neben γδ T-Zellen ILC3s als einer der Hauptproduzenten von IL-22 angesehen.[17] Eine IL-22^+ Nicht-T-Zellpopulation war in der vorliegenden Arbeit nicht detektierbar. Da die ILC3s auch nur einen sehr geringen Teil der Gesamtleukozyten-Anzahl in der murinen Niere ausmachen, spielt die Produktion von IL-22 durch ILC3s in der Niere eine untergeordnete Rolle.[47]

Der beobachtete Anstieg der IL-22 Expression in der experimentellen RPGN lässt vermuten, dass IL-22 an der Initialisierung oder Kontrolle des glomerulären Schadens

beteiligt ist. Mit Hilfe von gendefizienten Mäusen und den entsprechenden Wurfgeschwistern als Kontrollen wurde daraufhin die Rolle von IL-22 in zwei verschiedenen Mausmodellen der glomerulären Schädigung (NTN-Modell und Adriamycin-Modell) untersucht. Die Ergebnisse zeigten, dass endogenes IL-22 weder einen Einfluss auf die Immunantwort noch auf den Verlauf des glomerulären Schadens in der Niere hat. Um mögliche Kompensationsmechanismen durch andere Zytokine in der IL-22-defizienten Maus auszuschließen, wurde geprüft, ob eine antikörpervermittelte Blockade von IL-22 einen Einfluss auf die RPGN hat. Auch dieses Experiment ergab keinen IL-22-spezifischen Phänotyp in der experimentellen RPGN.
Die mehrheitlich negativen Ergebnisse der hier vorliegenden Arbeit lassen den Schluss zu, dass IL-22 in den zwei Modellen der immunvermittelten glomerulären Schädigung keine maßgebliche Funktion zu übernehmen scheint. Es sind eher die zuvor beschriebenen pro-inflammatorischen Prozesse, ausgelöst durch die IL-23/IL-17-Achse, und die T_H1-Immunantwort, die hauptsächlich zur Schädigung der Glomeruli und zum rasch fortschreitenden Funktionsverlust der Niere beitragen.

Die Funktion von IL-22 in Mausmodellen von weiteren Nierenerkrankungen wurde bereits von anderen Arbeitsgruppen untersucht, wenngleich unterschiedliche, zum Teil widersprüchliche Beobachtungen gemacht wurden. Eine Studie zeigte, dass eine Hemmung von IL-22 zu einer geringeren Proteinurie und einer Reduktion des glomerulären Schadens in einem Modell der Lupus Nephritis (MRL/MPJ-*Fas*^{lpr}) führt.[48] Demgegenüber wies eine andere Studie mit Hilfe einer IL-22 Gentherapie in einem Mausmodell der Diabetischen Nephropathie nach, dass IL-22 durch die Inhibition des „NOD-, LRR- and pyrin domain-containing 3“ (NLRP3)-Inflammasoms eine Fibrose verhindert.[49] Der Grund für die in der Literatur beschriebene gegensätzliche Funktion von IL-22 liegt im Zytokinmilieu, das entscheidend für die Rolle von IL-22 ist. Im systemischen Autoimmunmodell der Lupus Nephritis ist eine pathogene Funktion von IL-22 wahrscheinlich, da IL-22 hier in Synergie mit pro-inflammatorischen Zytokinen wirkt. In der Diabetischen Nephropathie hingegen ist IL-22 an lokalen Entzündungsmechanismen beteiligt und kann daher eine protektive Funktion besitzen.
Im speziellen Fall der IL-22 Gentherapie sollte bedacht werden, dass durch die systemische Expression von IL-22 nierenunspezifische Prozesse, wie z. B. eine

verbesserte Blutzucker-Kontrolle im Modell der Diabetischen Nephropathie aktiviert werden und diese einen positiven Effekt auf die Erkrankung der Niere haben können.

Die Rolle von IL-22 in der Initialisierung von chronischen Nierenerkrankungen im Menschen ist weiterhin unklar. Eine Studie zeigte, dass ein Einzelnukleotidpolymorphismus im Gen des IL-22 Rezeptors mit dem Auftreten einer IgA Nephropathie bei Kindern assoziiert ist. Ebenso wurden vermehrt IL-22-sekretierende $CD4^+$ T-Zellen und ein erhöhtes Level an IL-22 im Blut bei Patienten mit IgA Nephropathie festgestellt.[50] In einer anderen Studie wurde eine Zunahme von $IL\text{-}22^+CD4^+$ T-Zellen in Patienten mit systemischem Lupus erythematodes (SLE) und renaler Beteiligung (Lupus Nephritis) beobachtet und mit der Schwere des Krankheitsverlaufs korreliert.[51] Im Gegensatz dazu wies eine andere Studie eine Abnahme von $IL\text{-}22^+CD4^+$ T-Zellen nach und zeigte eine geringere Menge von IL-22 im Urin von Patienten mit aktiver Lupus Neprhitis.[52]

In der hiesigen Studie wurde eine weitere interessante Beobachtung im Hinblick auf die mRNA Expression des natürlichen Inhibitors IL-22BP gemacht. In der experimentellen RPGN, nahm die IL-22BP Expression parallel zur IL-22 Expression zu. Dieses Ergebnis deutet daraufhin, dass die durch IL-22 induzierten Prozesse einer starken Kontrolle unterliegen, um bspw. eine Überreaktion von IL-22 in inflammatorischen Prozessen der Niere zu verhindern. Mit Hilfe einer IL-22BP-defizienten Maus wurde untersucht, welche Folgen ein ungehindertes Agieren von IL-22 in der experimentellen RPGN hat. Die Ergebnisse zeigten, dass IL-22 auch ohne natürliche Regulation durch IL-22BP keine relevante Funktion in der Initialisierung und Progression der RPGN besitzt.

Zusammenfassend konnte die hier vorliegende Studie eingehend darlegen, dass das immunregulatorische Zytokin IL-22 trotz nachweislicher Hochregulation weder eine entscheidende Funktion in der Entstehung noch in der Protektion einer Entzündungsreaktion in der experimentellen GN übernimmt. Diese Information ist relevant im Hinblick auf zurzeit getestete IL-22-Therapien,[18] die zwar vermutlich keinen therapeutischen Effekt in der Glomerulonephritis zeigen, aber ebenso höchstwahrscheinlich keine Nebenwirkungen in der Niere induzieren werden.

6.2 Nachweis der ILC-Subtypen in der humanen Niere und ihre Rolle in der Glomerulosklerose

Adaptive Typ-2-Immunantworten werden klassischerweise als Abwehrmechanismen gegen Wurminfektionen verstanden. Neuere Erkenntnisse belegen allerdings, dass Typ-2-Zytokine darüber hinaus eine äußerst wichtige Rolle in der Gewebereparatur und -regeneration übernehmen. Studien zeigten, dass in Abwesenheit eines Pathogens Typ-2-Immunantworten in der Haut, im Dickdarm und in der Leber für den Erhalt des dortigen Epithelgewebes notwendig sind. Diese Daten weisen darauf hin, dass sich die Typ-2-Immunantwort nicht initial als Abwehrmechanismus gegen mehrzellige Parasiten entwickelt hat, sondern evolutionär in erster Linie für den Erhalt der natürlichen Barriere des Körpers wichtig war.[28,53]

Die Typ-2-Immunantwort wird maßgeblich von den Typ-2-Zytokinen IL-4, IL-5, IL-9 und IL-13 vermittelt, die für eine Infiltration von Eosinophilen, Mastzellen und Basophilen sorgen, die Stimulation von alternativ aktivierten Makropagen induzieren und die Produktion von IgE seitens der B-Zellen anregen. Es ist also ein komplexes Zusammenspiel zwischen Entzündungsmediatoren, Wanderung und Reifung von Immunzellen und vielschichtigen intrazellulären Prozessen.[28]

Die Entdeckung der ILC2s änderte das Verständnis der Typ-2-Immunantwort grundlegend. Die mehrheitlich gewebsresidenten ILC2s sekretieren IL-5 und IL-13 und repräsentieren die vorherrschende Quelle der Typ-2-Zytokine in der angeborenen Immunantwort.[31] ILC2s sind also in der Lage Typ-2-Immunantworten zu initiieren und zu erhalten. Durch die Expression des Wachstumsfaktors Amphiregulin können ILC2s direkt auf Epithelzellen wirken und tragen so zur Gewebereparatur bei.[32] Des Weiteren sorgen ILC2s für eine Hemmung der gewebsschädigenden Typ-1-Entzündungsreaktionen und tragen so zum Erhalt der Gewebeintegrität an Grenzflächen bei.[54]

Im Hinblick auf immunvermittelte glomeruläre Nierenerkrankungen, könnten Typ-2-ILCs eine tragende Rolle bei der Reparatur der geschädigten glomerulären Zellkompartimente einnehmen. Die Fragen, ob ILCs in der Niere zu einem nennenswerten Anteil vertreten sind und inwieweit die regenerative Funktion von ILCs in der Niere manipuliert werden kann, wurden in der zweiten hier vorliegenden Studie adressiert.

Es ist bekannt, dass durch exogene Stimuli angeregte oder geschädigte Epithelzellen die Alarmine IL-25, IL-33 und das „thymic stromal lymphopoietin“ (TSLP) sekretieren und dadurch die Typ-2-abhängige Gewebereparatur in der Lunge, im Darm und in der Haut induzieren.[55] Ob in der Niere ähnliche gewebsregenerierende Prozesse durch die Expression von Alarminen aktiviert werden und ILC2s an diesen Mechanismen beteiligt sind, wurde bis dato nur in einer Studie näher untersucht. Nach Applikation von IL-25 wurde eine starke Expansion der ILC2-Population in der Niere beobachtet und alternativ aktivierte Makrophagen wurden induziert. Im Tiermodell für das akute, ischämische Nierenversagen sorgte ebenjene Applikation von IL-25 für eine reduzierte Inflammation in der Niere und durch die Expression von Proliferationssignalen unterstützten die aktivierten ILC2s die Regeneration von Tubulusepithelzellen.[56]
Bisher wurde die ILC-Zellpopulation in der Niere noch nicht vollständig charakterisiert und die Funktion von ILCs bei der Etablierung des Nierenschadens oder der Geweberegeneration in glomerulären Erkrankungen war unbekannt.

In der hier vorliegenden Studie wurde mit Hilfe einer durchflusszytometrischen Analyse zum ersten Mal gezeigt, dass die helper-like ILCs 0,5 - 3 % aller Leukozyten in der humanen und murinen Niere ausmachen. Dabei sind die IL-33R$^+$ ILC2s mit 80 % die größte Population der ILCs in der Maus.
Eine wichtige Grundlage für die Untersuchung der Typ-2-Immunantwort und ihrer potentiellen gewebsregenerierenden Funktion in der Niere ist die spezifische Expansion von ILC2s. Die Tatsache, dass Nieren-ILC2s fast ausschließlich IL-33R$^+$ sind, erlaubte es durch Applikation von IL-33 (4 x 400 ng/Tag) die ILC2s in der Niere zu expandieren. Damit einhergehend wurde eine starke Zunahme der Typ-2-Zytokine in der Niere beobachtet. Die IL-33-abhängige Expansion der ILC2s hielt bis zu acht Wochen in der Niere an, in der Milz hingegen war der Effekt nur transient.

Die durch die Applikation von IL-33 hervorgerufene massive Expansion der ILC2s sorgte in der hier vorliegenden Arbeit für eine signifikante Reduktion des glomerulären Schadens im Mausmodell der Glomerulosklerose.
IL-33 ist maßgeblich an der Induktion der Typ-2-Immunantwort in verschiedenen Geweben beteiligt und kann, abhängig vom Organ, verschiedene Zelltypen wie z. B. ILC2s, Eosinophile, Mastzellen, Basophile und Subtypen von T-regulatorischen Zellen

(Tregs) aktivieren.[57] Im Mausmodell der glomerulären Schädigung konnten hier gezeigt werden, dass der IL-33-abhängige Effekt in erster Linie durch ILC2s ohne Beteiligung von T-Zellen vermittelt wird. T- und B-Zell-defiziente *Rag2*$^{-/-}$ Mäuse, aber nicht *Rag2*$^{-/-}$*IL2rg*$^{-/-}$ Mäuse, die neben B- und T-Zellen auch keine ILCs besitzen, zeigten eine starke Reduktion des glomerulären Schadens nach IL-33-Therapie. Aufgrund dieser Ergebnisse kann nicht ausgeschlossen werden, ob IL-33 eine direkte Wirkung auf Tregs besitzt und diese eventuell ebenfalls an der Verminderung des Schadens in Wildtyp-Mäusen beteiligt sind.

Häufig wird der gewebsprotektive Mechanismus zwar durch ILC2s initiiert, aber andere Immunzellen vermitteln die eigentliche Wirkung auf die Zielzellen.[32] Bei der Induktion der ILC2-abhängigen Reparaturprozesse spielen die Typ-2-Zytokine IL-5 und IL-13 eine entscheidende Rolle. Während IL-5 in erster Linie für die Rekrutierung von Eosinophilen verantwortlich ist, induziert IL-13 unter anderem alternativ aktivierte Makrophagen (AAMs). Dass Eosinophile an der Geweberegeneration beteiligt sind, wurde schon in der Leber und in Muskelzellen beschrieben.[58,59] Für AAMs konnte ebenso eine regenerative Funktion im Nierengewebe festgestellt werden.[60] Welche Zellen in dem hier untersuchten Mausmodell den ILC2-vermittelten Effekt ausüben, wurde eingehender untersucht. Nach Gabe von IL-33 wurde ein eindeutiger Anstieg der Eosinophilen beobachtet und aufgrund dessen eine Beteiligung dieses Zelltyps an der Gewebereparatur vermutet. In ΔdblGATA Mäusen, die keine Eosinophilen besitzen, konnte der protektive Effekt von IL-33 in der Glomerulosklerose nicht beobachtet werden. Dieses Ergebnis belegt, dass der beobachtete Effekt der ILC2s auf das Gewebe überwiegend durch Eosinophile vermittelt wird.

Auf welche Zelle IL-33 wirkt ist stark vom Entzündungsumfeld und Zytokinmilieu abhängig und unterscheidet sich bei verschiedenen Erkrankungen wie bei einer Virusinfektion,[61] einer Sepsis,[62] im akutem Nierenschaden[63] und sterilen Entzündung wie z. B. chronischen Nierenerkrankungen[64] stark voneinander. Aus diesem Grund gilt es bei einer systemischen Gabe von IL-33 potentielle Nebenwirkungen zu beachten und die Dauer und Dosis zu bedenken. Eine chronische IL-33-Exposition kann entweder zu einer allergischen Reaktion führen oder, wie in der Leber gezeigt, durch

übermäßige Umstrukturierung oder Erneuerung bestehender Gewebe zu einer Fibrose führen.[65]

Zusammenfassend kann festgehalten werden, dass eine substantielle ILC2-Population in der Niere vorhanden ist und diese Population nach Aktivierung durch IL-33 in der Lage ist Typ-2-Immunantworten zu induzieren und so den Schaden im Nierengewebe einzudämmen. Die Applikation von IL-33 sorgt für eine rasche, spezifische Akkumulation der ILC2s in der Niere, die lange nach der Gabe von IL-33 persistiert.
Diese Studie weist darauf hin, dass eine ILC2-spezifische Zytokin-Therapie eingesetzt werden könnte, um progressive chronische Nierenerkrankungen zu behandeln. Inwieweit einzelne ILC-Subpopulationen in der humanen Niere auf andere Zytokine reagieren und ob es eine Korrelation zwischen verschiedenen Entitäten von Nierenerkrankungen und Häufigkeit von ILC-Subpopulationen im Menschen gibt, bleibt noch offen.
Es bedarf weiterer Untersuchungen, um diese komplexe angeborene Immunzellpopulation der ILCs in der humanen Niere und ihre Bedeutung in verschiedenen Nierenerkrankungen zu verstehen.

6.3 Die Rolle und Regulation von nierenresidenten Typ-2-ILCs in der autoimmunen renalen Inflammation

Der systemische Lupus erythematodes (SLE) ist eine chronische Autoimmunerkrankung, an der vorrangig junge Frauen im gebärfähigen Alter erkranken. Charakteristisch für diese Erkrankung sind die Bildung von Autoantikörpern und eine anschließende Ablagerung von Immunkomplexen im Gewebe, die eine chronische Entzündung induzieren. Bei der Entstehung der komplexen Erkrankung sind sowohl die zelluläre als auch die humorale Immunantwort beteiligt, die in einem engen Zusammenspiel mit anderen immunpathogenen Faktoren agieren. Die B- und T-Zell-aktivierenden Antigene sind nukleäre Strukturen, die durch eine unzureichende Phagozytose von apoptotischen Zellen an ihre Oberfläche gelangen und dort in erster Linie von B-Zellen als „fremd" erkannt werden.[66,67] Dieser Verlust der B-Zell-Toleranz führt zu einer Typ-III-Hypersensitivitätsreaktion, die durch Zirkulation und Ablagerung von Antigen-Antikörper-Komplexen zu einer lokalen Hyperinflammation und schwerwiegenden irreversiblen Zerstörung des Gewebes führt.[68]
Bei SLE-Patienten sind häufig die Haut, das Nervensystem, die Muskeln und Gelenke sowie die Niere von dem immunvermittelten Schaden im Gewebe betroffen. Typischerweise verläuft die Erkrankung in Schüben und aufgrund ihrer heterogenen Art bei jedem Patienten anders.[68]
Die Ursachen für die Entstehung dieser komplexen Form der Autoimmunerkrankung sind bis heute noch unklar. Neben einer genetischen Komponente spielen verschiedene Umwelteinflüsse wie UV-Strahlung, Infektionen und Stress eine große Rolle bei der Bildung der Autoantikörper.[68] Im Verlauf der Erkrankung tritt in 80 % der Fälle eine Manifestation in der Niere (Lupus Nephritis, LN) auf, die verantwortlich für die hohe Morbidität und Mortalität der SLE-Patienten ist.[66] Die derzeitigen Therapieoptionen für SLE-Patienten beruhen meist auf einer unspezifischen Immunsuppression mit Glucocorticoiden und zytotoxischen Agenzien, die mit starken Nebenwirkungen einhergehen.[66] Aus diesem Grund ist es ein wichtiges Ziel der aktuellen Forschung, die der SLE und LN zugrundeliegenden molekularen und zellulären Mechanismen zu verstehen, um spezifischere und wirkungsvollere Therapien zu entwickeln.

Grundlage für funktionelle Untersuchungen der immunologischen Abläufe in der SLE sind geeignete Mausmodelle, die die pathologische Situation im Menschen widerspiegeln. Ein geeignetes Mausmodell für die Untersuchung einer Lupus Nephritis stellt die MRL/MPJ-*Fas*^{lpr} (MRL-*lpr*) Maus dar. Durch eine spontane Mutation in dem Gen für den Fas Rezeptor, der nach Aktivierung für die Apoptose von autoreaktiven B- und T-Zellen verantwortlich ist, kommt es zur Anreicherung eben jener Lymphozyten und zur starken Lymphadenopathie und Milzvergrößerung.[69] Anhand von experimentellen Untersuchungen in diesem und anderen Mausmodellen konnte in den letzten Jahren gezeigt werden, dass eine fehlgeleitete T-Zellaktivierung und eine Überproduktion von Typ-1-IFN-regulierten Genen, die sogenannte Typ-I-IFN-Signatur, die Hauptursachen für die Entwicklung des SLE sind.[70]

Klassischerweise sind Typ-1- und Typ-3-Immunantworten für die Entstehung einer chronischen Entzündungsreaktion in Autoimmunerkrankungen wie zum Beispiel Multipler Sklerose, Arthritis und Glomerulonephritis verantwortlich. Typ-1- und Typ-3-Immunantworten aktivieren Makrophagen und sorgen für die Infiltration von Neutrophilen, die dann den Gewebeschaden induzieren. In diesem Zusammenhang konnte schon gezeigt werden, dass Typ-2-Immunantworten in der Lage sind die pathogenen Typ-1- und Typ-3-Immunantworten zu hemmen, und so den Gewebeschaden eindämmen.[71,72]
Inwieweit ILC2s an der Regulation von Autoimmunerkrankungen beteiligt sind, war bisher noch weitestgehend unverstanden. Ein erster Hinweis für eine protektive Rolle von ILC2s in Autoimmunerkrankungen, lieferte eine kürzlich publizierte Studie, in der die geschlechtsspezifische Prädisposition in einem bestimmten Mausstamm (SJL) für die experimentelle Autoimmunenzephalitis (EAE), ein Mausmodell für Multiple Sklerose, mit der Abnahme von ILC2s einhergeht.[73] In männlichen Versuchstieren, die resistent gegen EAE waren, konnte eine starke Zunahme der ILC2s im entzündeten Gewebe festgestellt werden, während in weiblichen Mäusen, die an EAE erkrankten, die ILC2-Population im betroffenen Gewebe abwesend war. In einer weiteren Studie wurde ein Zusammenhang zwischen EAE-Prädisposition und Schwere des Krankheitsverlaufs in Mäusen mit einer genetischen Defizienz des IL-33R, der für die Aktivierung von ILC2s verantwortlich ist, hergestellt.[74,75]

In der hier vorliegenden Studie konnte gezeigt werden, dass mit zunehmender Manifestation der Lupus Nephritis die Anzahl und die Proliferationsfähigkeit der ILC2s in der Niere abnahm. Gleichzeitig war eine Zunahme der Infiltration von $CD4^+$, $CD8^+$ und $CD4^-$ $CD8^-$ T-Lymphozyten in die Niere zu beobachten, was kennzeichnend für eine proliferative Lupus Nephritis ist.[70,76] Diese eindeutige Abnahme der ILC2-Population bei gleichzeitiger Zunahme der T-Lymphozyten lässt eine direkte Regulation der ILC2s durch T-Zellen in der Niere vermuten.

Im Zusammenhang mit der pathogenen Rolle von ILC2s in Allergien, wurde in den letzten Jahren ein komplexes Netzwerk an negativen Regulatoren der ILC2-Achse entdeckt.[77-80] Typ-1- und Typ-2-IFN und IL-27 wurden als wirkungsvolle Inhibitoren von ILC2s in Wurminfektionen, allergischen und virus-induzierten Atemwegserkrankungen beschrieben.[77-80] Die Ergebnisse der Studien beruhten auf der systemischen Applikation von IL-27 und den Typ 1- und Typ 2-IFN und wiesen somit eine Inhibition der ILC2s mit einer nicht-physiologischen Dosis nach. Dass endogene IFN-Antworten in gleicher Weise ILC2-Populationen hemmen können zeigten zwei Studien von *Listeria monocytogenes*- oder Influenza-A-Virus-Infektionsmodellen in der Lunge.[57,77] Ob ähnliche Zytokin-vermittelte regulatorische Mechanismen für die Hemmung der ILC2s in Gegenwart einer Autoimmunreaktion verantwortlich sind, war bisher unklar.

In der vorliegenden Studie konnte gezeigt werden, dass die Expression von IFN-γ und IL-27 in der Niere im Verlauf der Lupus Nephritis in den MRL-*lpr* Mäusen deutlich zunimmt. Dass die starke Reduktion der ILC2s in der Niere mit dem beobachteten Anstieg der zwei Zytokine zusammenhängt, konnte mit *in vitro* Stimulationsexperimenten belegt werden. Aus der Niere isolierte und mit IL-33-stimulierte ILC2s zeigten eine starke Sensibilität gegenüber der IFN-γ- und IL-27-vermittelten Inhibition. Diese Beobachtungen bestätigen die Ergebnisse einer anderen Studie, in der eine systemische Aktivierung von NK T-Lymphozyten zur Sekretion von IFN-γ führt, was eine verminderte ILC2-Effektorzytokinproduktion in der Lunge und Niere zur Folge hat.[81]

Die zellulären Quellen der potentiellen ILC2-Inhibitoren in Tiermodellen von entzündlichen Erkrankungen sind nur unzureichend untersucht. Eine kürzlich

erschienene Studie konnte zeigen, dass aktivierte NK-Zellen durch Sekretion von IFN-γ ILC2s und von ihnen sekretierte Typ-2-Zytokine in Mausmodellen mit T-Zell-unabhängigen akuter Leber- und Lungenentzündungen hemmen.[79] Allerdings unterscheiden sich akute von chronischen Entzündungsreaktionen, was eine andere zelluläre Quelle der ILC-Inhibitoren in Autoimmunerkrankungen vermuten lässt. In dem hier verwendeten MRL-*lpr* Mausmodell zeigte sich, dass $CD4^+$ T-Zellen die Hauptproduzenten von IFN-γ sind, während IL-27 hauptsächlich von myeloiden Zellen produziert wird. Diese Beobachtung lässt vermuten, dass beide Zelltypen in der Lage sind im Mausmodell der Lupus Nephritis ILC2s zu hemmen, sie dies aber durch verschiedene Signalwege tun.

Einen Hinweis für eine protektive Rolle von ILC2s in der Pathogenese der SLE lieferte eine Studie, in der IL-27-rezeptordefiziente MLR-*lpr* Mäuse eine erhöhte Überlebensrate zeigten. Damit einhergehend wiesen diese Mäuse im Verlauf der Erkrankung neben einer systemischen Typ-2-Immunantwort eine reduzierte Manifestation der Lupus Nephritis auf.[82] Zum Zeitpunkt der Veröffentlichung dieser Studie waren die ILCs noch nicht bekannt, sodass diese Ergebnisse nur einen indirekten Nachweis über die Beteiligung der ILC2s an der schützenden Typ-2-Immunantwort liefern und die zelluläre Quelle der Typ-2-Immunantwort unbekannt bleibt.

In humanen Studien zeigten Blutanalysen von Patienten mit Rheumatoider Arthritis und ANCA-Vaskulitis, zwei Autoimmunerkrankungen, keine signifikante Verminderung der ILC2-Anzahlen.[83,84] Interessanterweise konnte ebenfalls gezeigt werden, dass nach Remission der Patienten mit Rheumatoider Arthritis, die ILC2s im Blut stark proliferierten.[85] Eine aktuelle Studie wies im Blut von SLE-Patienten reduzierte ILC2-Zahlen bei gleichzeitig erhöhter Anzahl an ILC1s und ILC3s nach.[84] Diese Ergebnisse stimmen mit den hier gemachten Beobachtungen überein.

Die Frage, ob eine Wiederherstellung der ILC2 Population in der Niere einen protektiven Effekt auf die Lupus Nephritis hat und damit eine neue therapeutische Strategie darstellt, wurde im letzten Teil der vorliegenden Arbeit adressiert. Einige Studien, inklusive dieser Arbeit zugrundeliegenden Studie, haben eine durch IL-33 hervorgerufene protektive Rolle der ILC2s in Mausmodellen der Multiplen Sklerose,

Arthritis und der Glomerulonephritis nachweisen können.[74,86] Aus diesem Grund wurde vermutet, dass eine kurzzeitige Gabe von IL-33 im MRL-*lpr* Modell einen positiven Effekt auf die Entstehung und den Verlauf der Lupus Nephritis haben könnte. In der Tat wurde eine reduzierte Immunzellinfiltration in den Glomeruli sowie eine geringere Schädigung der Niere in IL-33-behandelten Tieren beobachtet. Aufgrund der massiven Expansion der ILC2s in der Niere ist es wahrscheinlich, dass der verminderte Schaden nicht durch andere anti-inflammatorische Zellpopulationen, wie z. B. Tregs, hervorgerufen wurde, sondern hauptsächlich von ILC2s vermittelt wird. Die hier beschriebenen Mechanismen sind in Abbildung 6 zusammengefasst.

Es ist wichtig zu erwähnen, dass der positive Effekt der IL-33-Therapie nicht über die vollständige Dauer des Experiments (bis Woche 22) aufrechterhalten werden konnte. Obwohl die Anzahl an ILC2s in der Niere konstant hoch blieb, war eine Zunahme der Zellinfiltration in den Glomeruli sowie eine erhöhte Proteinurie am Ende der Erkrankung zu verzeichnen. Diese Beobachtung deutet darauf hin, dass der erwünschte, protektive der ILC2s durch andere Immunzellpopulationen überlagert wurde. Nichtsdestotrotz zeigte die IL-33-behandelte Tierkohorte eine signifikant höhere Überlebensrate. Grund hierfür kann ein verringerter immunvermittelter Schaden in anderen Organen sein, die in der hier vorliegenden Studie nicht näher untersucht wurden. Ebenso wurde eine verringerte Expression von IL-27 in den Nieren von IL-33-behandelten MRL-*lpr* Mäusen beobachtet, was darauf hindeutet, dass ILC2-abhängige -Zytokine und Typ-1-Zytokine sich gegenseitig negativ regulieren.

Zusammenfassend kann festgehalten werden, dass im Mausmodell der SLE die von T-Zellen und myeloiden Zellen sekretierten Zytokine IFN-γ und IL-27 nierenresidente ILC2s unterdrücken und ihren protektiven Effekt aufheben. Daher könnte die Anreicherung von ILC2 und die Aktivierung ihrer protektiven Eigenschaften eine mögliche therapeutische Strategie gegen Autoimmunkrankheiten wie SLE darstellen.

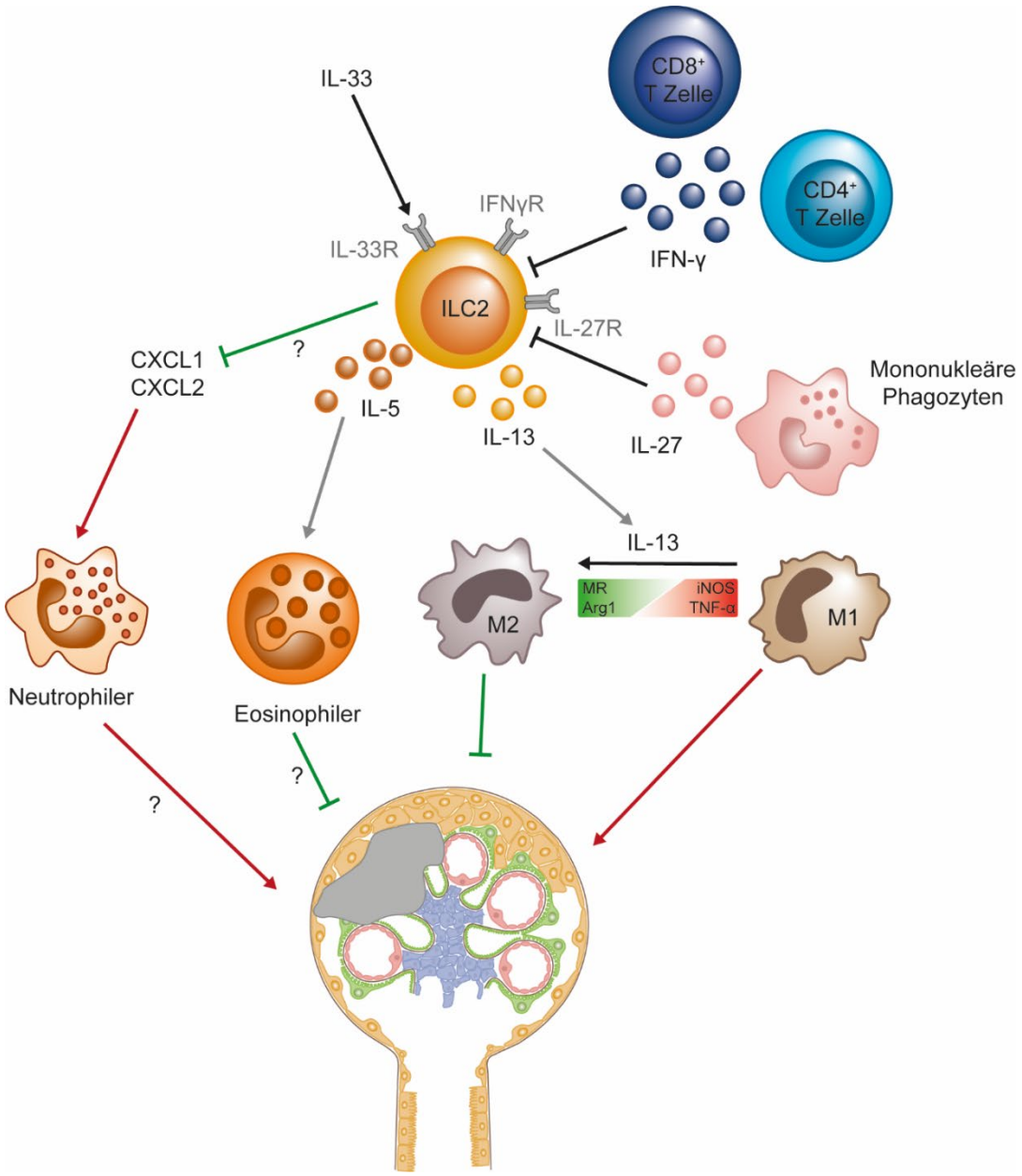

Abbildung 6: Funktion von ILC2s in glomerulären Erkrankungen. Das Alarmin IL-33 aktiviert ILC2s, während von CD4+ und CD8+ T-Zellen sekretiertes IFN-γ und von Myeloiden Zellen stammendes IL-27 die ILC2-Aktivität inhibiert. ILC2s sekretieren nach Aktivierung die Effektorzytokine IL-5 und IL-13, die zu einer Akkumulation von Eosinophilen führt und die Umwandlung der Makrophagen von einem pro-inflammaotorischen Phänotyp (M1) zu einem anti-inflammatorischen Phänotyp (M2) induziert. Die Aktivierung der ILC2s führt ebenso zu einer Reduktion der Chemokine CXCL1 und CXCL2 in der Niere, die die Rekrutierung der pathogenen Neutrophilen verhindert. Fragezeichen weisen auf bisher unverstandene Mechanismen hin. Grüne Linien geben protektive Effekte und rote Pfeile geben pro-inflammatorische Effekte an. (Arg1 = Arginase 1; iNOS = Inducible nitric oxide synthase; MR = Mannose Receptor; M1 = Klassischer Makrophage; M2 = alternativ aktivierter Makrophage; TNF-α = Tumor Nekrosefaktor α).

7 LITERATURVERZEICHNIS

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8 ANGABEN ZUM EIGENANTEIL

Die Funktion von IL-22 in immunvermittelten glomerulären Nierenerkrankungen

Die projektbezogene Fragestellung dieser Arbeit hat Priv.-Doz. J.-E. Turner entwickelt. Zusammen mit Priv.-Doz. J.-E. Turner habe ich den Projektplan erstellt und im Anschluss die Experimente selbstständig durchgeführt, ausgewertet und zusammen mit Priv.-Doz. J.-E. Turner kritisch bewertet und interpretiert. Bei der Durchführung von aufwändigen Experimenten hatte ich Unterstützung von M. Wunderlich. Das Manuskript habe ich gemeinsam mit Priv.-Doz. J.-E Turner verfasst.

Nachweis der ILC Subtypen in der humanen Niere und ihre Rolle in der Glomerulonephritis

Für diese Arbeit hat Priv.-Doz. J.-E. Turner den konzeptionellen Plan der Studie entwickelt. Ich habe Dr. J-H. Riedel und Dr. Martina Becker bei der Durchführung der Experimente unterstützt und weiterführende Analysen durchgeführt und selbstständig ausgewertet.

Die Rolle und Regulation von nierenresidenten Typ-2-ILCs in der autoimmunen renalen Inflammation

Für diese Arbeit hat Priv.-Doz. J.-E. Turner die Fragestellung entwickelt und ich habe zum Ende des Projektes wichtige Experimente selbstständig geplant, durchgeführt und ausgewertet. Das Manuskript habe ich korrigiert und kritisch kommentiert.

9 EIDESSTATTLICHE ERKLÄRUNG

Hiermit versichere ich, Ann-Christin Gnirck, dass ich die vorliegende Dissertation selbstständig und ohne fremde Hilfe verfasst und keine anderen als die hier kenntlich gemachten Quellen und Hilfsmittel genutzt habe. Außerdem erkläre ich, dass die vorgelegte Abhandlung in dieser oder ähnlicher Form noch nicht anderweitig als Promotionsleistung vorgelegt und bewertet wurde.

Hamburg, den

Ann-Christin Gnirck

11 DANKSAGUNG

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12 ANHANG

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RESEARCH ARTICLE

Endogenous IL-22 is dispensable for experimental glomerulonephritis

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Gnirck AC, Wunderlich M, Becker M, Xiong T, Weinert E, Meyer-Schwesinger C, Dumoutier L, Renauld JC, Huber S, Panzer U, Turner JE. Endogenous IL-22 is dispensable for experimental glomerulonephritis. *Am J Physiol Renal Physiol* 316: F712–F722, 2019. First published February 6, 2019; doi:10.1152/ajprenal.00303.2018.—In recent years, the cytokine interleukin (IL)-22 attracted considerable attention due to its important immunoregulatory function in barrier tissues, such as the gut, lung, and skin. Although a regenerative role of IL-22 in renal tubular damage has been demonstrated, the role of IL-22 in the immunopathogenesis of glomerular injury is still unknown. Here, we demonstrate that the IL-22 receptor is expressed in the glomerular compartment of the kidney and that IL-22 expression increases in the renal cortex after induction of glomerular injury in a mouse model for crescentic glomerulonephritis (cGN, nephrotoxic nephritis). We identified γδ T cells and T_H17 cells as major sources for IL-22 in the nephritic kidney. However, neither genetic or antibody-mediated deletion of IL-22 nor genetic deficiency in its endogenous inhibitor IL-22Rα2 (IL-22 binding protein) resulted in substantial phenotypic differences in mice with cGN with respect to crescent formation, tubulointerstitial damage, and kidney function impairment. Similarly, we did not observe significant differences between wild-type or IL-22-deficient mice in a mouse model of secondary focal and segmental glomerulosclerosis (adriamycin-induced nephropathy). As shown previously, we detected concomitant upregulation of IL-17A and IFN-γ production by T cells during the course of cGN, providing alternative cytokine pathways that mediate glomerular injury in this model. In conclusion, we show here that endogenous IL-22 expression is redundant in different forms of glomerular injury, indicating that the IL-22-directed therapies that are being tested in various human diseases might not affect the kidney in patients with glomerular disease.

cytokines; glomerulonephritis; IL-22; IL-22 receptor; T cells

INTRODUCTION

Immune-mediated glomerular diseases are a heterogeneous group of disorders that are a major cause of end-stage renal diseases in the Western world (5). The common underlying mechanisms of glomerulonephritis encompass a disturbed immune reaction leading to pathogenic inflammation in the glomerulus of the kidney. Although important discoveries in this research field were made in recent years, current treatment options for glomerulonephritis patients still rely on unspecific immunosuppressive agents that can cause severe side effects and are only partially effective. To overcome these therapeutic restrictions, the development of more effective and specific individual treatment strategies is crucial (9).

Cytokines are central mediators of tissue responses in inflammation and under homeostatic conditions. In recent years, the cytokine interleukin (IL)-22 received considerable attention due to its important function as an immunoregulatory cytokine at barrier surfaces (4, 18). Depending on the tissue location and type of immune response, the cellular source of IL-22 can vary. In most settings, IL-22 production has been attributed to T_H17 cells, γδ T cells, and group 3 innate lymphoid cells (ILC3s) (4, 18), but nonlymphoid cells, such as macrophages and neutrophils, have also been described as possible sources of IL-22 (6, 8, 29).

In contrast to other interleukins, IL-22 acts exclusively on nonhematopoietic, epithelial, and stromal cells via the heterodimeric IL-22 receptor (IL-22R) complex, consisting of the IL-22Rα1 and the ubiquitously expressed IL-10Rβ2 chain. The receptor complex is expressed on various barrier surfaces, with highest expression levels in the skin, colonic mucosa, and pancreas followed by liver, lung, and kidney (20, 26). Importantly, IL-22 function is also regulated by an endogenous inhibitor, the soluble IL-22-binding protein (IL-22bp or IL-22Rα2), adding another layer of complexity to IL-22 signaling biology (7).

Depending on the context, IL-22 can mediate pro- or anti-inflammatory functions and is therefore often described as a double-edged sword. In homeostasis and after acute injury (e.g., of the intestine), IL-22 expression promotes barrier integrity by induction of anti-apoptotic genes and anti-microbial peptides in epithelial cells, resulting in enhanced tissue regeneration and improved protection against invading pathogens (18). Conversely, IL-22 function has been linked to inflammatory tissue pathology in immune-mediated diseases, such as psoriasis. Here, the coexpression of IL-22 with other cytokines, such as IL-17A, induces proinflammatory molecules in parenchymal cells and thereby contributes to the recruitment of pathogenic effector cells promoting tissue inflammation (4).

The considerable expression of IL-22R mRNA in human and murine kidney tissue (20, 26) suggests a potential involvement of IL-22 in renal diseases. In line, two independent studies recently detected IL-22R expression on proximal tubu-

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lar epithelial cells in the murine kidney and observed that IL-22 signaling promotes tubular regeneration after ischemia- and reperfusion-induced acute kidney injury (8, 27).

Because studies addressing a potential involvement of endogenous IL-22 in glomerular diseases are still lacking (25), here, we focus on a potential role of IL-22 in glomerular injury.

MATERIALS AND METHODS

Animals. All animals were raised under specific pathogen-free conditions. IL-22-deficient mice on the C57BL/6J background were kindly provided by J.-C. Renauld (Ludwig Institute for Cancer Research, Brussels, Belgium). To generate BALB/c IL-22-deficient mice, the same animal strain was backcrossed for at least 10 generations to the BALB/c background.

IL-22Rα2-deficient mice (7) on C57BL/6J background were provided by S. Huber with permission of Regeneron Pharmaceuticals. Sex- and age-matched C57BL/6J and BALB/c wild-type littermate controls were bred in the animal facility of the University Medical Centre Hamburg-Eppendorf. All animal experiments were performed according to national and institutional animal care and ethical guidelines and were approved by the local committees.

Induction of experimental glomerulonephritis, experimental focal and segmental glomerulosclerosis, and functional studies. Crescentic glomerulonephritis (cGN) was induced by intraperitoneal injection of nephrotoxic sheep serum (2.5 mg/g body wt) in 8- to 12-wk-old mice as previously described (2). For induction of experimental focal and segmental glomerulosclerosis (FSGS), BALB/c mice were injected intravenously with 12 μg adriamycin/g body weight (Cell Pharm). For urine sample collection, mice were housed in metabolic cages for 5 h. Urinary albumin excretion was determined by standard ELISA (Mice-Albumin Kit; Bethyl Laboratories, Montgomery, TX). Urinary creatinine levels were measured with Creatinine Jaffé Fluid (Hengler Analytik, Steinbach, Germany). Plasma cholesterol and blood urea nitrogen (BUN) were analyzed by standard laboratory procedures.

Antibody-mediated neutralization of IL-22. The IL-22-blocking antibody (AM22.1) used in this study was previously described (23). IgG2a was used as an isotype control (no. BE0085; clone C1.18.4; BioXcell). IL-22-blocking antibody and its isotype control (both 100 μg/mouse) were administered intraperitoneally 1 day before and at *day 3* after induction of crescentic glomerulonephritis (cGN).

Histopathology and immunohistochemistry. Formalin-fixed paraffin-embedded kidney sections were stained with periodic acid-Schiff (PAS) reagent according to standard laboratory procedures. Crescent formation in the cGN model was assessed in 30 glomeruli per mouse in a blinded fashion. Tubular injury was assessed by using photographs of nonoverlapping cortical areas from PAS-stained kidney sections. In the glomerulonephritis (GN) model, percentage of interstitial area was determined by superimposition of the photographs with 40 apportioned dots, and subsequently interstitially located dots were counted. In the progressive glomerulosclerosis model, percentage of tubular injury was assessed by counting of the area displaying dilated, atrophic, or cast-filled tubules after superimposition of a grid.

For detection of glomerular sheep IgG and mouse IgG deposition, paraffin-embedded kidney sections were stained with anti-mouse and anti-sheep antibodies (Jackson ImmunoResearch) and developed with a polymer-based secondary antibody alkaline phosphatase kit (POLAP; Zytomed, Berlin, Germany). Glomerular deposition of mouse IgG was scored from 0 to 3 in 30 glomeruli per mouse. Scoring was performed with the Axioskop light microscope, and photographs were taken with an Axiocam MRc camera (both Zeiss, Jena, Germany).

For staining of deposited C3 complement, paraffin-embedded kidney sections were stained with a FITC-conjugated anti-C3 antibody (Cappel Research Reagents). Stained sections were evaluated by confocal microscopy using the Laser Scanning Microscope 800 and the appropriate software (all Zeiss).

Antigen-specific humoral immune response. Total mouse anti-sheep IgG titers were measured by ELISA using plasma of mice 7 and 21 days after induction of cGN. In brief, ELISA microtiter plates were coated with sheep IgG (100 μg/ml; Sigma, St. Louis, MO) overnight at 4°C. After blocking with 1% bovine serum albumin (BSA) Tris-buffered saline (Sigma), the plates were incubated with serial dilutions of mouse plasma (1:100 to 1:12,500) for 1 h, shaking at room temperature. Total bound mouse IgG was detected using peroxidase-conjugated goat anti-mouse IgG (R&D) in a 1:1,000 dilution, 3,3′,5,5′-tetramethylbenzidine (TMB) peroxidase substrate, and absorbance readings (at 450 nm) on a spectrophotometer.

Cell isolation. For isolation of renal leukocytes, mouse kidneys were cut into small pieces and digested in complete medium (RPMI 1640, 10% fetal bovine serum, 1% HEPES, and 1% penicillin/streptomycin; all GIBCO) with collagenase D (0.4 mg/ml; Roche, Basel, Switzerland) and DNase I (100 μg/ml; Roche) for 45 min while rotating on a MACSmix tube rotator (Miltenyi, Bergisch Gladbach, Germany) and then further dispersed by using gentleMACS dissociation (Miltenyi). Further leukocyte purification was achieved by Percoll gradient centrifugation (37.5%) (GE Healthcare, Chicago, IL). After subsequent erythrocyte lysis with ammonium chloride, cell suspensions were filtered through a 50-μm strainer and used for further analyses.

Flow cytometry. Nonspecific staining was prevented by incubation with 10% normal mouse serum (Jackson ImmunoResearch Laboratories, West Grove, PA). To characterize leukocyte subsets, cell suspensions of mouse kidneys were stained with fluorochrome-coupled antibodies against CD45 (30-F11), $Thy^{1.2}$ (CD90.2; 30-H12), CD3 (145-2C11), CD4 (RM4-5), CD8 (53-6.7), CD11b (M1/70), CD11c (HL-3), TCR-γδ (H57-597), TCR-αβ (GL3), Ly6G (1A8), SiglecF (E50-2440), and F4/80 (BM8) (all Biolegend; eBioscience; BD Biosciences). Intracellular staining was performed with IL-22 (Poly5164; Biolegend), IL-17A (TC11-18H10.1; BD Biosciences), and IFN-γ (XMG1.2; eBioscience). Isolated leukocytes were restimulated with phorbol 12-myristate,13-acetate (1 μg/ml; Sigma) and ionomycin (1 μg/ml; Calbiochem) in the presence of brefeldin A (10 μg/ml; Sigma) for 2.5 h. Subsequently, surface staining was performed as described above. Next, cells were fixed with formalin (3.7%; Sigma), permeabilized with IGEPAL CA-630 (0.1%; Sigma), and stained with the above-mentioned intracellular fluorochrome-coupled antibodies. Dead cell staining was performed using the LIVE/DEAD Fixable Read Dead Stain Kit (Invitrogen) or Zombie Dye (Biolegend). Absolute numbers of $CD45^+$ cells in kidney cell suspension were determined by staining with flourochrome-coupled anti-CD45 combined with cell count beads (Countbright; Invitrogen). All samples were acquired on a LSRII flow cytometer (BD Biosciences) and analyzed with the FlowJo Software (Treestar).

Isolation of glomeruli and tubules. The isolation of glomeruli from the murine kidney was performed as described previously (21). In brief, mice were perfused with Dynabeads (Thermo Fischer), and, after extraction of the kidney, the inner and outer medulla were removed and discarded. Subsequently, after digestion of the kidney with collagenase I for 20 min at 37°C, the glomeruli, containing the magnetic beads, were separated from the renal tubules by magnetic separation using the DynaMag-2 magnet (Invitrogen).

Real-time RT-PCR analysis. Total RNA of the renal cortex was prepared according to standard laboratory methods. After reverse transcription to cDNA, real-time PCR was performed for 45 cycles (initial denaturation at 95°C for 10 min, followed by cycles of denaturation at 95°C for 15 s and primer annealing and elongation at 60°C for 1 min) with 1 μl of cDNA samples in the presence of 0.5 μl of specific murine primers. TaqMan Gene Expression Assays and a StepOnePlus Real-Time PCR system (both Thermo Fisher Scientific) were used for quantification of the housekeeping gene (*Hprt1*) and the genes of interest. All samples were run in duplicates.

Statistics. Student's *t*-test was used for comparison between two groups. In cases of three or more groups, one-way ANOVA was used

followed by a post hoc analysis with Newman-Keuls test for multiple comparisons. A P value <0.05 was considered to be statistically significant.

RESULTS

Expression of IL-22 and its receptor in experimental cGN. To investigate the role of IL-22 in glomerular injury, we first wanted to address whether the IL-22R is expressed in the glomerular compartment of the kidney. In earlier studies, low constitutive expression of IL-22R was identified in the murine kidney (20), which was later located to proximal tubular epithelial cells (8, 27). Performing quantitative analysis of IL-22Rα1 and IL-10Rβ mRNA expression in the tubular and glomerular tissue isolated from the kidney from naïve C57BL/6 mice, we could confirm dominant IL-22Rα1 expression in the tubulointerstitial compartment. However, our data also revealed substantial expression of both IL-22R subunits in the glomerular compartment (Fig. 1*A*). Next, we addressed the question whether induction of glomerular inflammation leads to upregulation of IL-22 expression. mRNA expression analysis of the renal cortex during the course of cGN induced in C57BL/6 wild-type mice by injection of "nephrotoxic" sheep IgG demonstrated elevated IL-22 expression levels as early as 12 h after induction of the model, followed by a rapid decline toward baseline levels (Fig. 1*B*). Induction of IL-22 expression was accompanied by upregulation of its natural inhibitor, the IL-22bp (IL-22rα2), and downregulation of the IL-22Rα1 receptor, indicating a tight regulation of IL-22 signaling in the kidney (Fig. 1*B*).

Flow cytometric analyses of leukocytes isolated from nephritic kidneys at the different time points verified induction of IL-22-producing cells in the kidney, which were mainly $CD3^+$ T cells (Fig. 1*C*). In line with the mRNA data, the abundance of IL-22-producing $CD3^+$ cells was elevated at 0.5 days but also revealed a second peak of IL-22 production at *day 7* after induction of cGN (Fig. 1, *C* and *D*). As demonstrated by using *Il22*$^{-/-}$ mice as a staining control, the apparent positivity of $CD3^-$ cells for IL-22 that was observed at several time points was primarily due to unspecific binding of the IL-22 antibody by $CD3^-Ly6G^+$ neutrophils (Fig. 1*E*), arguing against a sig-

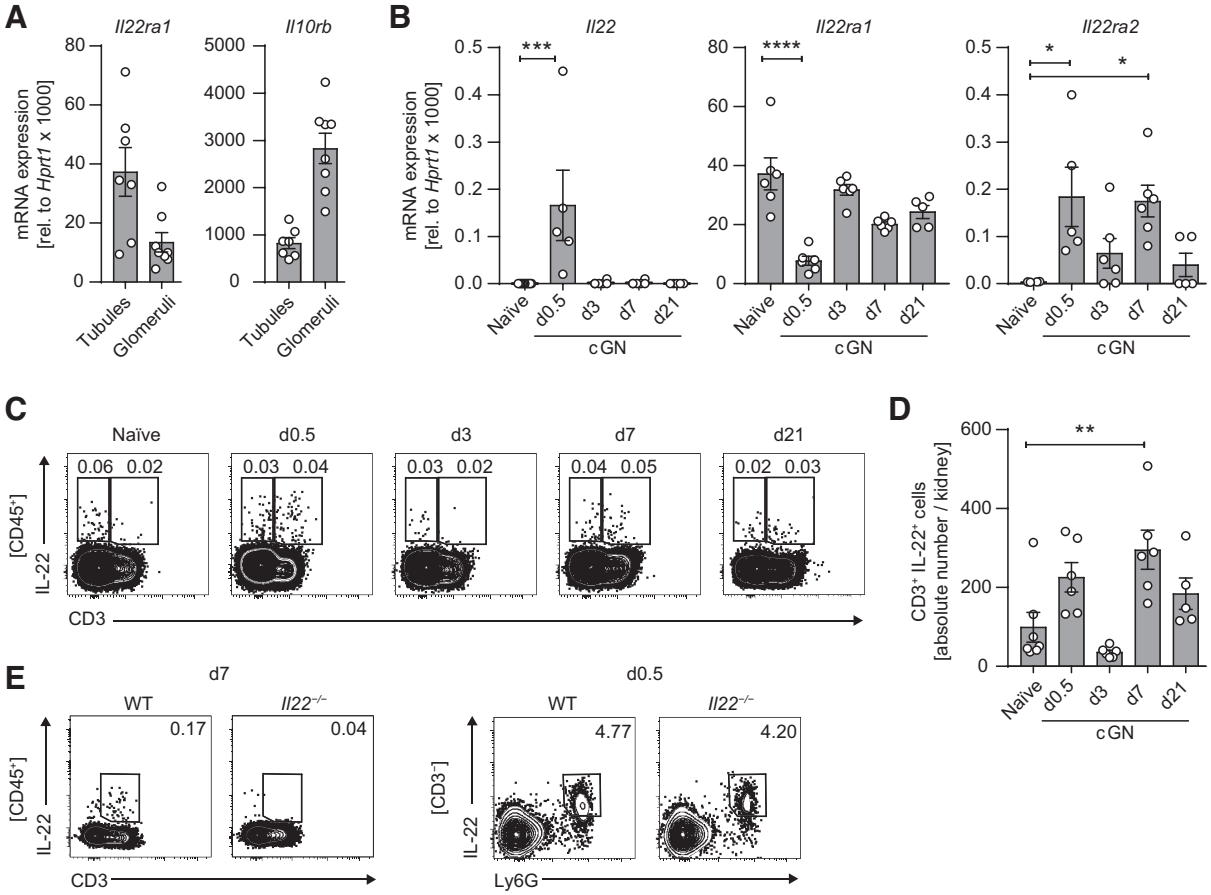

Fig. 1. Expression of IL-22 and its receptor in the kidney of naïve and nephritic mice. *A*: quantitative RT-PCR analysis of IL-22 receptor subunit transcripts in the tubular and glomerular compartment of the kidney in naïve wild-type (WT) C57BL/6 mice (n = 7–8). *B*: quantitative RT-PCR analysis of IL-22, IL-22Ra1, and IL-22bp (IL-22Ra2) transcripts in the renal cortex of naïve WT mice and nephritic WT mice at different time points after induction of crescentic glomerulonephritis (cGN) (n = 5–6/group). *C*: representative flow cytometric analysis of kidney leukocytes after stimulation with phorbol 12-myristate,13-acetate (PMA) and ionomycin at the different time points of cGN. Numbers indicate the percentage of cells in the respective gates. *D*: quantification of IL-22-producing T cells per kidney (n = 5–6). *E*: specificity of IL-22 staining was examined by flow cytometric analysis of kidney leukocytes from WT mice and IL-22-deficient littermates at *days 0.5* and *7* of cGN. Representative plots show a specific $CD3^+IL22^+$ population and unspecific IL-22 antibody binding by $Ly6G^+$ neutrophils. Numbers indicate the percentage of cells in the gates. Gating strategy is specified in brackets. All data are pooled from at least 2 independent experiments with similar results. Bars represent means ± SE; open circles represent individual animals (****$P < 0.0001$, ***$P < 0.001$, ** $P < 0.01$, and *$P < 0.05$).

nificant contribution of myeloid cells and ILC3s to IL-22 production in this model of cGN.

Taken together, these data illustrate the presence of the IL-22 receptor in the glomerular compartment and an inflammation-dependent upregulation of IL-22 in the murine kidney in cGN.

Cellular sources of IL-22 in experimental cGN. To further characterize the cellular sources of IL-22 in experimental cGN, we performed more detailed flow cytometric analyses of the IL-22$^+$CD3$^+$ cells at *days 0.5* and *7* after induction of the model (Fig. 2). These analyses revealed that ~70% of IL-22-producing cells at the early IL-22 expression peak were γδ T cells, whereas, during the second expression peak at *day 7*, IL-22 was preferentially produced by CD4$^+$ T cells (Fig. 2, *A* and *B*). Furthermore, coexpression analysis of IL-22 with the CD4$^+$ T helper cell lineage-defining cytokines IL-17A and IFN-γ showed that IL-22 was mainly expressed by T_H17 cells and IL-17A$^+$ γδ T cells, but not by IFN-γ$^+$ T_H1 cells (Fig. 2, *C* and *D*). In summary, these data show a time-dependent cellular source of IL-22, switching from γδ T cells as the main IL-22$^+$ subset in the early phase to T_H17 cells in later stages of the model. As shown previously (12, 22), IL-17A- and IFN-γ-producing T cell subsets were significantly upregulated during the course of cGN (Fig. 2, *E* and *F*), providing alternative cytokine pathways that can mediate glomerular injury in this model.

Humoral and cellular immune responses in Il22$^{-/-}$ mice with cGN. To investigate the potential role of IL-22 in experimental cGN, we induced the nephrotoxic nephritis model in C57BL6/J *Il22$^{-/-}$* mice and their wild-type littermates. In this cGN model, administration of nephrotoxic sheep IgG directed against components of the glomerular basement membrane (GBM) induces complement activation in the glomeruli leading to early neutrophil recruitment and glomerular damage in the heterologous phase (9). To exclude differences between *Il22$^{-/-}$* and wild-type mice with respect to sheep IgG and complement C3 binding at the GBM, we analyzed kidney sections of the groups of nephritic mice by immunohistochemistry and immunofluorescence staining. These analyses revealed similar amounts of glomerular sheep IgG and comple-

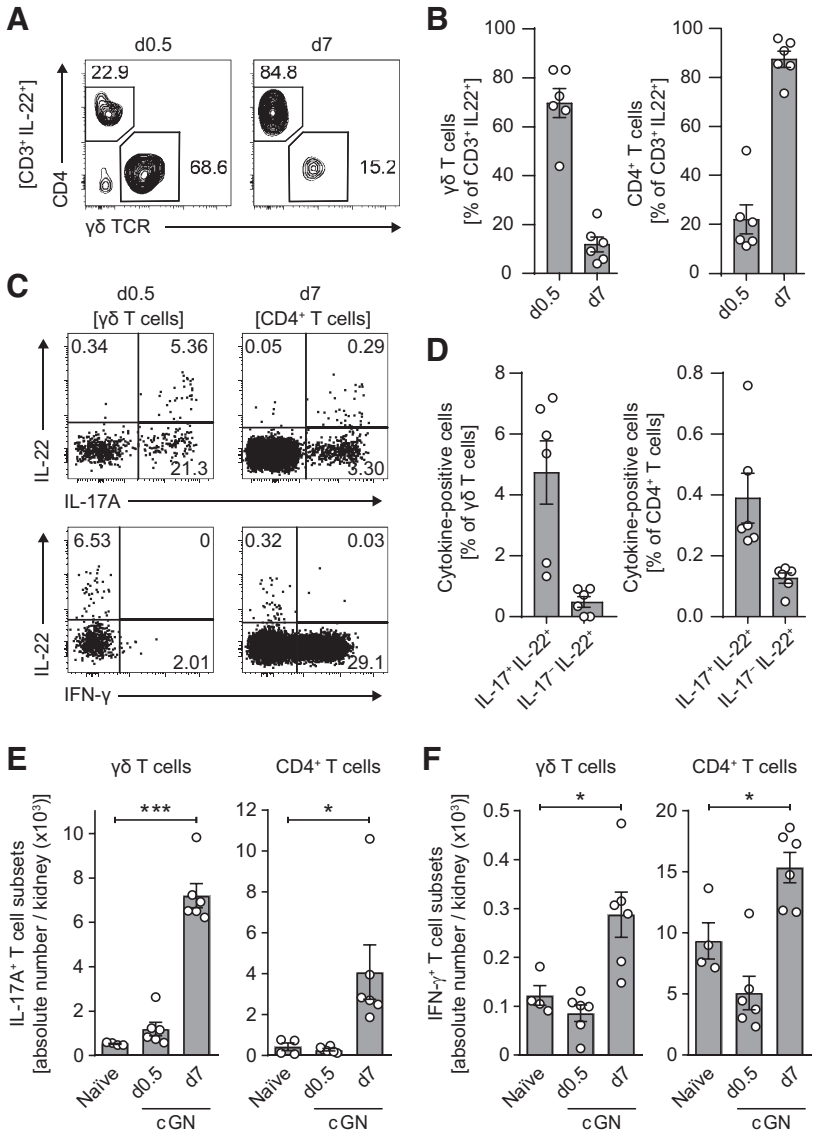

Fig. 2. Time-dependent cellular source of IL-22 during crescentic glomerulonephritis (cGN). *A*: representative flow cytometric analysis of IL-22-producing T cells in wild-type (WT) C57BL/6 mice at 0.5 and 7 days after induction of cGN. Numbers indicate the percentage of cells in the gates. *B*: frequency of γδ T and CD4$^+$ T cells within the IL-22$^+$CD3$^+$ gate at the respective time points (n = 6). *C*: representative flow cytometric analysis of kidney leukocytes stimulated with phorbol 12-myristate,13-acetate (PMA)/ionomycin shows coexpression of IL-22 with IL-17A. Numbers indicate the percentage of cells in the quadrants. *D*: frequency of IL-17A$^+$IL-22$^+$ and IL-17A$^-$IL-22$^+$ cells in the γδ T and CD4$^+$ T cell population of the murine kidney at *days 0.5* and *7* of cGN (n = 6). Gating strategies are specified in brackets. *E* and *F*: quantification of IL-17A- and IFN-γ-producing T cell subsets per kidney (n = 5–6). All data are pooled from at least 2 independent experiments with similar results. Bars represent means ± SE; open circles represent individual animals (***$P < 0.001$ and *$P < 0.05$).

ment C3 deposition in *Il22*$^{-/-}$ and wild-type mice after cGN induction at *day 7* (Fig. 3, *A* and *B*). In the subsequent autologous phase of the cGN model, a humoral immune response against the deposited sheep IgG results in glomerular mouse IgG deposition, further recruitment of immune cells, and immune-mediated glomerular damage. To examine if IL-22 deficiency influences the humoral immune response against sheep IgG as the nephritic antigen, we performed immunohistochemistry for mouse IgG deposition in the glomeruli and measured serum levels of sheep IgG-specific total mouse IgG in wild-type and *Il22*$^{-/-}$ mice at different time points of cGN. However, we did not detect differences in these parameters between the two groups of mice (Fig. 3, *C–E*).

Infiltration of immune cells in the kidney is the crucial driver of tissue damage in human and murine cGN (3). As IL-22 has been shown to influence leukocyte recruitment by inducing production of chemoattractants in epithelial cells (17), we assessed if IL-22 deficiency alters immune cell infiltration during the course of

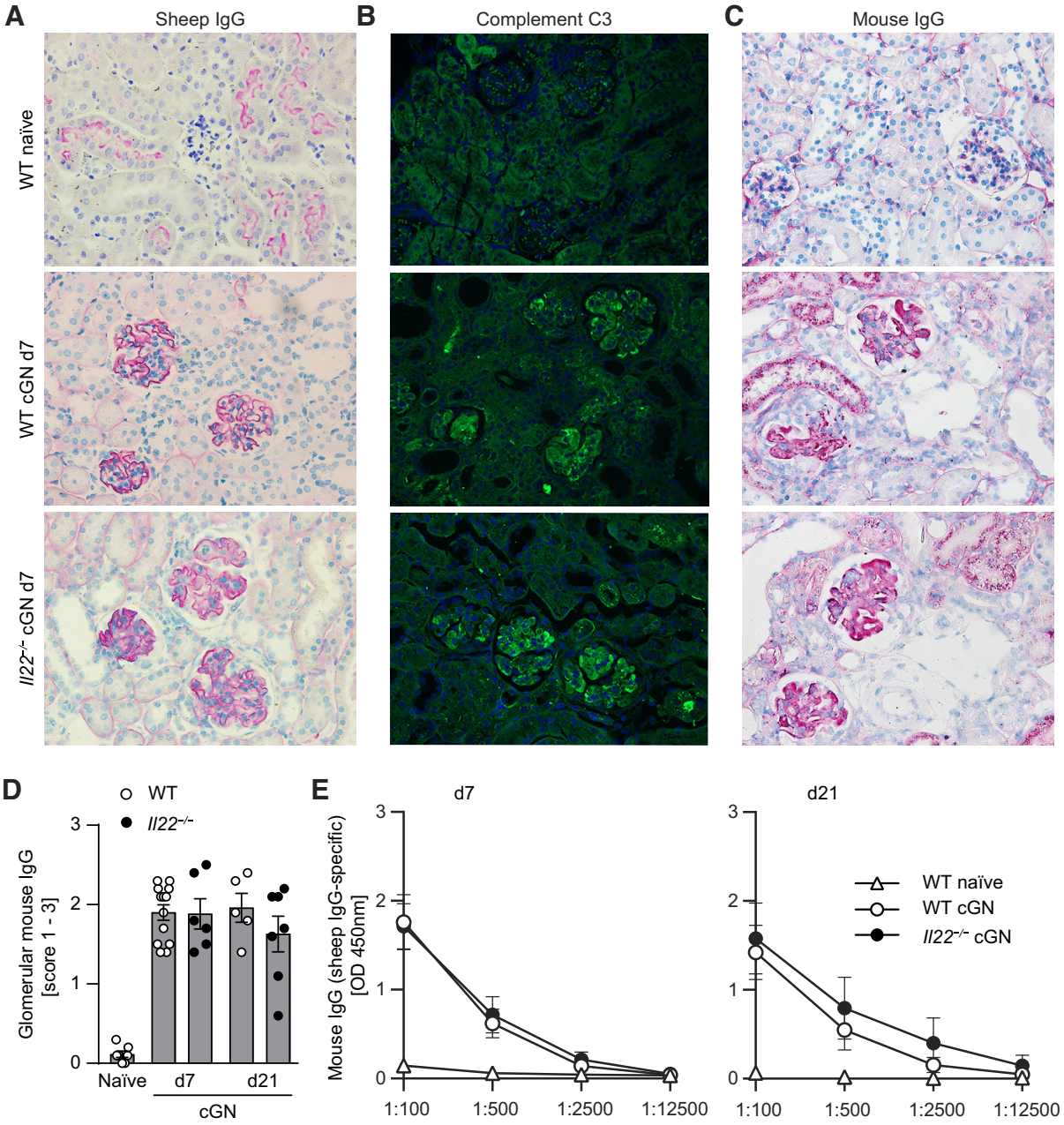

Fig. 3. Humoral immune response in *Il22*$^{-/-}$ mice during the course of crescentic glomerulonephritis (cGN). *A–C*: representative photographs of kidney sections stained immunohistochemically with anti-sheep IgG (*A*), anti-complement C3 (*B*), and anti-mouse IgG (*C*) (original magnification ×400) of wild-type (WT) and *Il22*$^{-/-}$ littermates 7 days after induction of cGN. *D*: quantification of glomerular mouse IgG deposition at *day 7* and *21* after induction of cGN in WT mice and *Il22*$^{-/-}$ littermates (naïve WT controls, $n = 7$; WT *day 7*, $n = 14$; WT *day 21*, $n = 5$; *Il22*$^{-/-}$ *day 7*, $n = 6$; *Il22*$^{-/-}$ *day 21*, $n = 7$). *E*: ELISA of sheep IgG-specific mouse IgG in the serum of WT mice and *Il22*$^{-/-}$ littermates at *day 7* and *21* of cGN (naïve WT controls, $n = 8$; WT *day 7*, $n = 7$; WT *day 21*, $n = 5$; *Il22*$^{-/-}$ *day 7*, $n = 15$; *Il22*$^{-/-}$ *day 21*, $n = 7$). Symbols in *D* and *E* represent means ± SE. Open and filled circles in *D* represent individual animals. Data are pooled from 3 individual experiments with similar results.

experimental cGN. To this end, we characterized the cellular infiltrates in *Il22*$^{-/-}$ and wild-type mice after induction of the model by flow cytometry (Fig. 4, *A–D*). These analyses showed a similar abundance of CD4$^+$ T cells, CD8$^+$ T cells, and γδ T cells, as well as the pathogenic T_H17 and T_H1 cell subsets, in the two groups of mice at early and late time points (Fig. 4, *A–C*). Similarly, the infiltration of the major myeloid cell subsets, e.g., neutrophils and mononuclear phagocytes, seemed to be unaffected by IL-22 deficiency in experimental cGN (Fig. 4, *A* and *D*). In summary, these data indicate a largely unaltered immune response in *Il22*$^{-/-}$ mice in this model for cGN.

IL-22 deficiency does not impact renal tissue damage in experimental cGN. IL-22 has been described to promote tissue protection at barrier surfaces by directly inducing epithelial cell regeneration (18). Therefore, we hypothesized that, although the immune response in *Il22*$^{-/-}$ mice was found to be unchanged, IL-22 expression might confer renal tissue protection in cGN by direct activation of IL-22R signaling in glomerular or tubular cells. To address this hypothesis, we analyzed renal histopathology and renal function parameters at *days 7* and *21* after induction of cGN in *Il22*$^{-/-}$ mice and their wild-type littermates.

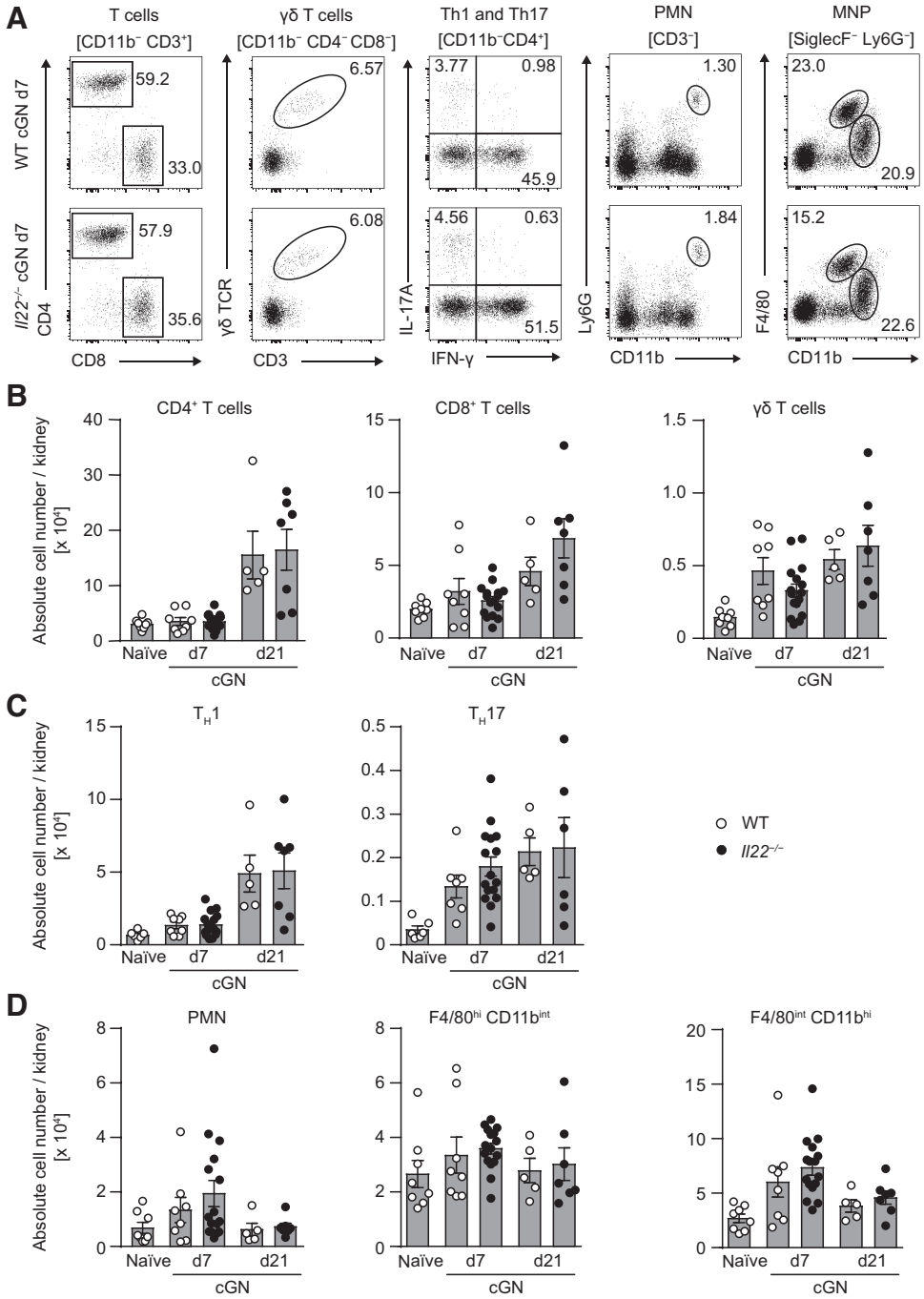

Fig. 4. Cellular immune response in the kidneys of wild-type (WT) and *Il22*$^{-/-}$ mice with crescentic glomerulonephritis (cGN). *A*: representative flow cytometry plots of renal leukocytes isolated from naïve and nephritic WT mice and their *Il22*$^{-/-}$ littermates at *day 7* of cGN. Numbers indicate the percentage of cells in the gates or quadrants. *B–D*: quantification of the absolute numbers of T (*B* and *C*) and myeloid (*D*) cell subsets per kidney at the indicated time points (naïve WT controls, $n = 8$; WT *day 7*, $n = 8$; WT *day 21*, $n = 6$; *Il22*$^{-/-}$ *day 7*, $n = 16$; *Il22−/− day 21*, $n = 7$). Symbols in *B–D* represent means ± SE; open and filled circles represent individual animals. Data are pooled from 3 individual experiments with similar results.

These analyses showed a similar degree of glomerular and tubulointerstitial tissue damage in wild-type and *Il22*$^{-/-}$ mice, as assessed by quantification of crescent formation and interstitial widening in periodic acid-Schiff (PAS)-stained kidney sections (Fig. 5, *A* and *B*). Moreover, albuminuria, hyperlipidemia, and renal function impairment observed in the cGN model were unaltered by IL-22 deficiency (Fig. 5*C*). In addition, we examined the long-term effects of IL-22 deficiency on repair mechanisms in the cGN model. These analyses 6 wk after induction of cGN showed that absence of IL-22 did not substantially alter renal histopathology, albuminuria, cholesterol levels, or BUN (Fig. 5, *D* and *E*, and data not shown). These analyses indicate that endogenous IL-22 is dispensable for experimental cGN.

Antibody-mediated depletion of IL-22 does not impact renal tissue damage in experimental cGN. To circumvent activation of alternate compensatory mechanisms potentially evoked by genetic deletion of *Il22*, we investigated the effect of antibody-mediated neutralization of IL-22 on the course of experimental cGN. Similar to the results obtained in *Il22*$^{-/-}$ mice, renal histopathology (Fig. 6*A*) and renal function parameters (Fig. 6*B*) in anti-IL-22-antibody-treated mice at *day 7* after induction of cGN were similar to those in mice treated with the isotype control.

Deficiency of IL-22Rα2 does not impact renal tissue damage in experimental cGN. Because expression of IL-22-binding protein (IL-22Rα2) was found to be upregulated in parallel with IL-22 in experimental cGN (see Fig. 1, *B* and *D*), we hypothesized that the IL-22 effect in this model might be strictly limited by its endogenous inhibitor. Therefore, to elucidate the effect of increased IL-22 signaling in the diseased kidney, we assessed mice that are genetically deficient in the endogenous inhibitor IL-22Rα2 at *day 7* after induction of cGN. In these analyses, neither renal histopathology (Fig. 7*A*) nor clinical parameters (Fig. 7*B*) were substantially different between wild-type and *Il22ra2*$^{-/-}$ mice, indicating that unrestricted signaling of endogenous IL-22 does not influence induction or repair mechanisms of experimental cGN.

IL-22 deficiency does not impact renal tissue damage in experimental focal and segmental glomerulosclerosis. Because kidney damage in the mouse model for cGN is primarily immune mediated, we asked the question whether IL-22 might play a role in a mouse model for glomerular injury that is based on direct injury of glomerular epithelial cells. Therefore, we induced adriamycin-induced nephropathy (AN), a mouse model for human secondary focal and segmental glomerulosclerosis (FSGS), in *Il22*$^{-/-}$ mice that were backcrossed to the BALB/c background for at least 10 generations and in their BALB/c wild-type littermates. At *day 14* after induction of AN, we could detect an upregulation of IL-22 expression by CD4^{+} T cells in the kidney of wild-type animals (Fig. 8, *A* and *B*). However, as in the cGN model, histopathological examination of PAS-stained kidney sections showed no difference between wild-type and *Il22*$^{-/-}$ mice in terms of glomerulosclerosis and tubular damage (Fig. 8, *C* and *D*). Furthermore, the analyses of albuminuria, cholesterol, and blood urea nitrogen levels did not reveal an impact of IL-22 deficiency on the renal outcome of the FSGS model (Fig. 8*E*).

Taken together, we can conclude that endogenous IL-22 does not show a regenerative or proinflammatory function in the mouse models for glomerular injury investigated in this study.

DISCUSSION

Depending on the tissue microenvironment and inflammatory context, the cytokine IL-22 can perpetuate inflammation or facilitate resolution of injury and promote restoration of tissue integrity. Especially, the beneficial effect of IL-22 with respect to epithelial regeneration after injury identifies it as a promising therapeutic target also in kidney diseases. In fact, the kidney is one of the organs that shows substantial baseline expression of the IL-22 receptor (20, 26), indicating that there might be a role for IL-22 in renal homeostasis and/or inflammation. In line with this concept, recent studies have linked IL-22 signaling to tubular regeneration after renal ischemia-reperfusion injury in mice (8, 27). In these studies, the IL-22 receptor was found to be expressed predominantly in tubular epithelial cells and to promote their survival by increasing expression of antiapoptotic genes and reducing expression of inflammatory mediators. However, studies addressing a potential role of IL-22 in immune-mediated glomerular diseases have not been reported so far.

We show here that the IL-22 receptor is expressed in the glomerular compartment of the kidney and that γδ T cells and T_H17 cells time dependently upregulate IL-22 expression in a mouse model for cGN. However, *Il22*$^{-/-}$ mice were able to mount an unaltered systemic and intrarenal immune response and did not show differences with respect to glomerular damage and kidney function impairment in two experimental mouse models of glomerular disease. Furthermore, neither antibody-mediated neutralization of IL-22 nor genetic deficiency in its endogenous inhibitor IL-22-binding protein resulted in a substantial alteration of the course of experimental cGN.

In the cGN model, we observed an early upregulation of IL-22 mRNA expression already 12 h after induction of injury, followed by a rapid decrease toward baseline levels. This expression pattern was similar to the IL-22 expression kinetic observed in acute tubular injury models (25), indicating that IL-22 might act as part of an immediate inflammatory response to different types of kidney damage. In line with the previous notion that γδ T cells are important early responders in experimental cGN (22), we found rapid induction of IL-22 expression in γδ T cells, whereas T_H17 cells were the main producers of IL-22 at later stages. Similar changes of the predominant source of IL-22 during the course of an inflammatory reaction have been described in the intestine and skin. For example, in the mouse intestine, ILC3s are known to be the main producers of IL-22 under homeostatic conditions and at the early stages of *Citrobacter rodentium* infection, whereas IL-22^{+}CD4^{+} T cells substantially expand at later stages (1). In a mouse model for psoriasis, IL-22^{+}CD4^{+} T cells expand together with γδ T cells when inflammation progresses, whereas in the steady state IL-22 is mainly provided by γδ T cells alone (1). In most of the studies, IL-22 production in CD4^{+} T cells was found to be associated with IL-17A expression and has therefore been attributed to the T_H17 cell subset while the evidence for a specialized "T_H22" (at least in the mouse) is still scarce (10). Production of IL-22 by nonlymphoid cells has also been described (6, 29), but, in contrast to previous reports from

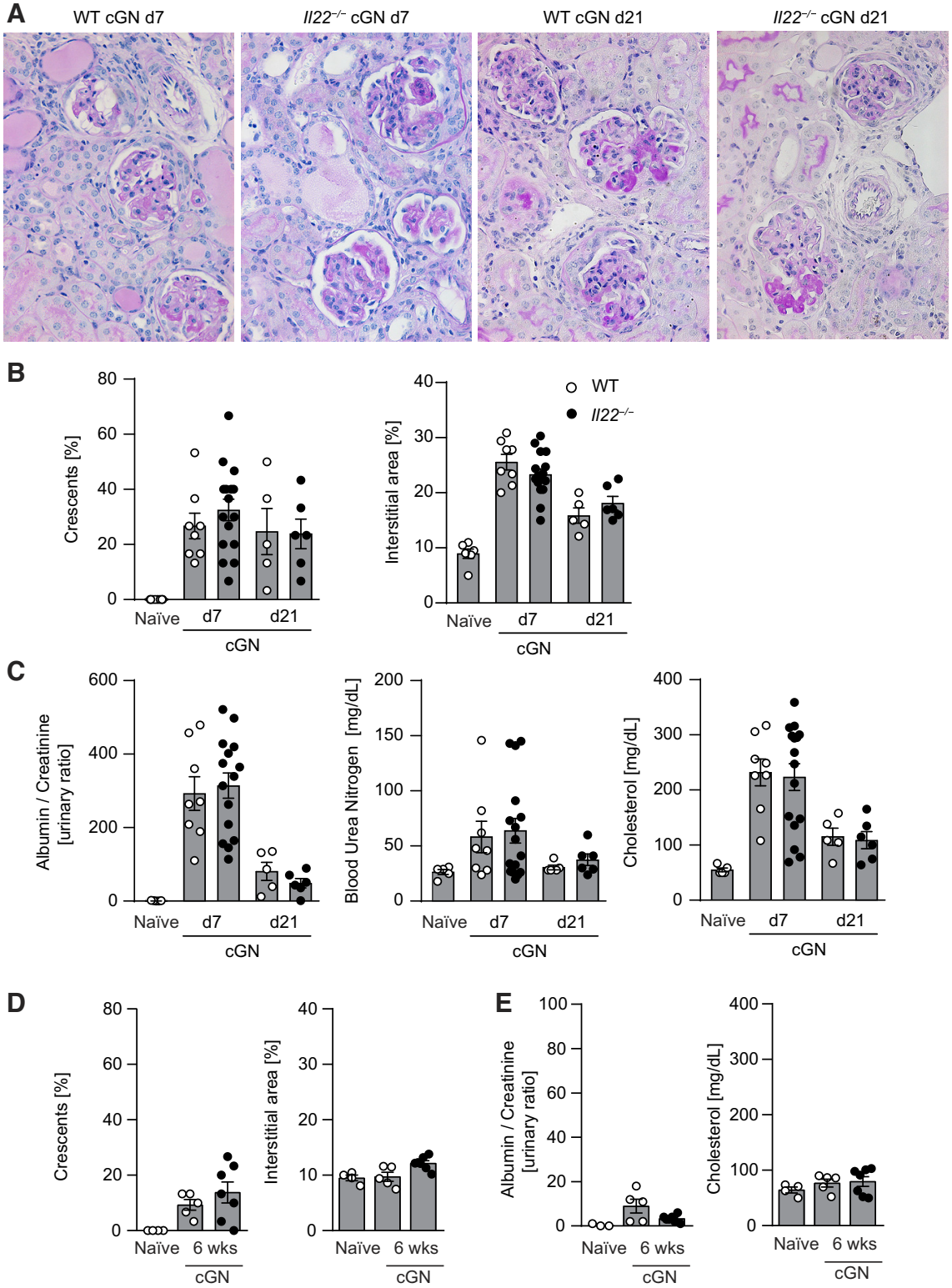

Fig. 5. Tissue damage and renal function parameters of wild-type (WT) and *Il22*$^{-/-}$ mice in crescentic glomerulonephritis (cGN). Representative photographs of periodic acid-Schiff-stained kidney sections (original magnification ×400) (*A*), histopathological quantification of glomerular and tubulointerstitial damage (*B*), and analysis of renal function parameters (*C*) in WT mice and *Il22*$^{-/-}$ littermates at 7 and 21 days after induction of cGN (naïve WT control, n = 5–8; WT *day 7*, n = 8; WT *day 21*, n = 5; *Il22*$^{-/-}$ *day 7*, n = 16; *Il22*$^{-/-}$ *day 21*, n = 4–6). Quantification of renal damage 6 wk after induction of cGN assessed by histopathological analysis of crescent formation and interstitial widening (*D*) and renal function parameters (*E*) in IL-22-deficient mice and WT animals. Symbols in *B*–*E* represent means ± SE; open and filled circles represent individual animals. Data are pooled from at least 3 individual experiments with similar results.

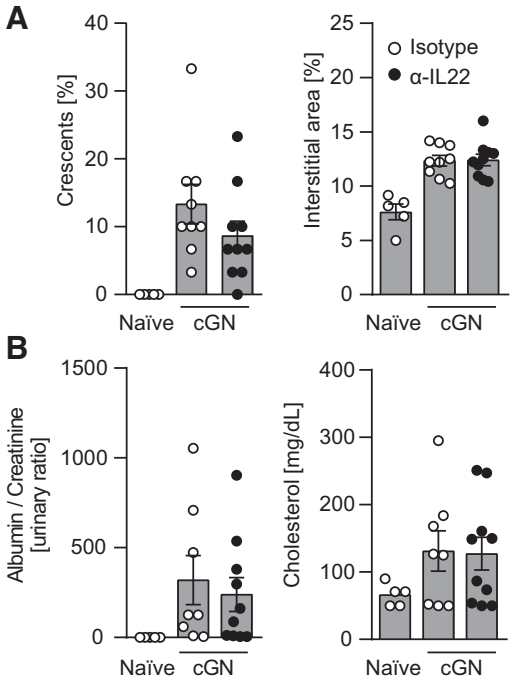

Fig. 6. Antibody-mediated neutralization of IL-22 in the crescentic glomerulonephritis (cGN) model. Histopathological quantification of glomerular and tubulointerstitial damage (*A*) and analysis of renal function parameters (*B*) in anti-IL-22 antibody- and isotype-treated C57BL/6 wild-type (WT) mice and naïve C57BL/6 WT animals 7 days after induction of cGN (naïve WT controls, $n = 5$; α-IL22, $n = 9$; α-IgGa2, $n = 10$). Symbols in *A* and *B* represent means ± SE; open and filled circles represent individual animals. Data are pooled from 2 individual experiments with similar results.

ischemia- and reperfusion-induced kidney injury (8), we were unable to detect specific IL-22 protein staining in the myeloid cell compartment of renal leukocytes in our study. Furthermore, we did not detect IL-22 production by CD3-negative lymphoid cells, excluding a major role of renal ILC3s as a potential source of IL-22, corroborating the previous observation that the ILC3 subset is very rare in the mouse kidney (15).

The evident expression of IL-22 in the inflamed kidney after induction of cGN raised the question whether it might serve as an important regulator of immune-mediated glomerular injury, and, so far, there were no detailed studies addressing this issue (25). Our comprehensive characterization of IL-22-deficient mice and their littermate controls in two well-established mouse models provides substantial evidence that endogenous IL-22 is redundant for initiation and control of glomerular injury. Potential compensatory mechanisms in mice with a genetic deletion of IL-22 were excluded by using anti-IL-22 antibody treatment, which did not influence immune-mediated renal injury in the cGN model, thereby supporting the data obtained with *Il22*$^{-/-}$ mice. Although the results presented here are mainly negative findings, they clearly underscore the central importance of alternative cytokine pathways, such as the IL-23/Th17 and Th1 axes that were both significantly upregulated in the cGN model, for initiation and progression of crescentic GN. However, one previous study that investigated the immunomodulatory function of a parasitic worm product in the MRL/MPJ-*Fas*^{lpr} model of lupus nephritis showed that IL-22 might be able to promote autoimmune kidney injury in this context (16). In a mouse model of diabetic nephropathy, in contrast, IL-22 overexpression by therapeutic gene transfer resulted in alleviation of glomerular injury (24), an effect that was in part mediated by IL-22-dependent suppression of the NOD-, LRR- and pyrin domain-containing 3 (NLRP3) inflammasome. These apparently divergent roles of IL-22 in glomerular injury may be explained by the different context of IL-22 expression, since synergistic proinflammatory effects of IL-22 can depend on coproduction of other proinflammatory cytokines, presumably being more pronounced in the lupus nephritis model than in experimental diabetic nephropathy. Moreover, excessive overexpression of IL-22 by gene transfer has systemic effects, e.g., on metabolic control, that might contribute to its beneficial effects in the diabetes model (24).

With respect to a potential involvement of IL-22 in human glomerulonephritis, the data are so far very limited and partially controversial. One study found increased frequencies of IL-22^{+}CD4^{+} T cells in patients with renal involvement of systemic lupus erythematosus and showed that they correlate with disease activity (14). Conversely, other studies described a decrease of IL-22^{+}CD4^{+} T cells (28) or urinary IL-22 mRNA levels (11) in patients with active lupus nephritis. Furthermore, a single nucleotide polymorphism in the IL-22R locus was found to be associated with the development of IgA nephropathy in children (19), and increased IL-22^{+}CD4^{+} T cells and IL-22 plasma levels in IgA nephropathy patients have also been reported (13). However, the functional significance of these findings remains to be elucidated.

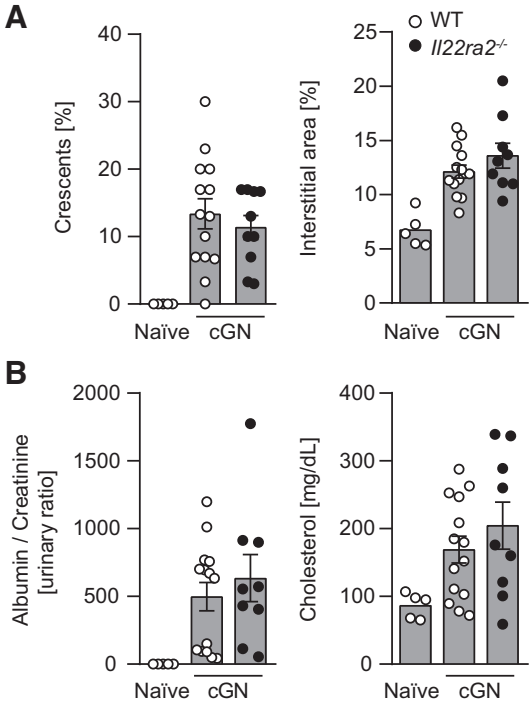

Fig. 7. Phenotype of *Il22ra2*$^{-/-}$ mice in the crescentic glomerulonephritis (cGN) model. Histopathological quantification of glomerular and tubulointerstitial damage (*A*) and analysis of renal function parameters (*B*) in naïve C57BL/6 wild-type (WT) mice and C57BL/6 WT and *Il22ra2*$^{-/-}$ mice at *day 7* after induction of cGN (naïve WT controls, $n = 5$; cGN WT, $n = 13$; cGN *Il22ra2*$^{-/-}$, $n = 9$). Symbols in *A* and *B* represent means ± SE; open and filled circles represent individual animals. Data are pooled from 2 individual experiments with similar results.

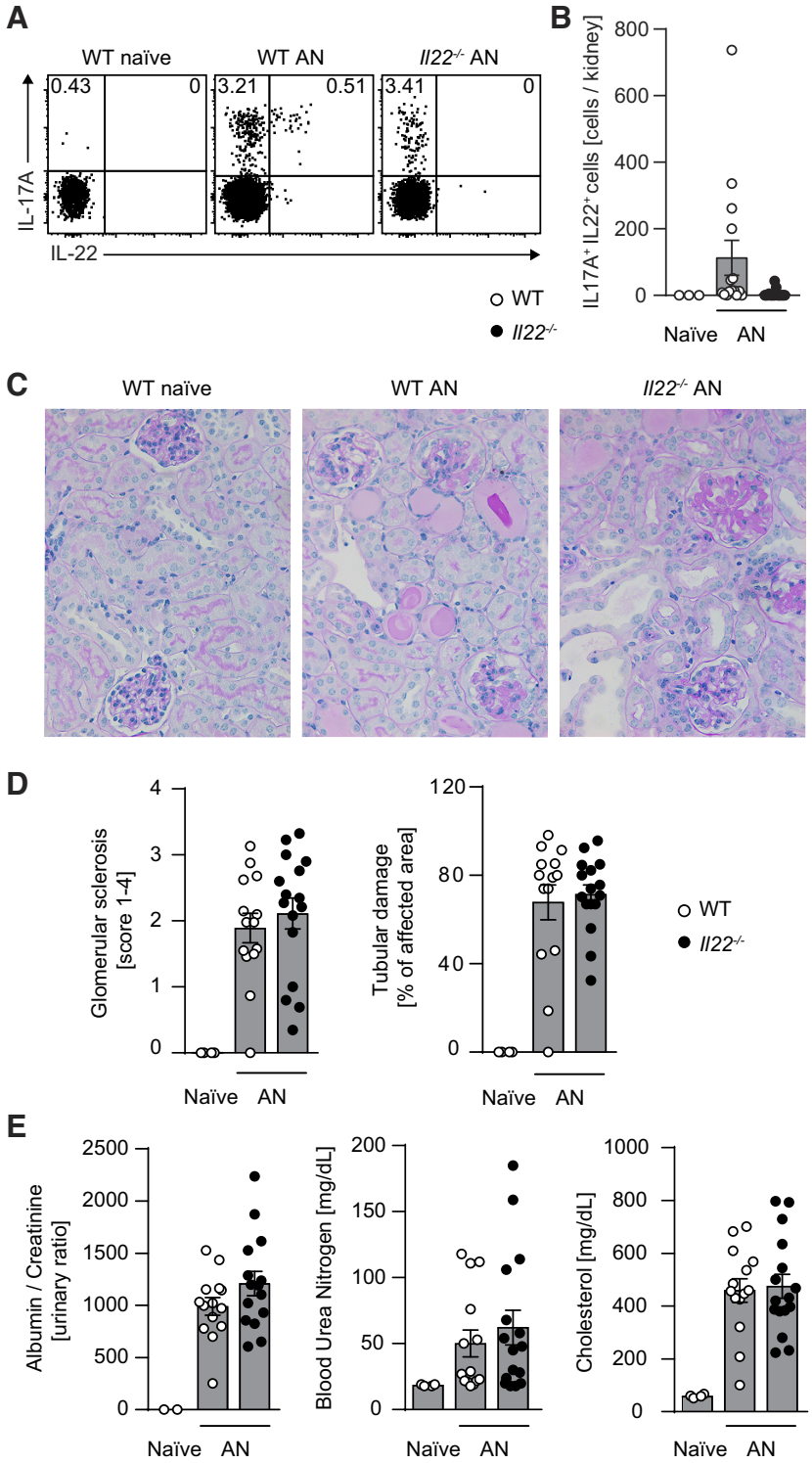

Fig. 8. Phenotype of *Il22*$^{-/-}$ mice in secondary focal and segmental glomerulosclerosis (FSGS). *A*: representative flow cytometric analysis of IL-22 expression by renal CD4$^+$ T cells at *day 14* after induction of adriamycin nephropathy (AN) in naïve BALB/c wild-type (WT) mice, AN WT mice, and AN *Il22*$^{-/-}$ littermates. Numbers indicate the percentage of cells in the gates. *B*: absolute number of IL-17A$^+$IL-22$^+$ CD4$^+$ T cells per kidney (AN WT, $n = 15$; AN KO, $n = 15$). *C*: representative photographs of periodic acid-Schiff-stained kidney sections (original magnification ×200) (*C*), histopathological quantification of glomerular and tubulointerstitial damage (*D*), and analysis of renal function parameters (*E*) in naïve BALB/c WT mice and BALB/c WT and *Il22*$^{-/-}$ littermates at *day 14* after induction of adriamycin nephropathy (AN; naïve WT controls, $n = 4$; AN WT, $n = 14$; AN *Il22*$^{-/-}$, $n = 16$). Symbols in *D* and *E* represent means ± SE; open and filled circles represent individual animals. Data are pooled from 2 individual experiments with similar results.

Another noteworthy observation in the present study is the substantial mRNA expression of the IL-22-binding protein (IL-22bp or IL-22Rα2), the endogenous inhibitor of IL-22, in the renal cortex. Interestingly, the upregulation of IL-22bp closely paralleled the kinetic of IL-22 expression in the cGN model (see Fig. 1, *B* and *D*), suggesting a tight control mechanism that prevents excessive IL-22 signaling in renal inflammation. To assess whether this regulatory pathway prevents endogenous IL-22 from exhibiting pro- or anti-inflammatory effects in glomerular injury, we analyzed IL-22bp-deficient mice in the cGN model. However, even in this setting of unrestricted signaling, we did not find a substantial function for endogenous IL-22 production in immune-mediated glomerular damage.

In conclusion, our data demonstrate that the immunoregulatory cytokine IL-22 is dispensable for promoting tissue inflam-

mation or protection in experimental glomerulonephritis, indicating that the IL-22-directed therapeutic approaches that are being tested in various human diseases (17) might not be beneficial in glomerulonephritis but might also not show detrimental renal side effects.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

A.-C.G., J.-C.R., S.H., U.P., and J.-E.T. conceived and designed research; A.-C.G., M.W., M.B., T.X., E.W., C.M.-S., L.D., J.-C.R., S.H., U.P., and J.-E.T. performed experiments; A.-C.G., M.W., M.B., T.X., E.W., C.M.-S., S.H., and J.-E.T. analyzed data; A.-C.G., M.W., M.B., L.D., J.-C.R., S.H., U.P., and J.-E.T. interpreted results of experiments; A.-C.G., M.B., S.H., and J.-E.T. prepared figures; A.-C.G., S.H., and J.-E.T. drafted manuscript; A.-C.G., M.W., M.B., T.X., C.M.-S., L.D., J.-C.R., S.H., U.P., and J.-E.T. edited and revised manuscript; A.-C.G., M.W., M.B., T.X., E.W., C.M.-S., L.D., J.-C.R., S.H., U.P., and J.-E.T. approved final version of manuscript.

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IL-33–Mediated Expansion of Type 2 Innate Lymphoid Cells Protects from Progressive Glomerulosclerosis

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ABSTRACT

Innate lymphoid cells (ILCs) have an important role in the immune system's response to different forms of infectious and noninfectious pathologies. In particular, IL-5– and IL-13–producing type 2 ILCs (ILC2s) have been implicated in repair mechanisms that restore tissue integrity after injury. However, the presence of renal ILCs in humans has not been reported. In this study, we show that ILC populations are present in the healthy human kidney. A detailed characterization of kidney-residing ILC populations revealed that IL-33 receptor–positive ILC2s are a major ILC subtype in the kidney of humans and mice. Short-term IL-33 treatment in mice led to sustained expansion of IL-33 receptor–positive kidney ILC2s and ameliorated adriamycin-induced glomerulosclerosis. Furthermore, the expansion of ILC2s modulated the inflammatory response in the diseased kidney in favor of an anti-inflammatory milieu with a reduction of pathogenic myeloid cell infiltration and a marked accumulation of eosinophils that was required for tissue protection. In summary, kidney-residing ILC2s can be effectively expanded in the mouse kidney by IL-33 treatment and are central regulators of renal repair mechanisms. The presence of ILC2s in the human kidney tissue identifies these cells as attractive therapeutic targets for CKD in humans.

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CKD affects around 10% of the Western population and is a major risk factor for cardiovascular mortality.[1] Regardless of the underlying cause, glomerular injury regularly results in proteinuria and progressive glomerulosclerosis with deteriorating kidney function.[2] In recent years, it has become evident that the immune system's response to the initial injury can critically contribute to the progression of kidney damage. However, regulatory components of the immune system can also promote resolution of kidney injury and limit chronic inflammation.[3] To develop novel therapeutic strategies for progressive CKD, it is therefore necessary to identify the immune cells and mediators that can shift the balance from chronic kidney inflammation toward resolution and restoration of tissue integrity.

Innate lymphoid cells (ILCs) are a newly described group of leukocytes that is defined by its lymphoid morphology and antigen-independent activation. According to a recently proposed nomenclature, ILCs can be subdivided into four groups that include conventional natural killer (NK) cells and three subsets of "helper-like" ILCs, differing in their tissue localization and functional characteristics.[4] The helper-like ILC subsets are defined by their distinct profiles of cytokine production and transcription factor usage and referred to as T-bet$^+$IFN-γ^+ ILC1s, GATA3$^+$IL-5$^+$IL-13$^+$

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ILC2s, and ROR-γt$^+$IL-17A$^+$ and/or IL-22$^+$ ILC3s. These ILC subtypes have mainly been studied in barrier organs, such as the gut, lung, and skin, where they promote tissue homeostasis and defense against different classes of pathogens, but also contribute to allergic and autoimmune diseases in mice and humans.[5,6] Recent evidence suggests that ILC2s especially are central regulators of repair mechanisms that are aimed at restoring homeostasis after acute injury in the lung,[7,8] intestine,[9] and skin.[10]

The question of whether helper-like ILC subsets reside in the kidney of humans and mice and, if so, whether they might be therapeutically exploited in chronic kidney inflammation, has not been addressed so far.

In this study, we identify and characterize resident ILC populations in the human kidney for the first time. We further show that IL-33 receptor-positive (IL-33R$^+$) ILC2s are a major ILC subtype in the healthy human and murine kidney. In line with their tissue regenerative capacity, expansion of ILC2s by short-term IL-33 treatment ameliorated progressive glomerulosclerosis and loss of kidney function induced by adriamycin injection in mice, a model of chronic kidney injury. ILC2s modulated the inflammatory response in the diseased kidney in favor of an anti-inflammatory milieu with a reduction of pathogenic neutrophils and mononuclear phagocytes and an increase of tissue-protective mediators, such as eosinophils and alternatively activated macrophages. We further show that the accumulation of eosinophils was required for the tissue-protective effect of ILC2s. Thus, we established that targeting ILC2s is a promising therapeutic strategy to promote tissue regeneration in progressive kidney disease.

RESULTS

Distribution of Helper-Like ILC Subtypes Residing in the Naïve Human and Murine Kidney

Helper-like ILCs (hILCs) are a largely tissue-resident population of lymphocytes that varies in frequency between different organs and shows a tissue-specific distribution of the ILC1, ILC2, and ILC3 subtypes.[11] So far, there are no reports on resident ILC populations in the human kidney. Flow cytometric analysis of leukocytes isolated from healthy human kidney cortex showed that approximately 0.4% of renal lymphocytes displayed an hILC phenotype, defined as lineage marker–negative (Lin$^-$), IL-7Rα–positive (CD127$^+$). The vast majority of the Lin$^-$CD127$^+$ ILC population in the human kidney expressed the pan-hILC marker CD161 (Figure 1, A and C). Further subtyping revealed that the kidney-residing Lin$^-$CD127$^+$CD161$^+$ population of hILCs contained CRTH2$^+$ ILC2s, NKp44$^+$, and NKp44$^-$ CRTH2$^-$CD117$^+$ ILC3s, as well as a smaller population of CRTH2$^-$CD117$^-$NKp44$^-$ ILC1s (Figure 1, A and D). Similar to the results obtained in human kidney tissue, flow cytometry of leukocytes isolated from the kidney of naïve C57BL/6 mice revealed a population of Lin$^-$CD127$^+$ hILCs that comprised around 0.8% of renal lymphocytes (Figure 1, B and C). Further characterization of hILCs residing in the naïve mouse kidney by transcription factor staining showed a distribution of ILC subtypes similar to the mouse lung,[8,12] with the majority of around 80% being GATA-3$^+$ ILC2s and only minor populations of the (NK cell receptor–negative) ROR-γt$^+$ ILC3s and NK1.1$^+$ and NK1.1$^-$ T-bet$^+$ ILC1 subsets (Figure 1, B and D). Of note, the T-bet$^+$ ILC1 subset was negative for the NK cell transcription factor Eomes, excluding a contaminating NK cell population in the ILC1 gate (data not shown). Consistent with the ILC subtype distribution defined by transcription factor staining and surface marker expression in mice, kidney ILCs restimulated with phorbol 12-myristate 13-acetate and ionomycin mainly produced the type 2 cytokines IL-5 and IL-13, whereas IFN-γ and IL-17A were produced by smaller fractions (Supplemental Figure 1). Similar to ILC2 populations from other tissue locations, human and mouse ILC2s in the kidney expressed the IL-33 receptor T1/ST2 and the IL-2 receptor high-affinity chain CD25 (Figure 1E). Thus, despite a discrepancy in the abundance of the ILC3 subsets between the healthy mouse and human kidney, IL-33R$^+$ ILC2s are a major kidney-residing hILC subset in both species.

Effective Expansion of Kidney-Residing ILC2s by Short-Term IL-33 Treatment

In order to investigate the function of ILC2s in a kidney disease model, we aimed at establishing a strategy to manipulate their abundance in the kidney. Thus, we addressed the question of how kidney IL-33R$^+$ ILC2s respond to short-term IL-33 treatment, a treatment that has been used to expand ILC2s residing in other tissue locations.[13–15] Analysis of leukocytes isolated from the kidneys of C57BL/6 mice 7 days after treatment with four daily doses of recombinant IL-33 (400 ng intraperitoneally each) showed a massive increase in GATA-3$^+$ Lin$^-$ ILC2 frequencies and numbers as compared with PBS-treated controls (Figure 2, A and B). IL-33–induced ILC2 accumulation in the kidney was maintained at a high level for up to 8 weeks after a single course of four IL-33 injections, whereas the ILC2 increase was more transient in the spleen (Figure 2C). In the kidney, ILC2 accumulation was accompanied by a sustained increase of *Il5* and *Il13* mRNA expression (Figure 2D). To assess a potential effect of IL-33 treatment on other kidney-residing leukocyte subsets, we analyzed IL-33R expression on various lymphocytic and myeloid cell populations in the naïve C57BL/6 kidney. These analyses showed that, among leukocyte populations in the murine kidney, high-level IL-33 receptor expression is unique to ILC2s (see Figure 1E, Supplemental Figure 2). However, although eosinophils lacked expression of the IL-33R, they were strongly expanded after IL-33 treatment (Figure 2E), suggesting an indirect effect mediated by ILC2-derived IL-5.[16] As compared with ILC2s and eosinophils, leukocyte populations in the kidney, spleen, and peripheral blood were only modestly altered in response to IL-33 treatment (Figure 2E, Supplemental Figures 2B and 3, A and B). Consistent with previous reports about IL-33

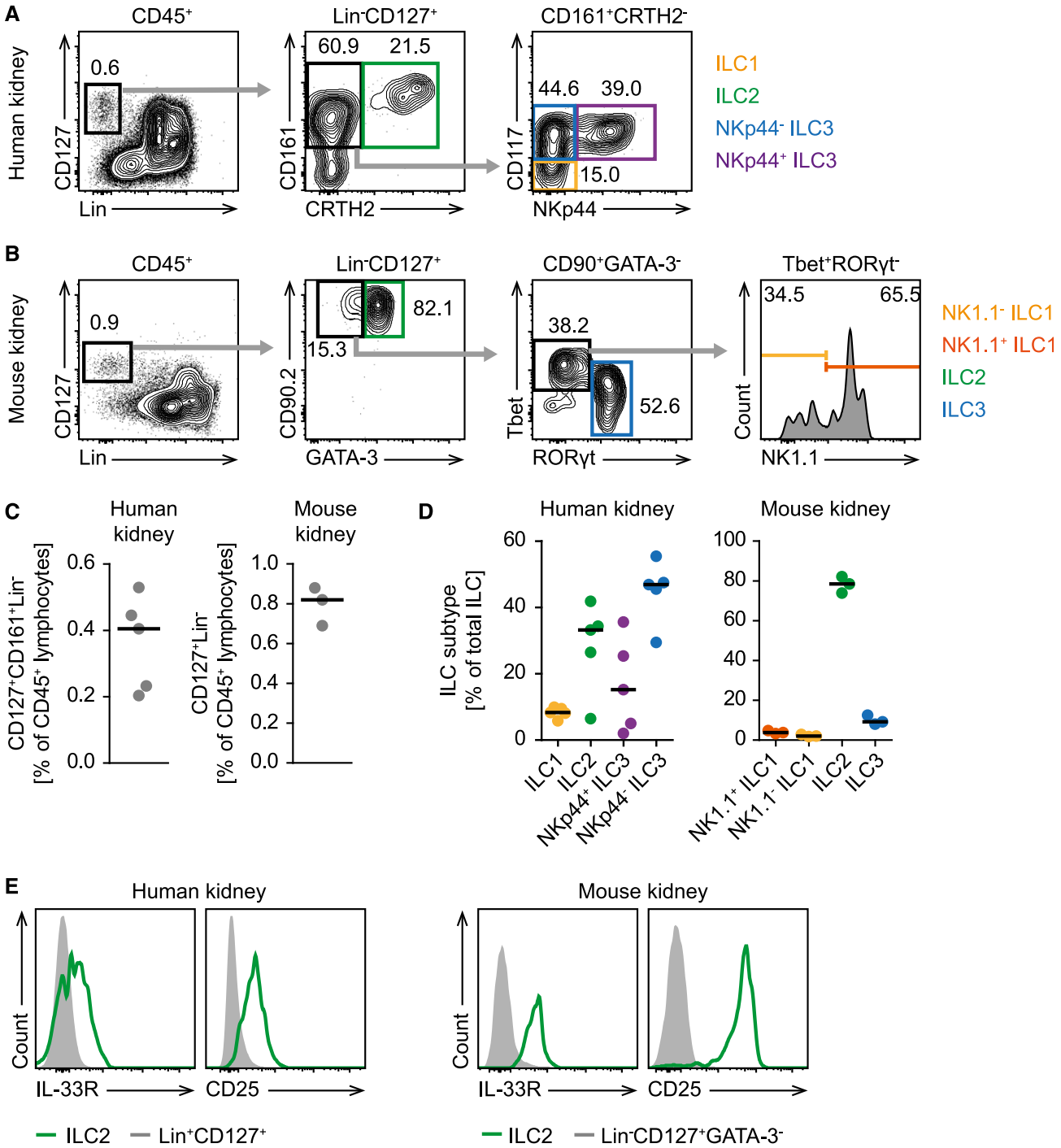

Figure 1. IL-33R$^+$ ILC2s are a major ILC population in the human and murine kidney. Flow cytometric analyses of leukocytes isolated from healthy human and mouse (C57BL/6) kidney tissue. (A and B) Representative plots of human kidney cells (A) stained for CD45, CD127 (IL-7Rα), CD161, CRTH2, CD117 (c-kit), NKp44, and lineage markers (Lin = CD1a, CD3, CD4, CD11c, CD14, CD16, CD19, CD34, CD56, CD94, CD123, $\gamma\delta$-TCR, $\alpha\beta$-TCR, FcεR1α, and BDCA1); and mouse kidney cells (B) stained for CD45, CD127, CD90.2, GATA-3, T-bet, ROR-γt, Eomes, NK1.1, and lineage markers (Lin = CD3, CD4, CD8, β-TCR, $\gamma\delta$-TCR, CD19, CD11b, CD11c, GR-1, CD49b, Ter119). T-bet$^+$ ILC1s were Eomes$^-$, excluding a contaminating NK cell population in the ILC1 gate. Numbers indicate the percentage of cells in each gate. (C) Frequency of human and mouse ILCs in percentage of total CD45$^+$ lymphocytes. (D) Frequency of the ILC1, ILC2, and ILC3 subtypes within the total ILC population (human total ILC: CD127$^+$Lin$^-$CD161$^+$; mouse total ILC: CD127$^+$Lin$^-$). (E) Representative flow cytometry analysis of IL-33R (T1/ST2) and CD25 expression on kidney-residing ILC2s in humans and mice. The mouse data represent at least three independent experiments with similar results. Symbols represent individual data points and the horizontal lines indicate the median.

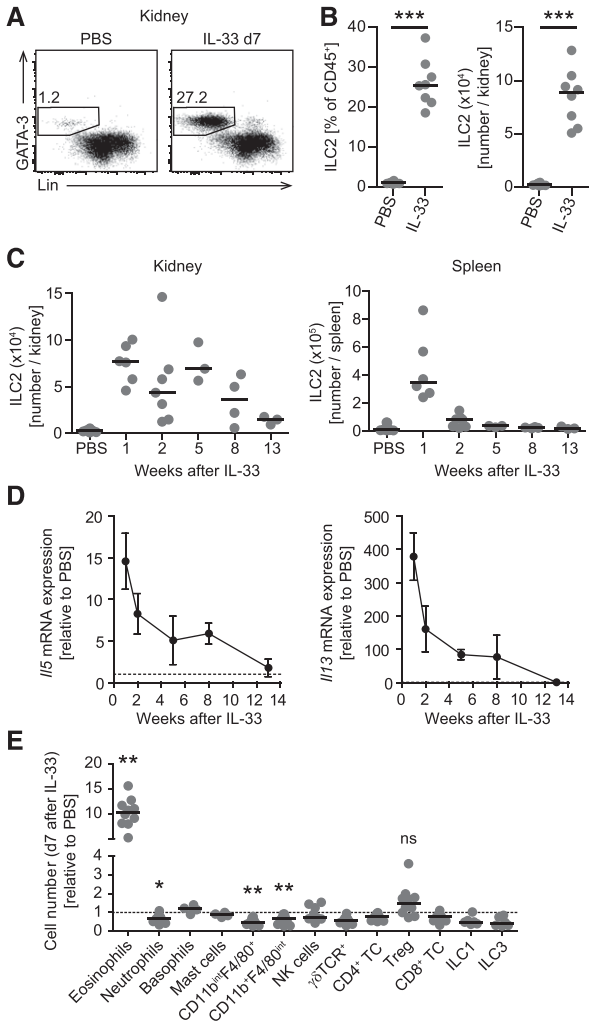

Figure 2. Sustained expansion of kidney-residing ILC2s after short-term IL-33 treatment. C57BL/6 mice were treated with IL-33 (400 ng intraperitoneally on four consecutive days) or PBS. (A) Representative flow cytometry of leukocytes isolated from the kidney at day 7 after start of treatment. Plots are gated for CD45$^+$ lymphoid cells and numbers indicate the percentage of events in the gate. (B) Frequency and absolute number of ILC2s (Lin$^-$GATA-3$^+$) in the kidneys at day 7 (n=8 per group). (C) Absolute numbers of ILC2s in the kidney and spleen in PBS-injected controls and at weeks 1–13 after start of IL-33 treatment (n=3–7 per IL-33–treated group, n=12 for controls). (D) Quantitative RT-PCR analysis of *Il5* and *Il13* mRNA transcripts in the kidneys of IL-33–treated mice relative to PBS-injected controls (numbers as in [C]). (E) Increase in absolute cell numbers of the indicated leukocyte subsets in kidneys of IL-33–treated mice relative to PBS-injected controls at day 7 (n=4–10 per IL-33–treated group, n=5 for controls). Data in (B–E) are pooled from two independent experiments with similar results. Symbols in (B, C, and E) represent individual data points and the horizontal lines indicate the median. Symbols in (D) represent mean±SEM (*P<0.05, **P<0.01, ***P<0.001).

responsiveness of a subset of regulatory T cells,[17] we also observed a moderate increase of Treg numbers in the kidney, spleen, and peripheral blood after IL-33 treatment (Figure 2E, Supplemental Figure 3, A and B). However, Tregs isolated from the spleens of IL-33–treated mice showed a similar suppressive capacity, as compared with Tregs from PBS-treated controls (Supplemental Figure 3C). Taken together, we were able to establish a 4-day IL-33 treatment course as an efficient tool to induce a sustained increase in ILC2 abundance in the murine kidney.

Localization of ILC2s in the Kidney

In the next step, we wanted to analyze the localization of ILC2s in the kidney. In flow cytometric analyses, we established that a combination of CD127 and GATA-3 reliably identifies ILC2s in the kidney, because other GATA-3$^+$CD127$^+$ cells (mainly CD4$^+$) were <5% of the total GATA-3$^+$CD127$^+$ cells in controls and IL-33–treated mice (Figure 3, A and B). Immunohistochemical costaining for GATA-3 and CD127 clearly identified GATA-3$^+$CD127$^+$ ILC2s in the tubulointerstitial compartment of the kidney of control and IL-33–treated wildtype mice and in IL-33–treated Rag1$^{-/-}$ mice (Figure 3C). More detailed analyses of the spatial distribution of ILC2s in IL-33–treated Rag1$^{-/-}$ mice by including lectin staining as an endothelial marker revealed that they can be found in the tubulointerstitial location (both distant from and close to peritubular capillaries), as well as in the glomerular tuft (Figure 3D).

IL-33–Mediated ILC2 Expansion Ameliorates Progressive Glomerulosclerosis

ILC2s have been described to maintain tissue integrity and promote repair functions in barrier organs, such as the lung, skin, and intestine.[7–10] In order to investigate if the IL-33–mediated expansion of ILC2s could be used as a therapeutic strategy to alter the course of CKD, we induced adriamycin-induced nephropathy (AN) in BALB/c mice, a model of progressive glomerulosclerosis with proteinuria.[18] IL-33 treatment was started at day 5 after disease induction (Figure 4A), a time point at which the initial glomerular damage is established. Flow cytometric analyses 2 weeks after disease induction confirmed the strong and persistent expansion of GATA-3$^+$ ILC2s in kidneys of IL-33–treated animals, whereas the diseased PBS-treated mice showed ILC2 frequencies and numbers comparable to naïve controls (Figure 4, B and C). To investigate if the IL-33–induced ILC2 accumulation affects the course of CKD, we assessed parameters of kidney damage at day 14 after disease induction. Periodic acid–Schiff (PAS)–stained kidney sections showed a severe glomerular and tubular pathology in PBS-treated mice that was significantly attenuated in the IL-33–treated group (Figure 4D), as shown by reduced glomerular and tubulointerstitial injury scores (Figure 4E). In line, analyses of kidney function parameters showed significantly reduced albuminuria and cholesterol levels, as laboratory markers of nephrotic syndrome, as well as significantly reduced blood urea nitrogen levels, as a marker of renal failure, in IL-33–treated mice (Figure 4, F and G). Thus, IL-33 administration leads to a sustained expansion of ILC2s and, even if initiated 5 days after induction of glomerular damage, significantly attenuates the course of progressive glomerulosclerosis.

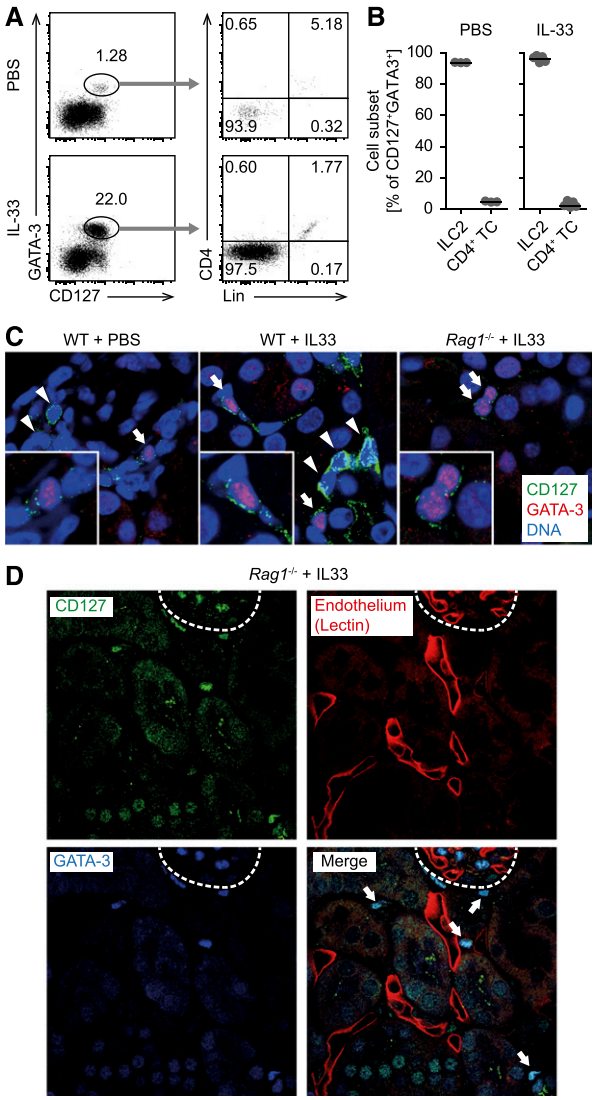

Figure 3. ILC2s are localized in the glomerular and tubulointerstitial comparment. (A) Representative flow cytometric analyses of kidney leukocytes isolated from wildtype C57BL/6 mice at day 7 after IL-33 (400 ng intraperitoneally on four consecutive days) or PBS treatment. Left panel is gated for CD45$^+$ lymphoid cells. Numbers indicate the percentage of cells in the gate or quadrants. (B) Percentage of ILC2s (Lin$^-$CD4$^-$) and CD4$^+$ Th2 cells (Lin$^+$CD4$^+$) in the CD127$^+$GATA-3$^+$ gate in PBS- (n=3) and IL-33–treated (n=5) mice. (C) Representative confocal microscopy images of kidney sections from PBS- and IL-33–treated wildtype mice, as well as from IL-33–treated *Rag1*$^{-/-}$ mice costained for CD127 and GATA-3. ILC2s (arrows) are found in wildtype and *Rag1*$^{-/-}$ mice, whereas CD127-single–positive T cells (arrowheads) are absent in *Rag1*$^{-/-}$ mice. (D) Representative confocal microscopy images of kidney sections of IL-33–treated *Rag1*$^{-/-}$ mice costained for CD127, GATA-3, and endothelial lectin binding. ILC2s (arrows) are found in the tubulointerstitial compartment and within the glomerular tuft (dotted line). Symbols in (B) represent individual data points and the horizontal lines indicate the median. Data in (A and B) are representative of at least three independent experiments with similar results.

IL-33 Treatment Induces a Tissue-Protective Type 2 Response in the Kidney

In order to investigate the mechanisms underlying IL-33–mediated tissue protection, we performed a detailed analysis of the kidney inflammatory milieu in IL-33– and PBS-treated mice with AN. In IL-33–treated animals, positivity of ILCs for IL-5 and IL-13 was >50% (Figure 5A) and the absolute number of IL-5$^+$IL-13$^+$ ILCs was significantly increased (Figure 5B). In line, levels of renal *Il5* and *Il13* mRNA transcripts were significantly elevated in IL-33–treated mice (Figure 5C), whereas IFN-γ, TNF-α, and GM-CSF expression was downregulated. We did not observe upregulation of the growth factor amphiregulin, which is secreted by ILC2 in other tissue locations, in the IL-33–treated group and, regardless of the treatment group, IL-4 (and also IL-17A) was undetectable in most samples. Consistent with the shift of the cytokine milieu toward a type 2 response, mRNA expression of macrophage activation markers in the kidney parenchyma was skewed toward an alternatively activated phenotype, with increased expression of arginase-1 and downregulation of the inducible nitric oxide synthase (Figure 5D). It is, however, noteworthy that arginase-1 can also be produced by ILC2s themselves,[19] which most likely contributes to the increased *Arg1* expression in IL-33–treated mice. mRNA expression analysis of a panel of chemokines showed a significant reduction of the neutrophil attractants CXCL1 and CXCL2, as well as of the T cell and monocyte attractant CCL5. Other chemokines involved in CD4$^+$ T cell (CCL20, CXCL10) and eosinophil (CCL11) migration remained unchanged (Figure 5E). The most striking change in the cellular infiltrate, beside the massive accumulation of ILC2s, was the significant increase in eosinophil abundance (Figure 5F). In accordance with the reduced levels of CXCL1, CXCL2, and CCL5, we observed reduced infiltration of neutrophils and F4/80^{int}CD11b^{hi} mononuclear phagocytes (Figure 5, G and H), which have both been implicated in the progression of renal tissue damage in models of inflammatory kidney diseases.[20,21] Quantification of neutrophils in kidney sections stained for GR-1 (Ly6G/C) confirmed their reduced accumulation in the IL-33–treated group (Figure 5I).

The abundance of total CD4$^+$ and CD8$^+$ T cells was unaffected by IL-33 treatment, but we observed a significant reduction in $\gamma\delta$ T cells, which have also been shown to be pathogenic in models of renal inflammation[22] (Supplemental Figure 4A). As described before for C57BL/6 mice (see Figure 2), we again observed a moderate accumulation of Tregs in the kidneys of nephritic mice treated with IL-33 (Supplemental Figure 4A). In addition, GATA-3$^+$ CD4$^+$ Th2 cells were significantly increased after IL-33 treatment. However, this subset was unlikely to contribute significantly to the overall amount of IL-5 and IL-13, because it was two orders of magnitude less abundant than ILC2s (Figure 4C, Supplemental Figure 4A). Further analyses of ILC and T cell subsets in the kidney showed no significant difference in the abundance of Th17 cells and ILC3s (Supplemental Figure

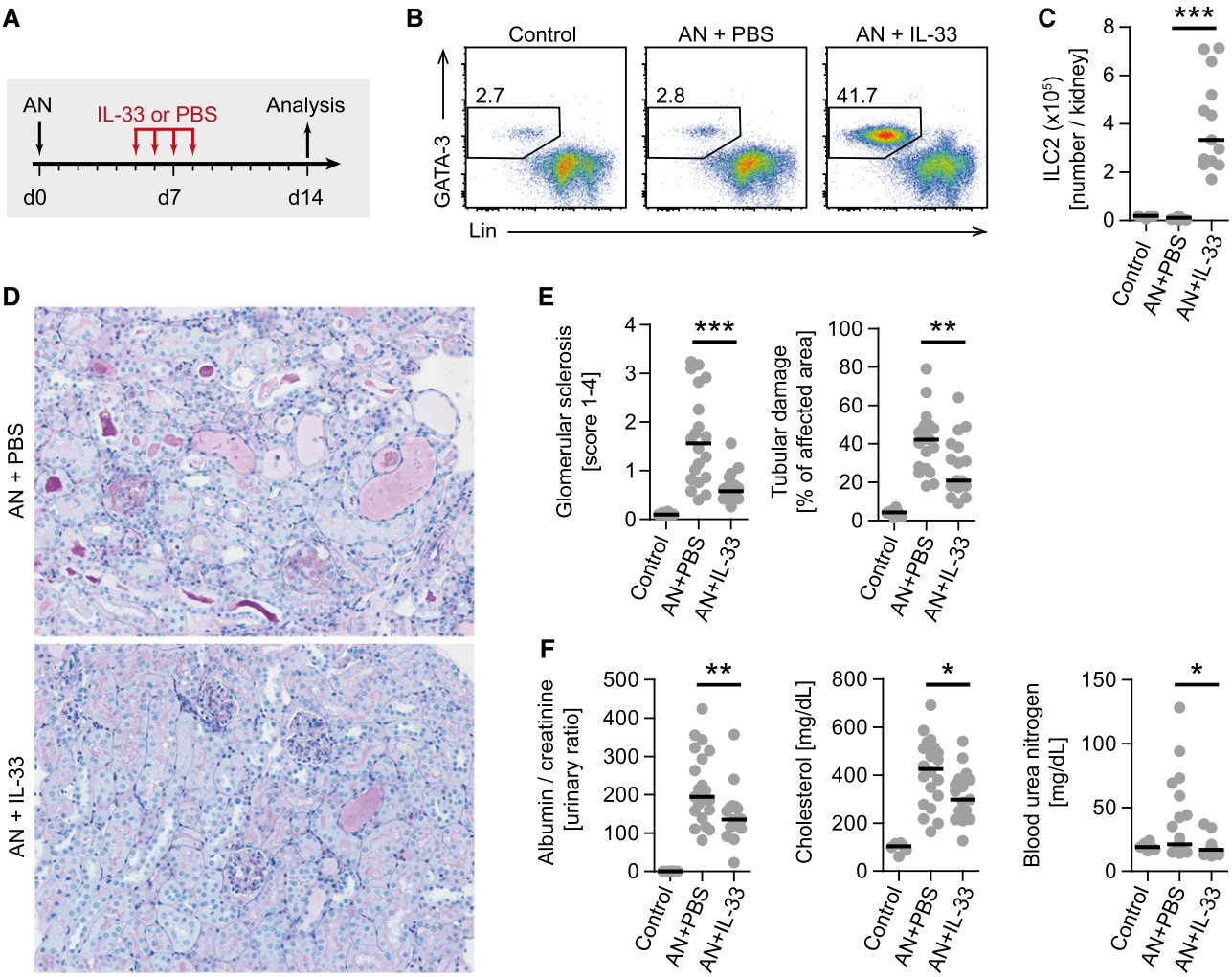

Figure 4. IL-33 treatment ameliorates the clinical course of AN. (A) PBS or IL-33 (400 ng) was injected intraperitoneally on days 5–8 after induction of AN in BALB/c mice. Mice were analyzed at day 14. (B) Representative flow cytometry plots and (C) absolute numbers of $Lin^{-}GATA\text{-}3^{+}$ ILC2s in the kidney of naïve control mice (n=4) and both AN groups (n=13–16). Numbers in B indicate the percentage of cells in the gate. (D) Representative photographs (original magnification, ×200) of PAS-stained kidney sections, (E) histopathologic quantification of glomerular and tubulointerstitial damage, and (F) analyses of renal function parameters in naïve control mice (n=6), PBS-treated mice with AN (n=20), and IL-33–treated mice with AN (n=17). Data are pooled from at least three independent experiments with similar results. Symbols represent individual data points and the horizontal lines indicate the median. (*P<0.05, **P<0.01, ***P<0.001).

4A). Finally, except for the expected increase in ILC2s and eosinophils, lymphocyte subsets in the spleen remained largely unchanged after IL-33 treatment of mice with AN (Supplemental Figure 4, B and C). In summary, IL-33 treatment modulated the inflammatory response in the diseased kidney in favor of an anti-inflammatory milieu with a reduction of pathogenic cell types and an increase of tissue-protective mediators.

IL-33–Mediated Tissue Protection Is ILC Dependent

In the next set of experiments, we addressed the question of whether ILCs are necessary and sufficient for the IL-33 effect on inflammatory mediators and cells of the myeloid lineage (eosinophils, neutrophils, mononuclear phagocytes), by subjecting BALB/c *Rag2*$^{-/-}$ mice (lacking T and B cells) and BALB/c *Rag2*$^{-/-}$*Il2rcg*$^{-/-}$ (lacking also all ILC populations) with AN to PBS or IL-33 treatment.

Flow cytometric analyses confirmed a marked expansion of ILC2s in the kidneys of IL-33–treated *Rag2*$^{-/-}$ mice, whereas ILCs were absent in *Rag2*$^{-/-}$*Il2rcg*$^{-/-}$ mice (Figure 6A). The IL-33–induced upregulation of *Il5* and *Il13* transcripts, the increased expression of arginase-1, and the accumulation of eosinophils were T cell independent and completely ILC dependent (Figure 6, C and D). Most importantly, the IL-33–induced reduction of neutrophil and mononuclear phagocyte infiltration into the kidney required the presence of ILCs,

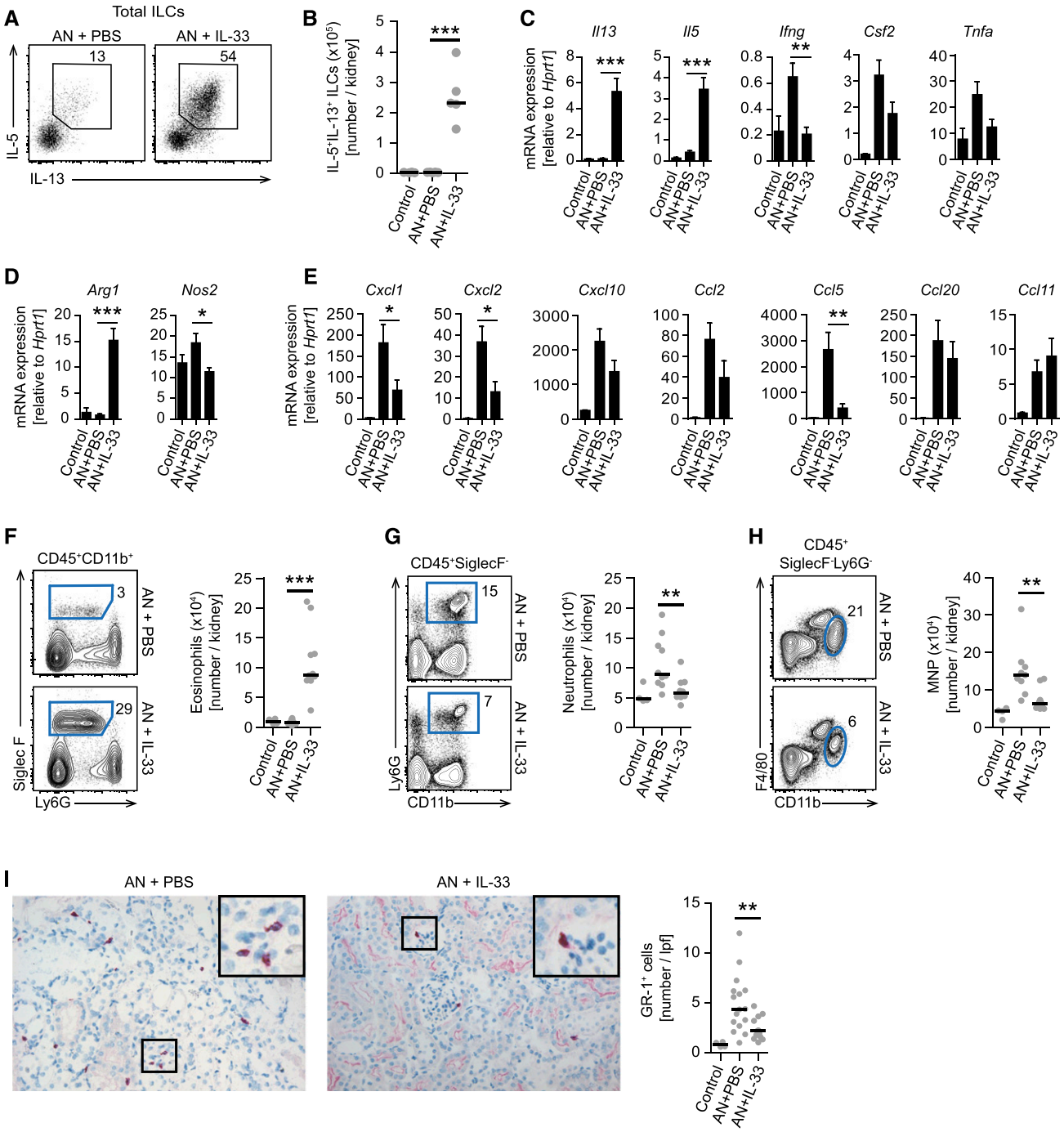

Figure 5. IL-33 treatment induces a protective type 2 response in the kidney. PBS or IL-33 (400 ng) was injected intraperitoneally on days 5–8 after induction of AN in BALB/c mice (see Figure 4A). Mice were analyzed at day 14. (A) Representative flow cytometric analyses of leukocytes isolated from the kidney, stimulated with phorbol 12-myristate 13-acetate and ionomycin, and stained intracellularly for IL-5 and IL-13. Plots are gated for $CD45^+CD90.2^+Lin^-$ total ILCs. Numbers indicate the percentage of cells in the gate. (B) Absolute numbers of $IL\text{-}5^+IL\text{-}13^+$ ILCs in the kidney of naïve controls (n=4) and both AN groups (n=5 per group). (C–E) Quantitative RT-PCR analyses of the indicated mRNA transcripts in the kidney of naïve control mice (n=3), PBS-treated mice with AN (n=10), and IL-33–treated mice with AN (n=8). (F–H) Representative flow cytometry plots and absolute numbers of (F) eosinophils, (G) neutrophils, and (H) mononuclear phagocytes (MNP) in the kidney of naïve control mice (n=4), PBS-treated mice with AN (n=11), and IL-33–treated mice with AN (n=10). (I) Representative photographs of kidney sections stained for the neutrophil marker GR-1 (original magnification, ×200) and histologic quantification of GR-1–positive neutrophils in the three groups (naïve controls: n=4; AN + PBS: n=16; AN + IL-33: n=13); lpf, low-power field. Data are pooled from two to three independent experiments with similar results. Symbols in (B and F–I) represent individual data points and the horizontal lines indicate the median. Symbols in (C–E) represent mean±SEM (*P<0.05, **P<0.01, ***P<0.001).

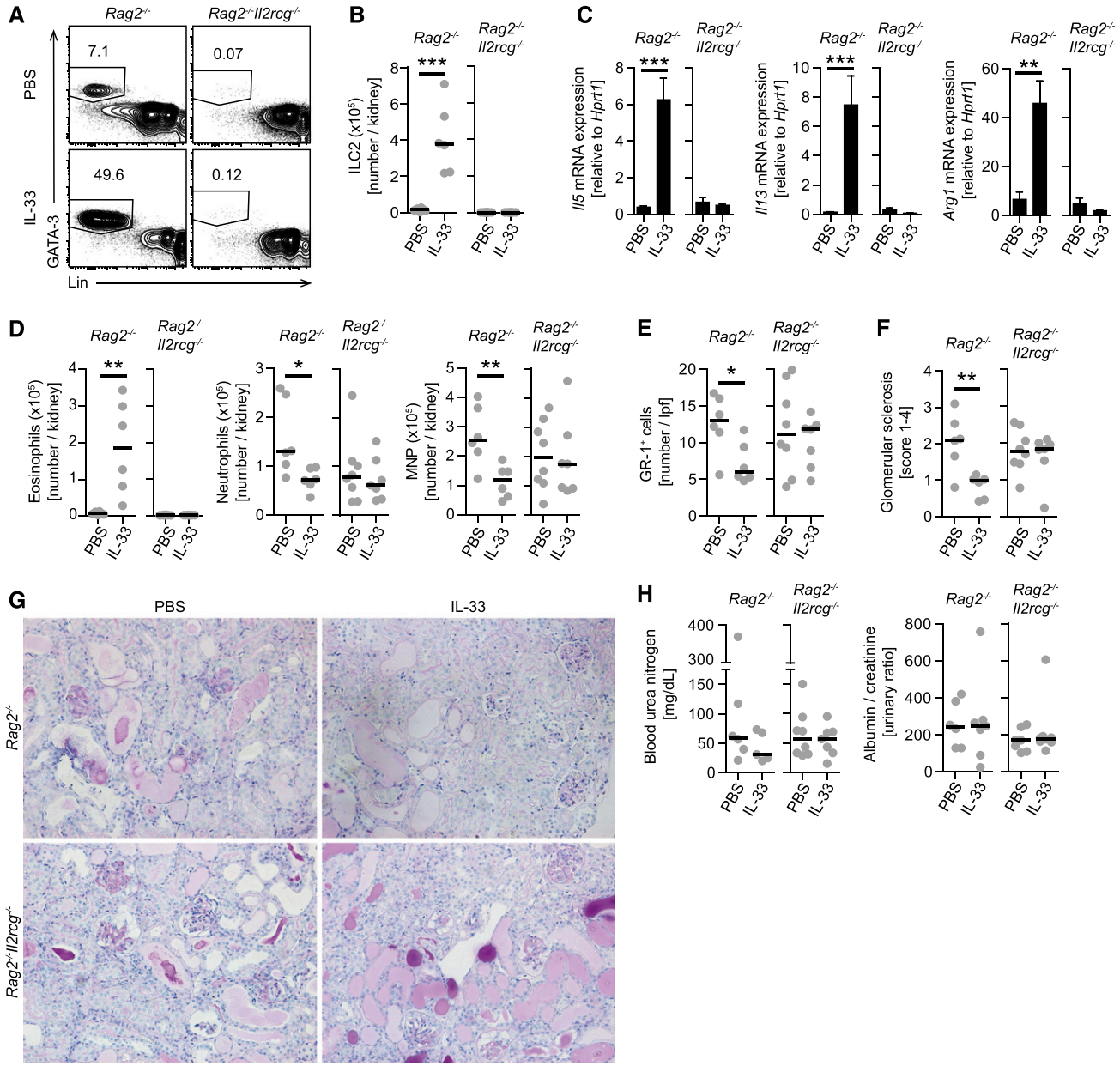

Figure 6. IL-33–mediated tissue protection is ILC dependent. PBS or IL-33 (400 ng) was injected intraperitoneally on days 5–8 after induction of AN in BALB/c *Rag2*$^{-/-}$ mice (PBS: *n*=8; IL-33: *n*=7) and BALB/c *Rag2*$^{-/-}$*Il2rcg*$^{-/-}$ (PBS: *n*=8; IL-33: *n*=7). Mice were analyzed at day 14. (A) Representative flow cytometric analyses of leukocytes isolated from the kidney of BALB/c *Rag2*$^{-/-}$ mice and BALB/c *Rag2*$^{-/-}$*Il2rcg*$^{-/-}$ with IL-33 or PBS treatment. Plots are gated for CD45^{+} lymphocytes. Numbers indicate the percentage of cells in the gate. (B) Absolute numbers of ILC2s in the kidney of the respective groups. (C) Quantitative RT-PCR analyses of the indicated mRNA transcripts in the kidney of BALB/c *Rag2*$^{-/-}$ mice and BALB/c *Rag2*$^{-/-}$*Il2rcg*$^{-/-}$ with IL-33 or PBS treatment. (D and E) Absolute numbers of eosinophils, neutrophils, and CD11b^{hi}F4/80^{int} mononuclear phagocytes (MNP) (D), as well as histologic quantification of GR-1–positive neutrophils (E), in the respective groups. (F and G) Histopathologic quantification of glomerular damage (F) and representative photographs (G) (original magnification, ×200) of PAS-stained kidney sections. (H) Analyses of renal function parameters in the four groups. Data are representative for three independent experiments with similar results. Symbols in (B, D–F, and H) represent individual data points and the horizontal lines indicate the median. Symbols in (C) represent mean±SEM (*P<0.05, **P<0.01, ***P<0.001).

because it was maintained in ILC-sufficient *Rag2*$^{-/-}$ mice, but absent in ILC-deficient *Rag2*$^{-/-}$*Il2rcg*$^{-/-}$ mice (Figure 6, D and E). In line, IL-33 was effective in reducing glomerular histopathology in ILC-sufficient mice, whereas it failed to do so in the ILC-deficient setting (Figure 6, F and G). However, as described before,[23] AN was exacerbated in these immunodeficient mice, leading to aggravation of renal function impairment in *Rag2*$^{-/-}$ animals that could only partially be rescued by ILC2

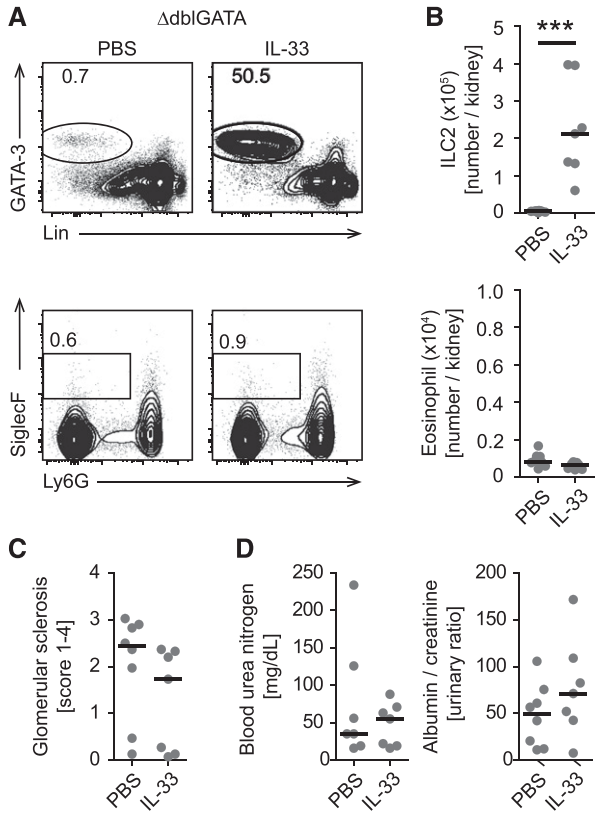

Figure 7. Eosinophils are required for IL-33–mediated tissue protection. PBS or IL-33 (400 ng) was injected intraperitoneally on days 5–8 after induction of AN in ΔdblGATA mice. Mice were analyzed at day 14. (A) Representative flow cytometry plots and (B) absolute numbers of Lin$^-$GATA-3$^+$ ILC2s and CD11b$^+$SiglecF$^+$ eosinophils in the kidney of ΔdblGATA mice treated with PBS or IL-33 (n=7–8). Numbers in (A) indicate the percentage of cells in the gate. (C) Histopathologic quantification of glomerular damage and (D) analyses of renal function parameters in the two groups. Data represent one of two independent experiments with similar results. Symbols represent individual data points and the horizontal lines indicate the median. (***P<0.001).

expansion (Figure 6H). Taken together, these data show that the beneficial effects of IL-33 treatment can be attributed to ILC2 expansion.

IL-33–Mediated Tissue Protection Requires Eosinophils

Because the accumulation of eosinophils in the kidneys of IL-33–treated mice was the most striking alteration of the inflammatory infiltrate, beside the expansion of ILC2s, we decided to address the role of eosinophils in IL-33–mediated protection from kidney injury in the AN model. To this end, we subjected eosinophil-deficient ΔdblGATA mice to AN with or without IL-33 treatment. Consistent with an important role for eosinophils in ILC2-mediated tissue protection, IL-33 treatment in the absence of eosinophils failed to protect mice from glomerulosclerosis, proteinuria, and renal function impairment, despite an unaltered expansion of ILC2s (Figure 7, A–D). It is therefore likely that the ILC2 effect is, at least in large parts, due to their role in regulating the abundance and function of other immune cells, such as eosinophils.

DISCUSSION

Accumulating evidence has led to the hypothesis that there is a link between type 2 immunity and the repair mechanisms that are initiated in response to tissue injury. Indeed, the type 2 immune response that is typically triggered by parasitic infections shares key mediators with the wound healing response aimed at restoring tissue integrity. From an evolutionary perspective this relationship seems advantageous, because the physical disruption of tissues often caused by migrating multicellular pathogens, such as helminthes, must be repaired rapidly in order to prevent microbial invasion at the sites of injury.[24]

Type 2 immunity is a highly complex system that involves multiple cell types and mediators, including the type 2 cytokines IL-4, IL-5, IL-9, and IL-13; Th2 cells; eosinophils; mast cells; and basophils, as well as alternatively activated macrophages (AAMΦ) and IgE-production by B cells.[24,25] In recent years, it has become evident that ILC2s are among the central regulators of the type 2 immune response in mice and men.[26] In the context of tissue damage, the type 2 response is thought to facilitate the restoration of tissue homeostasis by inhibiting tissue destructive type 1 inflammation and promoting matrix deposition and remodeling.[25,27] Only in the last few years has it been appreciated that therapeutic targeting of the type 2 response, and especially of ILC2s,[28] might not only be of relevance in the setting of helminth infections and allergic disease, but could also be exploited to enhance endogenous mechanisms of regeneration after different forms of injury.[7–10,25–27,29]

In this study, we apply this emerging concept to develop novel therapeutic strategies for CKD, a global health burden that affects around 10% of the Western population and is a major risk factor for cardiovascular mortality.[1] For the first time, we identify IL-33R$^+$ ILC2s as a major ILC population in the human and mouse kidney and show that therapeutic expansion of ILC2s by IL-33 treatment shifts the inflammatory milieu after kidney injury toward a type 2 response that ameliorates the course of progressive, proteinuric CKD induced by injection of adriamycin in BALB/c mice.

In the last years, it has been recognized that the cytokines IL-25, IL-33, and thymic stromal lymphopoietin that are released after epithelial cell damage are potent inducers of ILC2-mediated type 2 responses and tissue repair mechanisms in the intestine, lung, and skin.[30] With respect to a potential role of ILCs in kidney disease, there is only one study that describes an expansion of ILC2s in the mouse kidney after IL-25 application. In a pre-emptive treatment approach, these IL-25–elicited ILCs were shown to ameliorate the course of ischemia/reperfusion-induced AKI by promoting alternative

activation of macrophages that downregulate the inflammatory response and provide survival and proliferation signals to damaged tubular epithelial cells in the kidney.[31] According to another report, a high dose of IL-33 (1 μg twice a day for 3 days) resulted in the aggravation of AKI in a model of cisplatin-induced tubular epithelial cell toxicity at a short time point (day 3).[32] However, in this study, ILCs were not addressed and the IL-33 effect was attributed to $CD4^+$ T cells. Thus, the evidence for a potential ILC-mediated protection from AKI is still controversial. The effect of IL-25 treatment was also investigated in AN, but again ILCs were not addressed in this study and the beneficial IL-25 effect depended on the presence of T cells.[33]

In summary, there are no published reports that provide a detailed characterization of kidney ILC populations in mice and the question of whether ILC populations can be found in the human kidney has not been addressed so far. Here, we show that helper-like ILCs constitute 0.5%–3% of leukocytes in the murine and human kidney and that ILC2s are a major subtype of kidney ILCs in both species. The high expression of the IL-33R on kidney-residing ILC2s prompted us to explore IL-33 therapy as a way to enhance the type 2 response in the kidney of mice by modulating ILC2 abundance. We found a remarkable responsiveness of kidney-residing IL-$33R^+$ ILC2s to a 4-day IL-33 treatment course (400 ng/d), resulting in a rapid accumulation of ILC2s in the kidney tissue that was accompanied by increased type 2 cytokine expression and was sustained for at least 8 weeks. Most importantly, the short-term IL-33 treatment, which was applied at a time point at which the glomerular damage is established, ameliorated parameters of histopathologic injury and renal function at later stages of adriamycin-induced proteinuric CKD in mice.

In the literature, there is robust evidence that IL-33 treatment can affect the function of a variety of different cell types,[34] and IL-33 treatment has been used in different experimental settings to modulate the inflammatory response in infection,[35,36] autoimmune disease,[37] and sterile inflammation.[9,10,38] Many of these studies were done before the emergence of ILCs as a separate cell subset and the unique responsiveness of ILC2s to IL-33 that we now know of makes it tempting to speculate that some of the IL-33 effects on other cell types described in *in vivo* models may indeed be attributed to indirect effects mediated by IL-33–elicited ILC2s. With respect to the kidney-residing leukocyte populations, we found no evidence for a significant stimulatory effect of IL-33 on cell types other than ILC2s, except for a mild increase of Tregs and Th2 cells. Moreover, we did not find evidence for an improved function of Tregs isolated from IL-33–treated mice, as described in other model systems.[39] The potential IL-33 effects on T cells, however, were not essential for the IL-33–induced protective type 2 response in the kidney, as illustrated by the fact that IL-33 treatment was able to reduce glomeruloclerosis in T cell–deficient *Rag2*$^{-/-}$ mice, but not in *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice that also lack ILCs. Of note, the IL-33–mediated protection in *Rag2*$^{-/-}$ mice was incomplete and we cannot fully exclude that a direct IL-33 effect on Tregs (or a potential ILC2-Treg interaction) may contribute to the amelioration of kidney damage observed in WT mice treated with IL-33. Moreover, the preferential cellular target of IL-33 action most likely depends on the inflammatory context in which IL-33 is used[34] and may therefore greatly vary between *e.g.*, viral infection,[36] sepsis,[35] AKI,[32] and a setting of chronic sterile inflammation, such as CKD. These considerations are also critical for assessing the risk for potential side effects of chronic IL-33 exposure, such as the development of allergic disease and the excessive activation of wound repair mechanisms resulting in tissue fibrosis,[40] which are likely to be determined by the dose, duration, and context of the cytokine application.[34]

The downstream mechanisms that ILC2s might use to protect from progressive kidney damage include IL-5–induced accumulation of eosinophils, a cell type that has been shown to promote tissue regeneration,[41] and IL-13–induced alternative activation of macrophages[31,42,43] that have been shown to promote kidney regeneration.[44] By subjecting eosinophil-deficient ΔdblGATA mice to AN with or without IL-33 treatment, we could show that IL-33 treatment in the absence of eosinophils failed to protect mice from AN, suggesting an important role for this cell type in ILC2-mediated tissue protection. It is therefore likely that the ILC2 effect is, at least in large parts, due to their role in regulating the abundance and function of other immune cells in the kidney tissue.

Taken together, we show here that ILC2s reside in the healthy kidney, proliferate vigorously in response to a short course of *in vivo* IL-33 therapy, have a long half-life in the kidney tissue, and are potent mediators of a type 2 response that promotes damage control in experimental CKD. Thus, we identify ILC2-directed cytokine therapy as a potential therapeutic strategy for chronic kidney inflammation. Further investigations of the different ILC subsets residing in the human kidney, their response to *ex vivo* cytokine stimulation, and a correlation of ILC subtype abundance with specific disease entities are needed to advance our understanding of this complex cell population in the human kidney.

CONCISE METHODS

Animals, Progressive Glomerulosclerosis, and IL-33 Treatment

C57BL/6 wildtype mice, C57BL/6 *Rag1*$^{-/-}$ mice, BALB/c wildtype mice, BALB/c *Rag2*$^{-/-}$ mice, and BALB/c *Rag2*$^{-/-}$*Il2rcg*$^{-/-}$ mice were bred in the animal facility of the University Medical Centre Hamburg-Eppendorf under specific pathogen-free conditions. ΔdblGATA mice were provided by S. Rausch (Berlin, Germany) or ordered from the Jackson Laboratory. Adult male and female mice with the appropriate sex- and age-matched controls were used in all experiments. For induction of progressive glomerulosclerosis, BALB/c mice were injected intravenously with 12 μg adriamycin (cell pharm GmBH, Germany) per gram body wt. For ILC expansion, mice were

injected intraperitoneally with 400 ng rmIL-33 (BioLegend) in 200 μl PBS on 4 consecutive days. Controls received 200 μl PBS. For urine sample collection, mice were housed in metabolic cages for 6 hours. Urinary albumin excretion was determined by standard ELISA analysis (Mice-Albumin Kit; Bethyl Laboratories, Montgomery, TX). Urinary creatinine, blood urea nitrogen, and serum cholesterol were measured using standard laboratory methods.

Human Material

Human kidney cortex specimens were obtained from patients that underwent partial nephrectomy because of suspected renal carcinoma. After evaluation by the pathologist, samples of macroscopic healthy, tumor-free kidney cortex were excised from the residual tissue that was not needed for diagnostic purposes and processed as described below.

Cell Isolation

For isolation of leukocytes from the mouse kidney, the tissue was cut into small pieces, digested in complete medium (RPMI 1640, 10% FCS, 1% HEPES, 1% penicillin/streptomycin; all Gibco) with collagenase D (0.4 mg/ml; Roche) and DNase I (100 μg/ml; Roche) for 45 minutes at 37°C while rotating on a MACSmix tube rotator (Miltenyi), and then further dispersed by using a gentleMACS dissociator (Miltenyi). For human kidney samples, collagenase VIII (0.5 mg/ml; Sigma-Aldrich) was used instead of collagenase D. Further leukocyte purification was achieved by Percoll gradient centrifugation (37.5%). Murine splenocytes were obtained by mashing spleens through a 70 μm strainer with PBS containing 1% FCS. After subsequent erythrocyte lysis with ammonium chloride, cell suspensions of kidneys and spleens were filtered through a 50 μm strainer and used for further analyses.

Flow Cytometry

To characterize ILC subsets, cell suspensions of mouse kidneys were stained with fluorochrome-coupled antibodies against CD45 (30-F11), IL-7Rα (CD127; A7R34), Thy1.2 (CD90.2; 30-H12), and a combination of lineage markers (Lin) including CD3 (145–2C11), CD4 (RM4–5), CD8 (53–6.7), CD11b (M1/70), CD11c (HL-3), CD19 (6D5), CD49b (DX5), TCR-β (H57–597), TCR-$\gamma\delta$ (GL3), GR-1 (RB6–8C5), and Ter119 (Ter119). Intranuclear staining, using antibodies against GATA-3 (L50–823), T-bet (4B10), ROR-γt (B2D), FoxP3 (FJK-16s), and Eomes (Dan11mag), was done with the Transcription Factor Staining Buffer Set (eBioscience) according to the manufacturer's instructions. For further characterization of ILC surface marker expression, antibodies against CD25 (PC61.5) and IL-33R (DJ8; MD Bioproducts) were used. For characterization of other leukocyte subtypes, antibodies against CD11b, Ly6G (1A8), SiglecF (E50–2440), CD11c, F4/80 (BM8), CD4, CD8, TCR-$\gamma\delta$, ckit (CD117; 2B8), CD49b (HMα2), and FcεRIα (MAR-1) were used. For intracellular cytokine staining, isolated leukocytes were restimulated with phorbol 12-myristate 13-acetate (1 μg/ml; Sigma-Aldrich) and ionomycin (1 μg/ml; Calbiochem) in the presence of brefeldin A (10 μg/ml; Sigma-Aldrich) for 2.5 hours, stained for surface markers as described above, fixed with formalin (3.8%; Sigma-Aldrich), permeabilized with IGEPAL CA-630 (0.1%; Sigma-Aldrich), and stained with a combination of fluorochrome-coupled antibodies against IL-5 (TRFK5), IL-13 (eBio13A), IL-17A (TC11–18H10), and IFN-γ (XMG1.2). To identify ILC subsets in the human kidney, a combination of the following antibodies was used: CD45 (2D1), IL-7Rα (CD127; A019D7), CD161 (HP-3G10), CRTH2 (CD294; BM16), NKp44 (P44–8), cKit (CD117; 104D2), CD25 (BC96), IL-33R (B4E6; MD Bioproducts), and lineage (Lin = CD1a [HI149], CD34 [581], CD123 [6H6], CD8 [RPA-T8], CD4 [RPA-T4], CD94 [DX22], TCR-$\gamma\delta$ [B1], CD3 [OKT3], TCR$\alpha\beta$ [IP26], CD19 [HIB19], CD14 [HCD14], FcER1α [AER-37], BDCA2 [AC144], CD56 [HCD56], CD11c [3.9], CD16 [3G8]). All antibodies were obtained from BioLegend, BD Biosciences, or eBioscience unless otherwise indicated. Absolute numbers of CD45$^+$ cells in kidney cell suspensions were determined by staining with fluorochrome-coupled anti-CD45 combined with the use of cell counting beads (Countbright; Invitrogen). All samples were acquired on an LSRII flow cytometer (BD Biosciences) and analyzed with the FlowJo software (Treestar Inc.).

In Vitro Suppression Assay

CD4$^+$ T cells from splenocytes of PBS- or IL-33–treated C57BL/6 mice were enriched *via* MACS (Miltenyi) according to the manufacturer's instructions using biotinylated antibodies and anti-biotin microbeads depleting all other cell types. Enriched CD4$^+$ T cells were stained with antibodies against CD3, CD4, CD25, and CD127. CD4$^+$ CD25$^-$ T_{eff} were sorted *via* FACS from the PBS group and CD4$^+$ CD25$^+$CD127$^-$ T_{reg} were sorted from PBS- and IL-33–treated animals using a FACSFusion (BD Biosciences). Five $\times 10^4$ T_{eff} were cocultured with titrated numbers of T_{reg} in complete medium (RPMI 1640, 10% FCS, 1% HEPES, 1% penicillin/streptomycin, 50 μM β-ME; all Gibco) in 96-well plates precoated with anti-CD3 mAb (5 μg/ml, clone 145–2C11; BD Biosciences) for 4 days. Cytokine concentrations in the supernatant were measured by using a bead-based cytokine array (LEGENDplex; BioLegend) according to the manufacturer's instructions.

Histopathology and Immunohistochemistry

Formalin-fixed, paraffin-embedded kidney sections were stained with PAS according to standard laboratory procedures and assessed for histopathology in a blinded fashion. Glomerular sclerosis (deposition of PAS-positive material) was scored from zero to four in 50 glomeruli per mouse. Tubular damage was assessed on the basis of formerly described methods.[45] In brief, photographs of nonoverlapping cortical areas from PAS-stained kidney sections were assessed for percentage of tubulointerstitial injury by superimposition of a grid and subsequent counting of the area displaying dilated, atrophic, or cast-filled tubules. For neutrophil quantification, paraffin-embedded sections were stained with an antibody directed against the neutrophil marker GR-1 (Ly6 G/C) (NIMP-R14; Hycult Biotech, The Netherlands) and developed with a polymer-based secondary antibody alkaline phosphatase kit (POLAP; Zytomed, Berlin, Germany). GR-1$^+$ cells were counted in at least ten low-power fields (original magnification, $\times$200) per section. Slides were evaluated with an Axioskop light microscope (Zeiss, Jena, Germany) and photographed with an Axiocam HRc camera (Zeiss). For immunofluorescence staining, 2 μm paraffin sections were deparaffinized and rehydrated to water.

Antigen retrieval was performed by constant boiling in DAKO antigen retrieval buffer pH 6. Unspecific binding was blocked with 5% horse serum (vector) and 0.05% triton-X100 in PBS. Primary antibodies (rabbit-CD127; LSBio; goat-GATA-3; R&D Systems) were incubated in 5% horse serum overnight at 4°C. GATA-3 antibody binding was visualized by using a Cy3- or AF647-coupled anti-goat and CD127 antibody binding by using an AF488-coupled anti-rabbit secondary antibody (Jackson ImmunoResearch Laboratories). DNA was counterstained with Draq5 (Molecular Probes). Staining of the endothelium was achieved by perfusion with lectin (40 μg/ml, DyLight 594 lycopersicon esculentum; Vector laboratories, Burlingame, CA), as previously described.[46] Stainings were evaluated with an LSM 510 Meta confocal microscope using the LSM software (Zeiss).

Quantitative Real-Time RT-PCR Analyses

Total RNA of the renal cortex was prepared according to standard laboratory methods. After reverse transcription, TaqMan Gene Expression Assays and a StepOnePlus Real-Time PCR system (both Thermo Fisher Scientific) were used for quantification of the housekeeping gene (*Hprt1*) and the genes of interest.

Statistical Analyses

The paired *t* test was used for comparison between two groups. In the case of three or more groups, one-way ANOVA was used followed by a *post hoc* analysis with Newman–Keuls test for multiple comparisons. A P value of <0.05 was considered to be statistically significant.

Study Approval

All animal experiments were performed according to national and institutional animal care and ethical guidelines and were approved by the local authorities (approval numbers G46/14 and G122/15; Behörde für Verbraucherschutz). For human kidney samples, written consent for the use of residual materials was obtained from all patients.

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J.-H.R. and M.B. planned and performed most of the experiments and helped with writing the manuscript. K.K., A.-C.G., M.A., and S.K. performed experiments. C.M.-S. performed double-immunofluorescence staining and confocal microscopy. S.R.B. and L.A.K. obtained human kidney samples. B.F. and R.A.K.S. provided mice and reagents and edited the manuscript. U.P. provided mice and reagents, helped to design experiments, and edited the manuscript. J.-E.T. performed experiments, planned and designed the study, and wrote the manuscript.

DISCLOSURES

None.

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Basic

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Research Article

T cell-derived IFN-γ downregulates protective group 2 innate lymphoid cells in murine lupus erythematosus

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Innate lymphoid cells (ILCs) are important regulators of the immune response and play a crucial role in the restoration of tissue homeostasis after injury. GATA-3$^+$ IL-13- and IL-5-producing group 2 innate lymphoid cells (ILC2s) have been shown to promote tissue repair in barrier organs, but despite extensive research on ILCs in the recent years, their potential role in autoimmune diseases is still incompletely understood. In the present study, we investigate the role of ILC2s in the MRL/MpJ-*Fas*^{lpr} (MRL-*lpr*) mouse model for severe organ manifestation of systemic lupus erythematosus (SLE). We show that in these MRL-*lpr* mice, progression of lupus nephritis is accompanied with a reduction of ILC2 abundance in the inflamed renal tissue. Proliferation/survival and cytokine production of kidney-residing ILC2s was suppressed by IFN-γ and, to a lesser extent, by IL-27 which were produced by activated T cells and myeloid cells in the nephritic kidney, respectively. Most importantly, restoration of ILC2 numbers by IL-33-mediated expansion ameliorated lupus nephritis and prevented mortality in MRL-*lpr* mice. In summary, we show here that development of SLE-like kidney inflammation leads to a downregulation of the renal ILC2 response and identify an ILC2-expanding therapy as a promising treatment approach for autoimmune diseases.

Keywords: Autoimmunity · Cytokines · Innate lymphoid cells · MRL-lpr model · Systemic lupus erythematosus

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Introduction

Systemic lupus erythematosus (SLE) is an aggressive, chronic autoimmune disease which mainly affects young women. SLE is characterized by development of cellular and humoral autoimmunity against nuclear structures, followed by formation of immune complexes that deposit in various organs and result in a type III hypersensitivity reaction with chronic tissue inflammation [1]. Involvement of the kidney (lupus nephritis) is very common (up to 80%) and a major prognostic factor for long term morbidity and mortality of these patients [1]. Current therapies for severe organ manifestations of SLE are based on unspecific immunosuppression by high-dose glucocorticoids and cytotoxic agents that have disabling and dose-limiting side effects. It is therefore necessary to identify novel therapeutic targets that are based on the specific mechanisms underlying disease pathogenesis.

According to current concepts of lupus pathogenesis, defects in apoptosis and clearance of dead cells act in conjunction with the loss of B cell tolerance to DNA and other nuclear structures to initiate production of anti-nuclear autoantibodies [1, 2].

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However, accumulating evidence indicates that cellular immunity, with aberrant T cell activation [3] and a type I interferon signature [4], is critical in the initiation and progression of tissue inflammation in SLE. Innate lymphoid cells (ILCs) are a recently identified group of immune cells that have been shown to be important regulators of inflammation in different settings [5, 6], but a potential role of ILCs in the pathogenesis of SLE has not been studied so far.

ILCs are defined by their lymphoid morphology and antigen-independent activation. According to a recently proposed nomenclature, ILCs can be divided into four groups (NK cells, or killer ILCs, and three subsets of helper-like ILCs, referred to as group 1 ILCs, group 2 ILCs and group 3 ILCs) that differ in their cytokine production, transcription factor usage, tissue localization and functional characteristics [5]. ILCs have been extensively studied in barrier organs, such as the gut, lung and skin, where they promote tissue homeostasis and defense against different classes of pathogens [6]. Especially, group 2 ILCs (ILC2s) that express the transcription factor GATA3 and produce high amounts of IL-5, IL-13, IL-9 and amphiregulin, can employ these mediators to promote resolution of inflammation and tissue repair after acute injury in the lung, skin and intestine [7–10]. However, the role of the different helper-like ILC subsets in the initiation of autoimmunity and progression or resolution of chronic tissue inflammation remains poorly understood [6].

A first indication that ILC2s could be protective in classical autoimmune diseases is provided by a recent report that linked the gender specific susceptibility of the SJL mouse strain to experimental autoimmune encephalomyelitis (EAE), a mouse model of multiple sclerosis, to a reduction in ILC2 numbers [11]. While male SJL mice that are resistant to EAE show substantial ILC2 accumulation in the inflamed CNS, female SJL mice that are susceptible to EAE were characterized by a lack of ILC2s at the site of inflammation. Moreover, male SJL mice became susceptible to neuroinflammation when carrying the Kit mutation (W/W^v), a phenotype that was shown to be independent from mast cells and again accompanied by an absence of ILC2s accumulation in the inflamed central nervous system, as seen in susceptible female mice [11]. Although indirect, this evidence is supported by other studies that demonstrated increased EAE susceptibility and disease severity in mice deficient for the IL-33 receptor ST2 that is crucial for ILC2 expansion and activation [12, 13].

Since ILC2s have recently been described to be a major helper-like ILC subtype in the healthy human and murine kidney [14], where they can mediate protection of the kidney tissue from acute and chronic injury in murine models [14, 15], we used the genetic MRL/MpJ-*Fas*^{lpr} (MRL-*lpr*) mouse model of SLE to study alterations in abundance and function of kidney-residing ILC2s during the progression of autoimmune renal inflammation. We identify a mechanism by which the production of IFN-γ by aberrantly activated T cells and of IL-27 by activated myeloid cells inhibit kidney ILC2 proliferation and cytokine production. We further show that restoration of ILC2s by IL-33 treatment can vice versa downregulate IL-27 production, ameliorate lupus nephritis and, most importantly, prevent mortality in MRL-*lpr* mice.

Results

Imbalance of ILCs and effector T cells in the kidney of MRL-*lpr* mice

Lupus-prone MRL-*lpr* mice spontaneously develop SLE-like organ inflammation and show prominent T cell infiltrates in the kidney, as well as glomerular pathology consistent with proliferative lupus nephritis at 20 weeks of age. In striking contrast to the accumulation of T cells in diseased MRL-*lpr* mice (Supporting Information Fig. S1), we observed a significant reduction in frequency and number of Lin^-CD127^+ total ILCs in the kidneys of diseased MRL-*lpr* mice, as compared to young MRL-*lpr* mice and MRL controls (Fig. 1A and B). We recently described ILC2s to be the major ILC subtype residing in the kidney of C57BL/6 and BALB/c mice [14]. Similarly, in MRL control mice, as well as in and young and in diseased MRL-*lpr* mice, almost 80% of total ILCs were characterized as $Lin^-CD127^+CD25^{hi}GATA3^+$ ILC2s. The remaining Lin^-CD127^+ cells contained small populations of T-bet$^+$ ILC1s and ROR-γt$^+$ ILC3s, while around 15% of the Lin^-CD127^+ cells remained undefined by the current gating strategy (Fig. 1C and D). In control MRL mice, up to 6% of total lymphocytes were Lin^-GATA3^+ ILC2s (Fig. 1E and F), a percentage that was considerably higher than in the C57BL/6 (~1%) and BALB/c (~2–3%) strains [14]. Similar to total ILC numbers, kidney ILC2s decreased in frequency and number with the progression of disease (Fig. 1D).

Similar to ILC2 populations in other organs, further characterization of kidney-residing $GATA3^+Lin^-$ cells in MRL-*lpr* mice showed a $CD127^+CD90.2^+Sca\text{-}1^{hi}CD25^{hi}$ phenotype (Fig. 1G). Flow cytometric assessment of the proliferation marker Ki-67 revealed a significant decline in frequency and numbers of renal Ki-67$^+$ ILC2s in diseased MRL-*lpr* mice, suggesting a reduction of ILC2 proliferation rate with the progression of kidney inflammation (Fig. 1H).

IFN-γ and IL-27 expression in the renal cortex is increased in lupus nephritis

Potential mechanisms to explain the failure of kidney ILC2s to expand with progressive lymphoproliferation at the onset of lupus nephritis include deficiency in cytokines that promote ILC2 proliferation (e.g. IL-33, IL-25, TSLP) and the upregulation of negative ILC2 regulators, some of which have been identified in recent studies (IFN-γ, IL-27, type I interferons) [16–18]. To address these mechanisms, we analyzed renal cortex mRNA expression of potential candidate cytokines in MRL-MpJ controls, young MRL-*lpr* mice before onset of renal disease and in nephritic MRL-*lpr* mice (Fig. 2). While mRNA expression of ILC2-stimulating cytokines was largely unchanged in MRL-*lpr* mice, we detected a significant increase in IFN-γ and both IL-27 subunits (IL-27p28 and EBI3) with progression of renal disease (Fig. 2). This suggests that the type 1 inflammatory response in SLE-like organ inflammation could be involved in downregulating ILC2 abundance in the nephritic kidney.

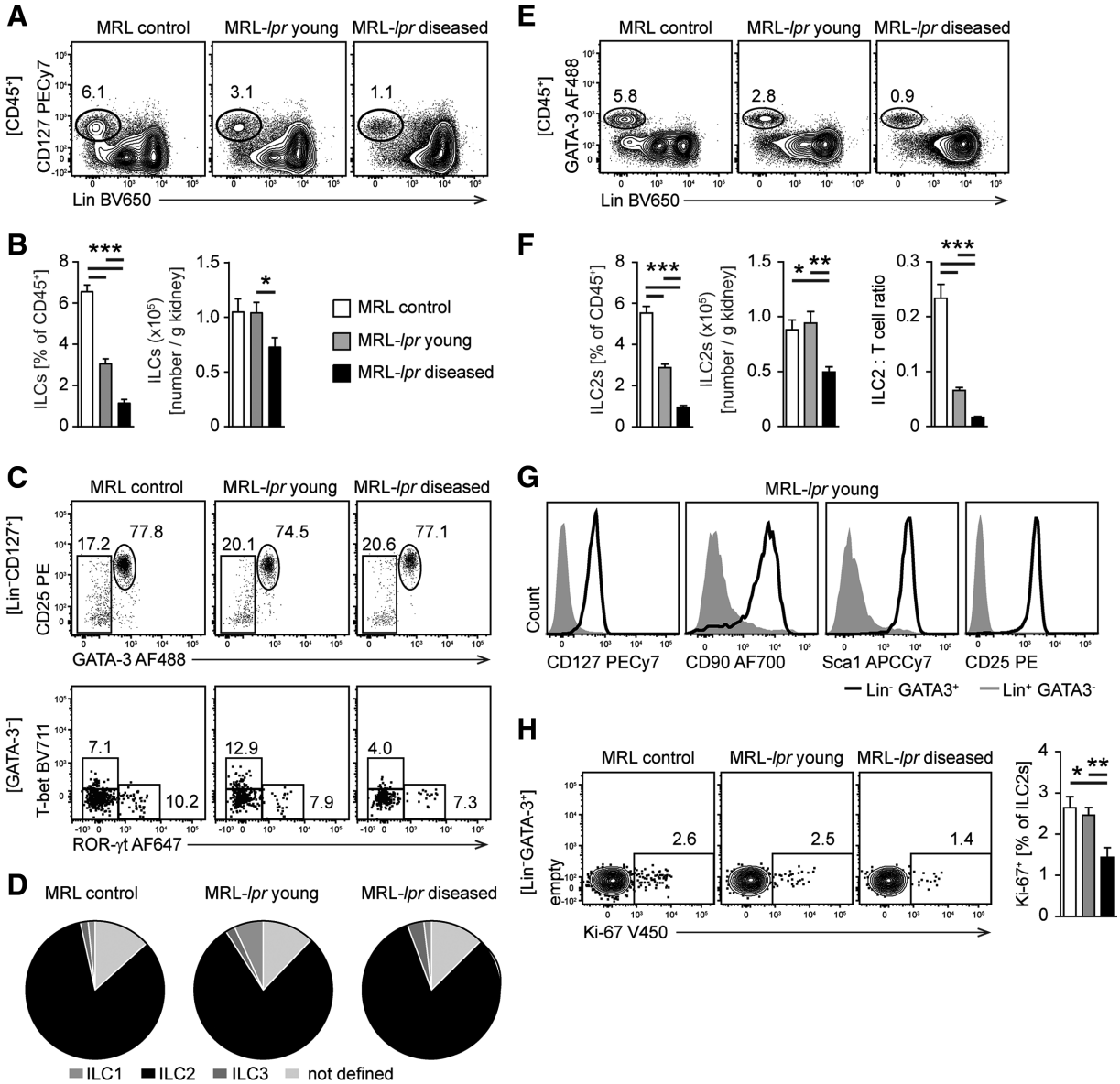

Figure 1. Characterization of ILCs in the kidney of MRL-*lpr* mice with lupus nephritis. (A) Representative flow cytometry of leukocytes isolated from the kidney of young MRL-*lpr* mice before onset of renal disease (~10 weeks-old), diseased MRL-*lpr* mice with lupus nephritis (~20 weeks-old) and MRL controls (~10 weeks-old). Cells were stained for CD45, CD127, CD25, GATA-3, T-bet, ROR-γt, CD90.2, Sca-1, Ki-67, and lineage markers (Lin = CD3, CD4, CD5, CD8, β-TCR, γδ-TCR, CD19, B220, CD11b, CD11c, GR-1, CD49b, NK1.1, Ter119). Gating strategy is specified in brackets and shown in Supporting Information Fig. S5. Numbers indicate the percentage of events in the respective gates. (B) Quantification of Lin$^-$CD127$^+$ ILCs as shown in (A). (C) Representative flow cytometry plots to identify different ILC subsets. (D) Composition of ILC subsets in the respective kidneys. (E) Representative flow cytometry plots and (F) quantification of Lin$^-$GATA-3$^+$ ILC2s in the kidney of the MRL-*lpr* mice and MRL controls. (G) Representative histograms of surface marker expression on kidney ILC2s in 10-weeks-old MRL-*lpr* mice. (H) Flow cytometry plots and quantification of Ki-67 expression in Lin$^-$GATA-3$^+$ ILC2s isolated from the kidney of MRL-*lpr* mice and MRL controls. ($n = 3$ for MRL controls and $n = 9$–10 for MRL-*lpr* mice). Data represent at least two independent experiments with similar results. Symbols represent mean ± SEM. ($^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ in a one-way ANOVA with post hoc analysis by Tukey´s test).

IFN-γ and IL-27 inhibit the IL-33-mediated response of kidney-residing ILC2s

To answer the question whether kidney-residing ILC2s could be a target of IFN-γ and IL-27, we purified Lin$^-$CD127$^+$Sca-1$^+$CD25$^+$ ILC2s by flow cytometry from the kidneys of young MRL-*lpr* mice (Fig. 3A). Renal CD4$^+$ T cells from the same animals were included as a control. mRNA expression analyses for the IFN-γ receptor and the heterodimeric IL-27 receptor (WSX-1 and gp130) revealed that both receptors are expressed in renal ILC2s (Fig. 3B). Next, we investigated the influence of IFN-γ and IL-27 on activation, proliferation/survival and cytokine production of renal ILC2s in

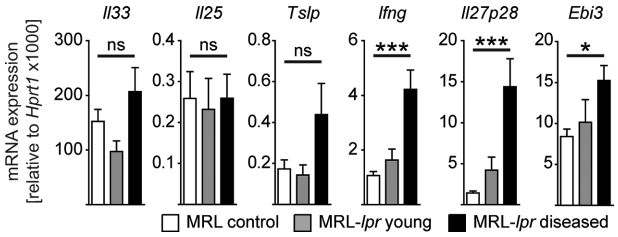

Figure 2. IFN-γ and IL-27 expression in the renal cortex is increased in lupus nephritis. Quantitative RT-PCR analyses of the indicated mRNA transcripts in the kidney of young MRL-*lpr* mice before onset of renal disease (~10 weeks-old), diseased MRL-*lpr* mice (~20 weeks-old) and MRL controls (~10 weeks-old) ($n = 10$ for MRL controls and $n = 6$–8 for MRL-*lpr* mice). Symbols represent mean ± SEM. ($^{*}p < 0.05$, $^{***}p < 0.001$ in a one-way ANOVA with post hoc analysis by Tukey´s test). Data are pooled from at least two independent experiments.

an ex vivo culture system of ILC2s freshly sorted from the kidneys of young MRL-*lpr* mice (Fig. 3C–E). It has been shown in numerous studies that the alarmin IL-33 induces proliferation, survival and cytokine production of ILC2s in vitro and in vivo [19, 20]. As expected, the addition of IL-33 to the culture medium resulted in activation of kidney ILC2s (as determined by a blast phenotype in forward / sideward scatter plots) and in increased ILC2 numbers retrieved from the cultures, indicative of ILC2 proliferation and/or increased survival (Fig. 3C and D). Furthermore, ILC2 activation by IL-33 induced strong secretion of IL-5 and IL-13 into the culture supernatant (Fig. 3E). The IL-33-mediated effects were completely inhibited by addition of IFN-γ to the culture medium, whereas the inhibitory effect of IL-27 was clearly detectable but less pronounced (Fig. 3C–E). None of the cytokine combinations used in these assays had an effect on activation, proliferation or cytokine production of $CD4^{+}$ T cells isolated from the kidneys of the same mice (Fig. 3D and data not shown). These data demonstrate that increased renal expression of IFN-γ and IL-27 in lupus nephritis can directly act on renal ILC2s to inhibit their activation, expansion and cytokine production in the kidney.

Cellular sources of IFN-γ and IL-27 in the kidney of MRL-*lpr* mice

To understand the mechanisms underlying the negative regulation of kidney ILC2s by other cell types accumulating in the kidney with progression of inflammation or by resident epithelial cells, we next investigated the cellular source of IFN-γ and IL-27 in MRL-*lpr* mice. In line with the mRNA expression data of the cytokines (Fig. 2), flow cytometry confirmed a significant increase of IFN-γ and IL-27 protein expression in leukocytes isolated from the kidneys of diseased MRL-*lpr* mice as compared to MRL-MpJ controls (Fig. 4).

Further characterization of the IFN-γ-producing cells showed that most of the IFN-γ is produced by $CD4^{+}$ and $CD8^{+}$ T cells and to a lesser extent by double-negative $CD4^{-}CD8^{-}$ T cells. In diseased MRL-*lpr* mice, contribution of $CD4^{+}$ T cells to total IFN-γ production further increased (Fig. 4A and C). In contrast to IFN-γ, IL-27p28 protein was mainly produced by $CD11b^{+}MHCII^{+/-}$

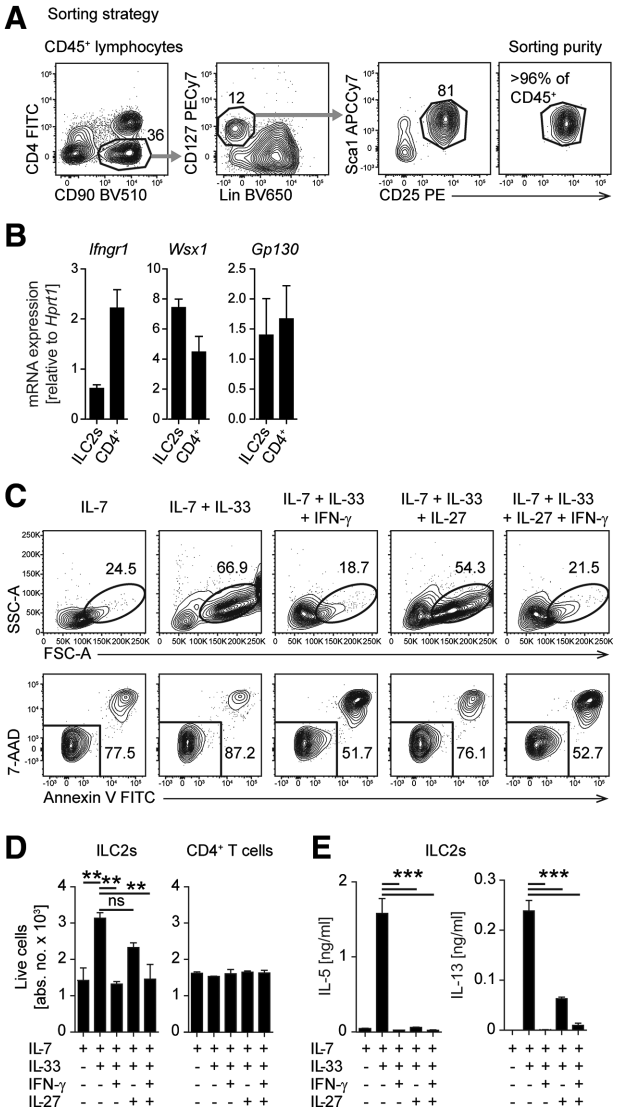

Figure 3. IFN-γ and IL-27 inhibit IL-33-mediated activation, survival and cytokine production of kidney-residing ILC2s. (A) Sorting strategy (left) with previous gating strategy as shown in Supporting Information Fig. S5 and purity check (right) for flow cytometric purification of ILC2s from the kidney of MRL-*lpr* mice (10-weeks-old). Cells were stained for CD45, CD4, CD127, CD25, CD90.2, Sca-1, and lineage markers. Numbers indicate the percentage of events in the respective gates. (B) Quantitative RT-PCR analyses for mRNA expression of the IFN-γ receptor (*Ifngr1*) and both components of the IL-27 receptor (*Wsx1* and *Gp130*) in ILC2s and $CD4^{+}$ T cells purified from the kidney as shown in (A). (Data are representative of three independent experiments with $n = 3$ per group) (C) Representative flow cytometry plots of the isolated kidney ILC2s after a 72 h culture period with different combination of cytokines. The upper row depicts the forward/sideward scatter properties, the lower row identifies live cells by 7-AAD/Annexin V staining. Numbers indicate the percentage of events in the respective gates. (D) Absolute number of 7-AAD / Annexin V-negative live ILC2s in the respective culture conditions after 72 h of culture. (E) Cytokine concentration in the ILC2 culture supernatant after 72 h. Data in C–E represent two independent experiments with similar results ($n = 3$ per group for each experiment). Symbols represent mean ± SEM. ($^{**}p < 0.01$, $^{***}p < 0.001$ in a one-way ANOVA with post hoc analysis by Tukey´s test).

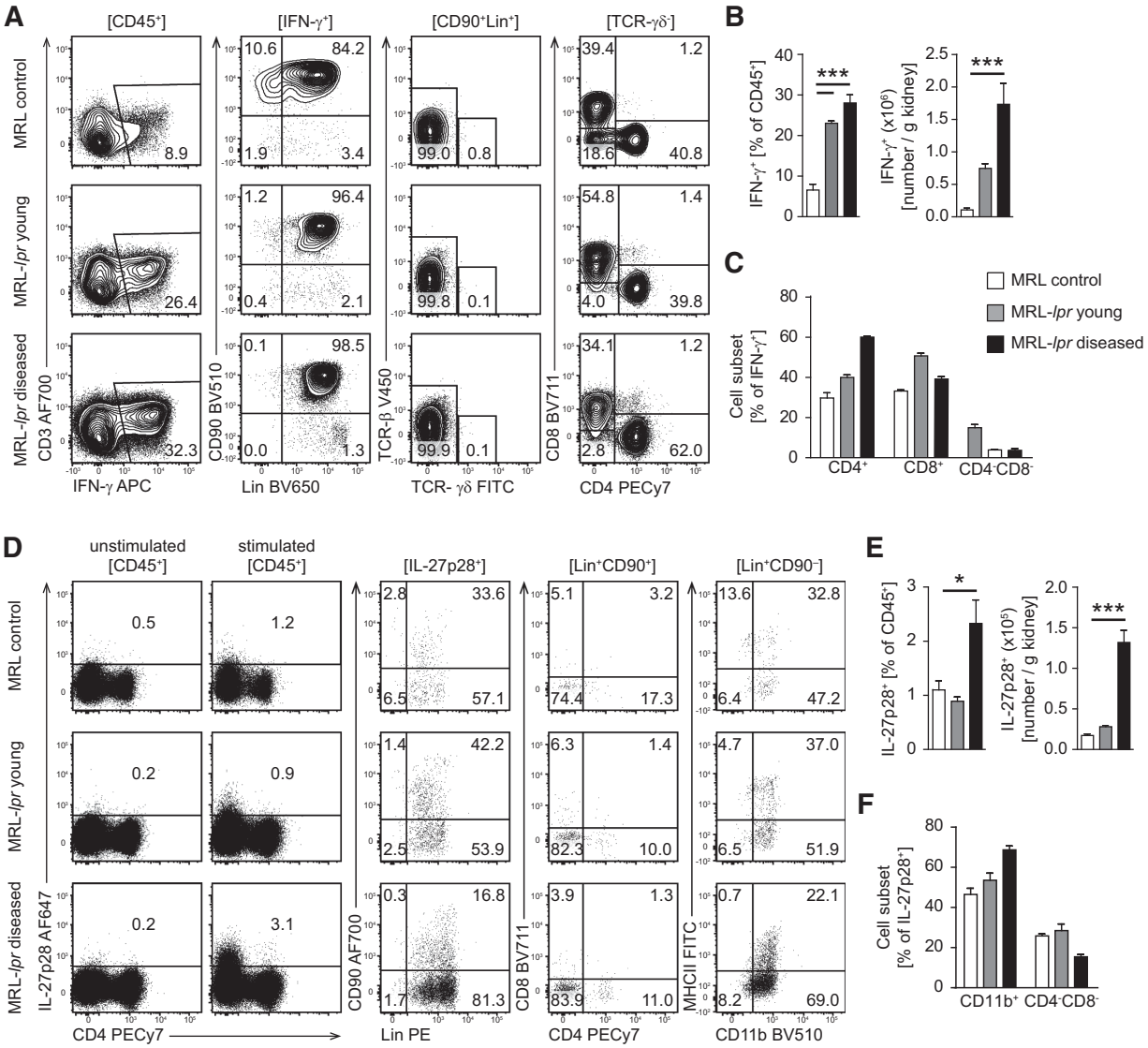

Figure 4. Cellular sources of IFN-γ and IL-27 in the kidney of MRL-*lpr* mice. (A) Representative flow cytometry of intracellular IFN-γ staining in leukocytes isolated from the kidneys of young MRL-*lpr* mice before onset of renal disease (~10 weeks-old), diseased MRL-*lpr* mice (~20 weeks-old) and MRL controls (~10 weeks-old). Before staining, cells were restimulated with phorbol 12-myristate 13-acetate and ionomycin for 2.5 h. Cells were stained for CD45, CD3, CD4, CD8, CD90.2, β-TCR, γδ-TCR, IFN-γ and lineage markers. Gating strategy is shown in Supporting Information Fig. S5 as specified in brackets and numbers indicate the percentage of events in the respective gates. (B) Quantification of the percentage and absolute number of IFN-γ$^+$ leukocytes in the kidneys of the three groups Data are representative of two independent experiments ($n = 3$ for MRL controls and $n = 4$–5 for MRL-*lpr* mice). (C) Contribution of CD4$^+$, CD8$^+$ and DN T cells to total INF-γ production in the three groups (group numbers as in B). (D) Intracellular IL-27p28 staining in leukocytes isolated from the kidneys of the three groups. Before staining, cells were restimulated with IFN-γ and the TLR7/8 agonist R848 for 26 h. Cells were stained for CD45, CD4, CD8, CD90.2, CD11c, CD11b, F4/80, MHCII, IL-27p28 and lineage markers. Unstimulated cells are shown as a control. Gating strategy is specified in brackets and numbers indicate the percentage of events in the respective gates. (E) Quantification of the percentage and absolute number of IL-27p28$^+$ leukocytes in the kidneys of the three groups (group numbers as in B). (F) Contribution of myeloid cells and DN T cells to total IL-27 production in the three groups (group numbers as in B). Data represent two independent experiments with similar results. Symbols represent mean ± SEM. (*$p < 0.05$, ***$p < 0.001$ in a one-way ANOVA with post hoc analysis by Tukey´s test).

myeloid cells. As described previously [21], baseline expression of IL-27p28 was also found in CD4$^-$CD8$^-$ T cells, but did not increase with progression of kidney inflammation in this subset. (Fig. 4D and F). To address a potential role of tubular epithelial cells (TECs) as a source of IFN-γ and/or IL-27, we purified TECs from the kidneys of young and diseased MRL-*lpr* mice by flow cytometry and assessed mRNA expression of the respective cytokines. These analyses revealed that expression of IFN-γ and the IL-27 subunits were very low in TECs, as compared to total kidney cortex mRNA expression, and did not significantly increase with progression of kidney inflammation in diseased MRL-*lpr* mice (Supporting Information Fig. S2). Together, these analyses identify distinct cytokine axes in myeloid cells and T cells that are dysregulated in autoimmune organ inflammation and lead to downregulation of a potentially

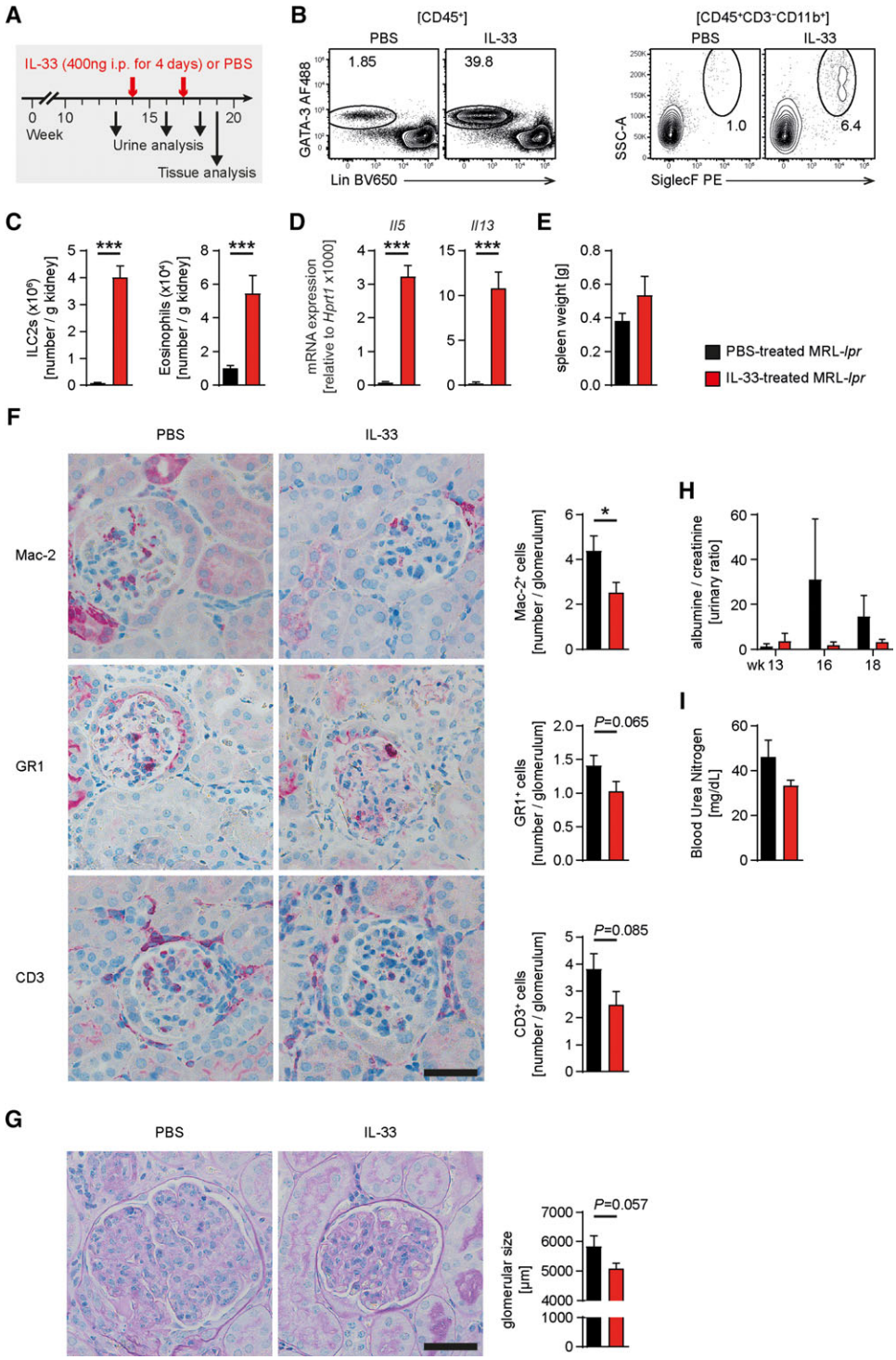

Figure 5. IL-33 treatment restores kidney ILC2s in vivo and ameliorates glomerular cell infiltration. (A) Treatment regimen for MRL-*lpr* mice. IL-33 (400 ng i.p.) or PBS was applied on four consecutive days at 14 and 17 weeks of age. Mice were sacrificed at week 19 for tissue analyses. (B, C) Representative flow cytometry plots (B) and absolute numbers (C) of Lin$^-$GATA-3$^+$ ILC2s and CD11b$^+$SiglecF$^+$SSc^{hi} eosinophils in the kidney of IL-33-treated and PBS-treated MRL-*lpr* mice at week 19. For identification of ILC2s, cells were stained for CD45, CD127, GATA-3, CD90.2, and lineage markers. For gating of eosinophils cells were stained for CD45, CD3, CD4, CD8, β-TCR, γδ-TCR, SiglecF, CD11c, CD11b, F4/80, Ly6G, NK1.1. Gating strategy is shown in Supporting Information Fig. S5 and specified in brackets. Numbers indicate the percentage of events in the respective gates. (D) Quantitative RT-PCR analyses of the indicated mRNA transcripts in the kidney of the two groups of mice. (E) Spleen weight in the two treatment groups. (F) Representative photographs (original magnification 400x) of kidney sections stained for the macrophage marker Mac-2, the T cell marker CD3, or the neutrophil marker GR-1 and histologic quantification of the respective positive cells in IL-33-treated and PBS-treated MRL-*lpr* mice at week 19. (G) Representative photographs (original magnification 400x) of Periodic Acid-Schiff-stained kidney sections of IL-33-treated and PBS-treated MRL-*lpr* mice at week 19 and quantification of glomerular size. (H, I) Analyses of albuminuria and blood urea nitrogen of IL-33-treated and PBS-treated MRL-*lpr* mice at the indicated time points (H) and at week 19 (I). (n = 13 for PBS- and n = 14 for IL-33-treated mice). Data are pooled from two independent experiments with similar results. Scale bars represent 50μm. Symbols represent mean ± SEM ($^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ in a two-tailed unpaired Student's t-test).

protective ILC2 response in the kidney during proliferative lupus nephritis.

IL-33 treatment restores kidney ILC2s in vivo and ameliorates glomerular cell infiltration

Next, we asked the question whether restoration of the renal ILC2 population in MRL-*lpr* mice would have an effect on the outcome of progressive SLE-like organ inflammation. ILC2s can be effectively expanded by a short treatment course of IL-33 injections in various organs, including the kidney [14]. To restore the number of renal ILC2s in the kidney, we treated cohorts of MRL-*lpr* mice with two courses of IL-33 injections (400 ng i.p. on four consecutive days) at 14 and 17 weeks of age and followed the clinical course of the disease (Fig. 5A). Analysis of mice two weeks after the second IL-33 treatment course at week 19 confirmed that

this regimen was sufficient to overcome reduced proliferation of ILC2s in MRL-*lpr* mice and resulted in a substantial increase of ILC2 numbers in the kidneys, as compared to PBS-treated MRL-*lpr* mice (Fig. 5B and C). Accumulation of ILC2s was accompanied by a significant increase in IL-5 and IL-13 mRNA expression and an increased abundance of eosinophils in the kidney (Fig. 5B–D). However, systemic lymphoproliferation, as assessed by spleen weight, was unaffected by IL-33 treatment (Fig. 5E).

To address compartment specific immune cell infiltration (glomerular versus tubulointerstitial) and glomerular pathology, we performed histologic analysis of the renal cortex of the two groups of mice (Fig. 5F and G). These analyses revealed a reduction of Mac-2$^+$ macrophage infiltration, and to a lesser extend of CD3$^+$ T cells and GR-1$^+$ neutrophils, in the glomerular compartment of IL-33 treated mice, as compared to their PBS-treated counterparts (Fig. 5F). In line with reduced glomerular cell infiltration, glomerular size, as a measure of glomerular proliferation, was also moderately decreased in IL-33-treated mice (Fig. 5G). Interestingly, this reduction in immune cell infiltration was specific to the glomerulus, since we did not observe such differences in the tubulointerstitial compartment (Supporting Information Fig. S3) and the overall infiltration of myeloid cell subsets and of effector and regulatory T cells into the kidney cortex, as assessed by flow cytometry, was also unaffected by IL-33 treatment (Supporting Information Fig. S3). Glomerular histopathology revealed glomerular proliferation consistent with lupus nephritis (Fig. 5G), but development of severe proliferative lupus nephritis with glomerular crescents was observed only in a small fraction of animals at this time point (PBS-treated group 2/14; IL-33-treated group 0/14). This was also reflected in the considerable variability regarding albuminuria and blood urea nitrogen levels, both showing a higher numerical mean in the PBS-treated group (Fig. 5H and I). Taken together these data indicate a moderate reduction in glomerular inflammation after IL-33 treatment of MRL-*lpr* mice at this early time point.

Prolonged IL-33 treatment improves survival of MRL-*lpr* mice

To answer the question, whether IL-33 treatment could have a prolonged effect on the clinical outcome of MRL-*lpr* mice, we treated cohorts of mice with two courses of IL-33 injections (400 ng i.p. on four consecutive days) at later time points (week 15 and 20) and followed the clinical course of the disease until week 25 (Fig. 6A). Analysis of a subgroup of mice two weeks after the second IL-33 treatment confirmed that this regimen was equally efficient to increase ILC2s, eosinophils and IL-5 / IL-13 mRNA expression in the kidneys of diseased MRL-*lpr* mice (Fig. 6B–D). Interestingly, IL-27p28 and, to a lesser extent, IFN-γ expression were decreased in the IL-33-treated group, suggesting a cross-regulation between ILC2s and the IFN-γ- and IL-27-producing cell types at this later time point in the kidney. Spleen weight and production of dsDNA-specific autoantibodies were unaffected by IL-33 treatment (Fig. 6E).

Next, we analyzed the effects of IL-33 treatment on renal outcome and overall survival of MRL-*lpr* mice. Similar to the data presented in Fig. 5, development of severe albuminuria over the time course of the disease was highly variable in both groups and mean albuminuria measured by ELISA was not significantly different between the two groups at any time point (data not shown). Mean dipstick proteinuria, as a clinical marker for lupus nephritis, tended to be reduced in the IL-33-treated group, but also failed to reach statistical significance (Fig. 6F). In line with early amelioration of nephritis by IL-33 treatment, the percentage of animals with overt nephritis (proteinuria grade 2 or higher in dipstick analysis) was significantly reduced among IL-33-treated animals four weeks after the first course of IL-33 injections (Fig. 6G). However, this beneficial effect of ILC2 expansion was less pronounced at the later time point (2 weeks after the second treatment course) and the reduction of glomerular immune cell infiltration observed with the early IL-33 treatment regimen (see Fig. 5), was not sustained at this later time point (data not shown). Taken together, this possibly indicates that the beneficial IL-33 effect on nephritis development is transient and outcompeted by the massive infiltration of pathogenic effector cells in the later stages of this genetic model.

Most importantly, however, survival of the IL-33-treated group until week 25 was significantly better than that of the PBS-treated group (Fig. 6H), suggesting that in addition to the moderate beneficial effects on renal outcome, IL-33 treatment might have kidney-independent effects on the progression of SLE-like disease in MRL-*lpr* mice.

Discussion

Since their initial identification as a separate cell population [19, 22–25], group 2 innate lymphoid cells (ILC2s) have gained a lot of attention in immunological research. They have been characterized as major players of the type 2 immune response that is induced by helminth infections and whose dysregulation leads to development of allergic diseases [26]. Another important feature of ILC2s is their ability to promote tissue regeneration by expressing type 2 mediators such as IL-13, IL-5, IL-9 and amphiregulin that initiate a repair program aimed at restoring tissue homeostasis after different types of injury [7–10].

While the role of ILC2s in helminth infections and allergic diseases has been extensively studied, a potential role of ILC2s in autoimmune disorders remains largely elusive. In classical settings of autoimmunity, such as multiple sclerosis, arthritis and glomerulonephritis, chronic inflammation is driven by a type 1/type 3 immune response that promotes classical activation of macrophages and infiltration of neutrophils, leading to tissue destruction [26, 27]. In this context, components of the type 2 response have been shown to suppress type 1/3 immunity on different levels, leading to amelioration of immune-mediated organ damage [26].

In the present study, we show that kidney-residing ILC2s, which were found at a relatively high frequency in the kidneys

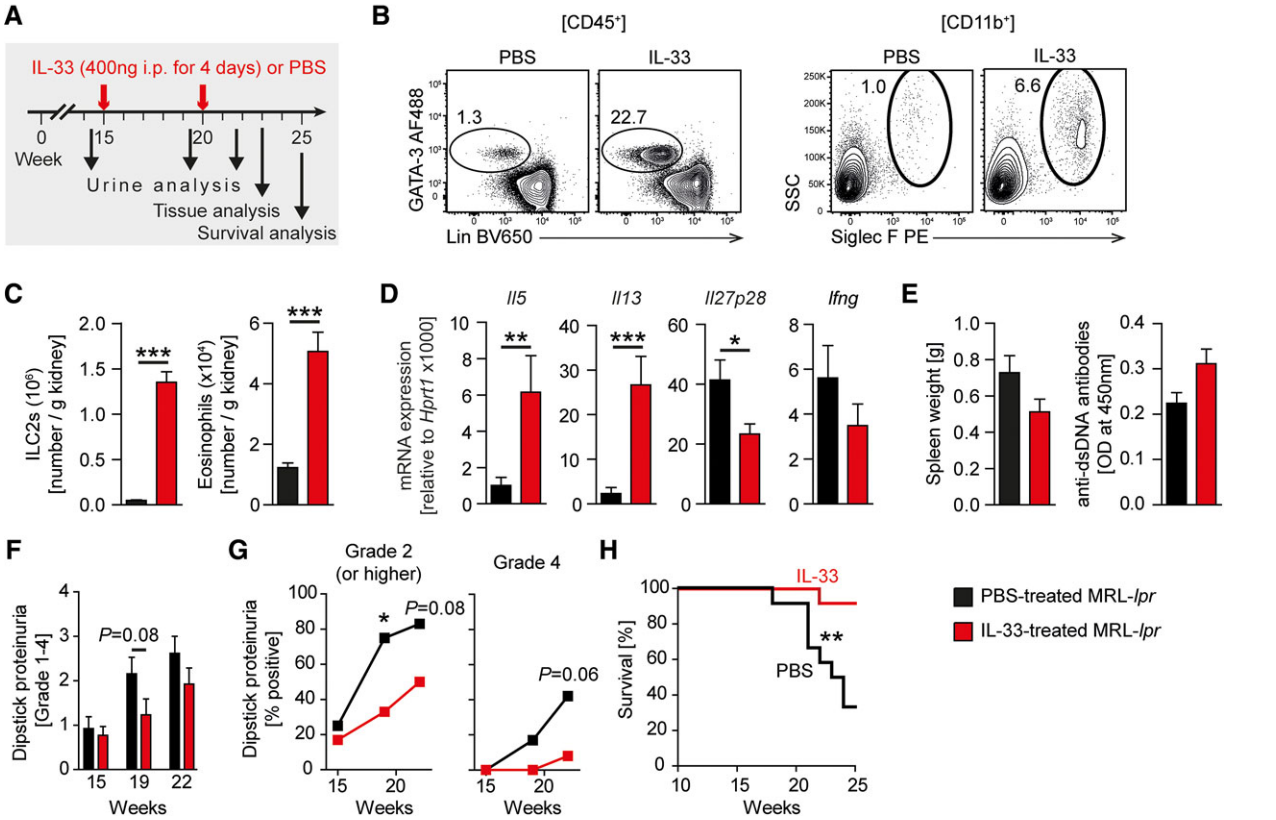

Figure 6. Prolonged IL-33 treatment improves survival of MRL-*lpr* mice. (A) Treatment regimen for MRL-*lpr* mice. IL-33 (400 ng i.p.) or PBS was applied on four consecutive days at 15 and 20 weeks of age. Mice were sacrificed at week 22 for tissue analyses or followed until week 25 for analysis of survival. (B, C) Representative flow cytometry plots (B) and absolute numbers (C) of Lin$^-$GATA-3$^+$ ILC2s and CD11b$^+$SiglecF$^+$SSc^{hi} eosinophils in the kidney of IL-33-treated and PBS-treated MRL-*lpr* mice at week 22. For gating of ILC2s, cells were stained for CD45, CD127, GATA-3, CD90.2, and lineage markers. For gating of eosinophils, cells were stained for CD45, CD3, CD4, CD8, β-TCR, γδ-TCR, SiglecF, CD11c, CD11b, F4/80, Ly6G, NK1.1. Gating strategy is specified in brackets and numbers indicate the percentage of events in the respective gates. Data are representative of at least two independent experiments (group numbers as specified below). (D) Quantitative RT-PCR analyses of the indicated mRNA transcripts in the kidney of the two groups of mice. (E) Spleen weight and anti-dsDNS antibody levels in the two treatment groups (OD = optical density at a serum dilution of 1:500). (F) Mean dipstick proteinuria in the two treatment groups before start of IL-33 treatment at week 15, at week 19 and at week 22. Differences between individual experimental groups in C-E were compared using a one-way ANOVA with post hoc analysis by Tukey's test for multiple comparisons. (G) Percentage of MRL-lpr mice with overt nephritis (≥ grade 2 dipstick proteinuria) and severe nephritis (grade 4 dipstick proteinuria) in the two groups. For contingency analysis of proteinuria a Chi-Square test was used. Group numbers in B-E are $n = 9$–12 for IL-33-treated MRL-*lpr* mice and $n = 11$–13 for the PBS-treated MRL-*lpr* mice. (H) Survival data for IL-33-treated MRL-*lpr* mice ($n = 12$) and PBS-treated MRL-*lpr* mice ($n = 12$), pooled from three independent experiments. Survival data was compared by using a Log-rank test (Mantel-Cox). Symbols in C–E represent mean ± SEM (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ in a two-tailed unpaired Student's *t*-test).

of young MRL-*lpr* mice before the onset of renal disease, were reduced in abundance and proliferative activity in mice with manifest lupus nephritis. This ILC2 reduction was paralleled by a strong infiltration of CD4$^+$, CD8$^+$ and DN T cells into the kidney, which is a hallmark of proliferative lupus nephritis [3, 28], leading to the assumption that there might be a direct effect of the infiltrating T cells on ILC2s in the kidney.

While initial reports that led to the identification of ILC2s as a separate cell population focused on epithelial cell-derived cytokines that promote expansion and activation of ILC2s [19, 22–25], such as IL-25 and IL-33, recent studies have revealed a network of negative ILC2 regulators that prevents overshooting ILC2 activation, thereby restricting type 2 immunopathology [16–18, 29, 30]. Especially type I and type II interferons [17, 18, 30], as well as IL-27 [17, 18, 29], have been identified as potent suppressors of ILC2 proliferation and activation in mouse models of helminth infections [16, 29], allergic airway disease [16–18, 29, 30], and viral respiratory tract infections [18]. Most available studies used exogenous cytokine application to demonstrate effects on ILC2s. However, two studies elegantly showed that the endogenous interferon response elicited by infection with *Listeria monocytogenes* (IFN-γ) or influenza A virus (type I interferons) inhibits ILC2 expansion in the lung [16, 18]. Whether a similar mechanism could lead to downregulation of a potentially protective ILC2 response in a setting where the endogenous Th1 response is a result of autoimmune dysregulation, had not been addressed so far.

Here, we show that IFN-γ and IL-27 expression in the kidney strongly increases with the progression of lupus nephritis in MRL-*lpr* mice. In vitro experiments revealed that IL-33-induced proliferation and cytokine production of purified kidney ILC2s are extremely sensible to inhibition by IFN-γ and IL-27, providing an

explanation for reduction in ILC2s in the inflamed kidney. This is in line with a report, indicating that artificial systemic activation of NKT cells by injection of α-galactosylceramide leads to reduced expression of IL-13 and IL-5 in lung and kidney ILC2s [31]. However, potential in vivo sources of negative ILC2 regulators in relevant models of inflammatory diseases had not been studied so far. A very recent study addressed this issue and clearly showed that IFN-γ production by activated NK cells inhibits ILC2s and leads to a reduced type 2 immune response in mouse models of T cell-independent acute lung and liver inflammation [30], but the consequences of this negative crossregulation between different groups of ILCs for chronic tissue immunopathology have not been addressed in this study.

While NK cells and NKT cells might be relevant producers of IFN-γ in acute inflammatory settings [30, 31], ILC2-inhibiting cytokines in chronic tissue inflammation, e.g. in autoimmune diseases, are presumably derived from other immune cell populations. In the MRL-*lpr* model of chronic SLE-like organ inflammation, we observed that $CD4^+$ T cells are the main producers of IFN-γ in the inflamed kidney, whereas IL-27, as another important negative ILC2 regulator, was mainly produced by cells of myeloid origin. This suggests that both lymphoid and myeloid cell subsets are equipped to downregulate ILC2 responses, but use distinct cytokine pathways to mediate this suppression in this relevant model of chronic autoimmunity.

A critical role for the type 1 response and especially for IFN-γ in development of proliferative lupus nephritis has been demonstrated in SLE patients [32] and the MRL-*lpr* model of SLE-like disease [33]. However, the pleiotropic effects of IFN-γ on cells of hematopoietic and non-hematopoietic origin and the lack of targeting strategies to delete the IFN-γ receptor specifically in ILC2s, make it difficult to determine the contribution of the IFN-γ effect on ILC2s in chronic inflammation models. Moreover, the final proof that T cell-derived IFN-γ and/or myeloid cell-derived IL-27 is responsible for in vivo ILC2 suppression in this context would require genetic deletion or efficient antibody blockade of these mediators which is difficult to achieve in the MRL-*lpr* model. Indirect evidence for a protective role of ILC2s in SLE pathogenesis is provided by a study demonstrating that MRL-*lpr* mice with a deficiency in the IL-27 receptor show improved survival accompanied with a systemic type 2 response and a less severe form of lupus nephritis [34]. However, this report was published long before ILC2s emerged as a separate cell population and the exact cellular source of the increased type 2 response in *Il27r*$^{-/-}$ MRL-*lpr* mice remained unclear [34]. A more recent study addressed the role of ILC2s in the development of joint inflammation in a mouse model and in patients with rheumatoid arthritis [35]. The authors provided evidence that IL-9 production by ILC2s promotes resolution of joint inflammation in mice via interaction with regulatory T cells. Most importantly, the reduced numbers of IL-9-producing ILC2s that were found in the blood and synovia of rheumathoid arthritis patients with active disease increased with disease remission, suggesting that ILC2s have an anti-inflammatory function in human autoimmune diseases [35]. In support of this hypothesis, ILC2s numbers were found to be reduced in the peripheral blood of patients with anti-neutrophil cytoplasmatic antibody-associated vasculitis, another important human autoimmune disease [36]. Furthermore, it has been shown that human ILC2s are sensible to interferon-mediated inhibition in vitro [18].

To approach the question if restoration of reduced ILC2 numbers is a valid therapeutic strategy in the MRL-*lpr* model of chronic SLE-like organ inflammation, we applied a four day course of the ILC2-activating cytokine IL-33 at 15 and 20 weeks of age. IL-33 treatment has been successfully used to promote protective, ILC2-dependent type 2 responses in mouse models of multiple sclerosis [12], arthritis [37] and in a mouse model of progressive chronic kidney disease induced by toxic-injury of glomerular podocytes [14]. In line with our hypothesis, early short-term treatment resulted in reduced glomerular immune cell infiltration and a reduced frequency of severe renal involvement in IL-33-treated MRL-*lpr* mice. Whether this amelioration of lupus nephritis under IL-33 treatment is exclusively mediated by ILC2s, or if other cell types participate, was not directly addressed in our study. However, the strong effect of IL-33 treatment on the ILC2 population in the kidney (more than 20-fold increase, see Fig. 5B and C; and Fig. 6B and C) and the absence of substantial changes of other potentially IL-33-responsive, anti-inflammatory cell populations in the kidney, such as Tregs (Supporting Information Fig. S4 and reference [14]), argue for a mainly ILC2-mediated effect. However, the fact that the beneficial effect of IL-33 treatment on glomerular immune cell infiltration and albuminuria was not sustained over the whole observation period until week 22, despite substantially increased kidney ILC2 numbers, indicates that the tissue protective properties of ILC2s might be outcompeted by other proinflammatory cell types in the kidney during later stages of renal disease. Importantly, we still observed a significant improvement of overall survival in the IL-33-treated group which might be a result of reduced immune-mediated injury in other organs not investigated in this study. We also found significantly reduced IL-27 expression in the kidneys of IL-33-treated MRL-*lpr* mice, indicative of a potential crossregulation between ILC2-mediated type 2 immunity and type 1 mediators that act as negative ILC2 regulators.

Although supported by a number of other studies mentioned above [12, 14, 37], our results are in disagreement to a recent report, suggesting that long-term anti-IL-33 antibody treatment ameliorates nephritis and improves survival in MRL-*lpr* mice [38]. While a number of other studies have shown promotion of Tregs [37, 39] and anti-inflammatory myeloid cell responses [40] by IL-33, Li at al. show a systemic reduction of pro-inflammatory cells types and a promotion of systemic Treg and myeloid-derived suppressor cell responses in IL-33-depleted MRL-*lpr* mice [38]. These discrepancies might be explained by the time course of IL-33 action (short-term IL-33 treatment vs. long-term IL-33 depletion), but the exact reasons for these conflicting results remain unresolved.

In summary, we show here that kidney-residing ILC2 functions are suppressed by T cell and myeloid cell-derived proinflammatory cytokines that are upregulated in a mouse model of SLE-like organ inflammation. Since ILC2s represent a major ILC subset in the healthy human kidney [14], we identify targeting of ILC2-inhibiting cytokines or direct activation of ILC2s

as a potential treatment approach for immune-mediated kidney injury in humans. Future studies in patients with SLE and other chronic inflammatory conditions will evaluate, whether ILC2-directed treatment, as a therapeutic strategy, can be translated to human autoimmune diseases.

Materials and methods

Animals and IL-33 treatment

MRL/MpJ-*Fas*^{lpr} (MRL-*lpr*) mice and MRL/MpJ (MRL control) mice were purchased from The Jackson Laboratory and bred in the animal facility of the University Medical Center Hamburg-Eppendorf. All mice were housed under specific pathogen-free conditions. For time course experiments of cell populations, we analyzed healthy 10-week-old MRL control mice, as well as young (10 weeks old) and diseased (20 weeks old) MRL-*lpr* mice. For ILC expansion, MRL-*lpr* mice were treated with 400 ng recombinant murine IL-33 (Biolegend) in 200 μL PBS. Controls received 200 μL PBS. Adult male mice with the appropriate age-matched controls, where applicable, were used in all experiments. For urine sample collection, mice were housed in metabolic cages for 6 h. Urinary dip stick analyses were performed by using Multistix® 10 SG according to manufacturer´s instructions (Siemens). Urinary albumin excretion was determined by standard ELISA analysis (Mice-Albumin Kit; Bethyl Laboratories, Montgomery, TX). Urinary creatinine and blood urea nitrogen were measured using standard laboratory methods.

Cell isolation

For isolation of leukocytes from the mouse kidney, the tissue was cut into small pieces, digested in complete medium (RPMI 1640, 10% FCS, 1% HEPES, 1% Penicillin/Streptomycin; all Gibco) with Collagenase D (0.4 mg/mL, Roche) and DNase I (100 μg/mL, Roche) for 45 min while rotating on a MACSmix® tube rotator (Miltenyi) and then further dispersed by using a gentleMACS® dissociater (Miltenyi). Further leukocyte purification was achieved by Percoll gradient centrifugation (37.5%). After subsequent erythrocyte lysis with ammonium chloride, cell suspensions were filtered through a 50 μm strainer and used for further analyses. For isolation of renal tubular cells from the kidney, the ureter was removed and the remaining tissue was cut into small pieces. Two kidneys were digested in 10 mL digestion medium (5 mL HBSS without $CaCl_2$ and $MgCl_2$ and 5 mL DMEM/F-12; both Gibco) containing 25 mg Collagenase V (Sigma) and 25mg BSA (Sigma) for 17 min at 37°C. Tubular epithelial cells were further enriched by filtration through a 250 μm strainer and subsequent Percoll gradient centrifugation (45%).

Flow cytometry and cell sorting

Before incubation of the cells with the antibody cocktail for 20 min at 4°C, nonspecific staining was blocked with 10% normal mouse serum. To exclude dead cells from analysis, cells were stained with Near-IR fluorescent reactive dye (Invitrogen) or Zombie dye (BioLegend) according to manufacturer's instruction. To characterize leukocyte subsets, cell suspensions of mouse kidneys were stained with fluorochrome-coupled antibodies against CD45 (30-F11), IL-7Rα (CD127; A7R34), Thy1.2 (CD90.2; 30-H12) and a combination of lineage markers (Lin) including CD3 (145-2C11), CD4 (RM4-5), CD5 (53-7.3), CD8 (53-6.7), CD11b (M1/70), CD11c (HL-3), CD19 (6D5), CD49b (HMα2), TCR-γδ (H57-597), TCR-αβ (GL3), NK1.1 (PK136), GR-1 (RB6-8C5), Ter119 (Ter119) and B220 (RA3-6B2). Intranuclear staining, using antibodies against GATA-3 (L50-823), T-bet (4B10), ROR-γt (Q31-378) and Ki-67 (B56) was done with the Transcription Factor Staining Buffer Set (eBioscience) according to the manufacturer's instruction. For further characterization of ILC surface marker expression, antibodies against CD25 (PC61.5) and Sca-1 (D7) were used. For characterization of other leukocyte subtypes, antibodies against CD3, CD4, CD8, TCR-β, TCR-γδ, CD90.2, Foxp3 (FJK-16s), CD11b, CD11c, MHCII, F4/80 (BM8), GR1, Ly6G (1A8) and SiglecF (E50-2440) were used. For staining of the renal proximal tubular cells, antibodies against Lotus Tetragonolobus Lectin (VEC-FL-1321, Biozol) and E-Cadherin (DECMA-1) were used. For intracellular IFN-γ staining isolated leukocytes were restimulated in standard medium (RPMI-1640 containing 10% FCS, 1% HEPES, 1% Penicillin/Streptomycin, and 2-Mercaptoethanol (50μM, Life Technologies)) with phorbol 12-myristate 13-acetate (50ng/mL, Sigma) and ionomycin (1μg/mL, Calbiochem) in the presence of brefeldin A (10 μg/mL, Sigma) for 2.5 h at 37°C. For intracellular IL-27p28 staining isolated leukocytes were first cultured in IFN-γ (0.1μg/mL, BioLegend) for 2 h at 37°C. Subsequently, brefeldin A (10 μg/mL) and R848 (TLR7/8 agonist, 2 μg/mL, Invivogen) were added and cells were cultured for another 24 h. After restimulation, cells were stained for surface markers as described above, fixed with formalin (3.8%, Sigma), permeabilized with IGEPAL® CA-630 (0.1%, Sigma), and stained with fluorochrome-coupled antibodies against IL-27p28 (MM27-7B1) and IFN-γ (XMG1.2). Samples were acquired on a Becton Dickinson (BD) LSRII system using the Diva software and data analyses were performed with FlowJo (Tree Star). Flow cytometry-based cell purification for cultures and mRNA expression analyses were done with a BD Fusion or Aria III. Full gating strategy reported in Supporting Information Fig. S5.

Innate lymphoid cell cultures

ILC2s were purified by FACS (Sorting strategy: $CD45^+$ $CD90.2^+CD4^-Lin^-CD127^+CD25^+Sca\text{-}1^+$, see Fig. 3A) from 10 week-old MRL-*lpr* mice and cultured for 72 h in RPMI-1640 containing 5% FCS, 1% HEPES, 1% Penicillin/Streptomycin, and 2-Mercaptoethanol (50 μM, Life Technologies), in the presence of IL-7 (10 ng/mL, BioLegend). Different combinations of the cytokines IFN-γ (50 ng/mL, BioLegend), IL-27 (50 ng/mL, BioLegend) and IL-33 (10 ng/mL, BioLegend) were added, respectively. Live dead staining was performed with the FITC

Annexin V Apoptosis Detection Kit with 7-AAD (BioLegend). Supernatants were analyzed for concentrations of the cytokines IL-5 and IL-13 with the Legendplex bead-based immunoassay according to the manufacturer's instructions (BioLegend).

Morphologic analysis

Light microscopy and immunohistochemistry were performed by routine procedures. In brief, paraffin-embedded sections (2 μm) were stained with an antibody directed against the T cell marker CD3 (A0452; Dako, Hamburg, Germany), the neutrophil marker GR-1 (Ly6 G/c, NIMP-R14; Hycult Biotech, Uden, The Netherlands), the glomerular monocyte marker MAC-2 (M3/38; Cedarlane Canada), or the macrophage/dendritic cell marker F4/80 (BM8; Dianova BMA, Augst, Switzerland) and developed with a polymer-based secondary antibody alkaline phosphatase kit (POLAP, Zytomed, Berlin, Germany). Glomerular $CD3^+$, $GR\text{-}1^+$ and $Mac\text{-}2^+$ cells in 30 glomerular cross-sections (magnification 400×), tubulointerstitial $GR\text{-}1^+$ cells in 20 low-power fields (magnification 200×), and tubulointerstitial $F4/80^+$ and $CD3^+$ cells in 30 high-power fields (magnification 400×) per kidney were counted in a blinded manner. Crescent formation was assessed in at least 30 glomeruli per mouse in a blinded fashion in Periodic Acid-Schiff-stained paraffin sections. Glomerular size was measured in 40 glomeruli per mouse (magnification 200×) with the contour tool of the AxioVision Software (Carl Zeiss Vision).

Real-time RT-PCR

Total RNA of renal cortex or sorted ILCs was prepared according to standard laboratory methods. After reverse transcription to cDNA, real-time PCR was performed for 40 cycles (initiating denaturation at 95°C for 10 min, denaturation at 95°C for 15 s, and primer annealing and elongation at 60°C for 1 min) with 1 μL of cDNA samples in the presence of 0.5 μL of specific murine primers. TaqMan® Gene Expression Assays and a StepOnePlus Real-Time PCR system (both ThermoFisher Scientific) were used for quantification of the housekeeping gene (*Hprt1*) and the genes of interest. All samples were run in duplicate.

Statistical analysis

The two-tailed unpaired Student's *t*-test was used for comparison between two groups. In the case of three or more groups differences between individual experimental groups were compared using a one-way ANOVA with post hoc analysis by Tukey´s test for multiple comparisons. For contingency analysis of proteinuria a Chi-Square test was used. Survival data was compared by using a Log-rank test (Mantel-Cox). A p-value of <0.05 was considered to be statistically significant.

Study approval

All animal experiments were performed according to national and institutional animal care and ethical guidelines and were approved by the local authorities (Behörde für Verbraucherschutz).

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Abbreviations: **ILC**: innate lymphpoid cells · **IL**: interleukin · **IFN**: interferon · **SLE**: systemic lupus erythematosus · **GATA-3**: GATA binding protein 3

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