Both Fc γ Receptor I and Fc γ Receptor III Mediate Disease in Accelerated Nephrotoxic Nephritis

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Recognition of immune complexes in glomeruli by activator Fc γ receptors (Fc γ RI and Fc γ RIII) is an important step in the development of glomerulonephritis. The low-affinity receptor (Fc γ RIII) has previously been shown to be important in passive heterologous immune complex glomerulonephritis. However, most forms of human glomerulonephritis involve an active immune response, and the relative importance of Fc γ RI (high-affinity receptor) and Fc γ RIII in an active model of glomerulonephritis is not known. We have now studied accelerated nephrotoxic nephritis in Fc γ RIII $^{-/-}$ mice and Fc γ RI/III double-deficient mice, and compared them with matched wild-type controls and FcR γ chain-deficient (FcR $\gamma^{-/-}$) mice. Mice were immunized against sheep IgG and injected with sheep anti-mouse glomerular basement membrane antibody 5 days later. Both Fc γ RI/III double-deficient mice and FcR $\gamma^{-/-}$ mice were strongly protected from renal injury. In contrast, Fc γ RIII $^{-/-}$ mice developed substantial nephritis, although there was a dose-dependent partial protection from glomerular crescents and thrombosis. Despite this histological protection from injury, the macrophage infiltrate was not reduced, implying a dissociation of macrophage accumulation from activation in the absence of activatory Fc γ RIII. Therefore, both Fc γ RI and Fc γ RIII play a role in this active model of glomerulonephritis, because both had to be deficient to protect markedly from disease. (*Am J Pathol* 2003, 162:1677-1683)

Most forms of human glomerulonephritis are associated with deposition of immune complexes in glomeruli. Immune complexes containing IgG communicate with cells via Fc γ receptors, an important link between the innate and adaptive immune systems. Increasing evidence implicates Fc γ receptors in immune-mediated inflammation¹ and the recognition of immune complexes deposited in glomeruli by cellular activator Fc γ receptors is an important step in the development of glomerulonephri-

tis.²⁻⁵ Blockade of this pathway is, therefore, an attractive therapeutic target but design of rational therapy requires a better understanding of the exact role of the various types of Fc γ receptor.

Murine phagocytic effector cells express three different classes of IgG receptors, Fc γ RI, Fc γ RII, and Fc γ RIII. Fc γ RI and Fc γ RIII are hetero-oligomeric activatory receptors in which a ligand-binding α chain is associated with a signal-transducing γ -chain. The γ -chain is required for receptor assembly and triggering of effector functions including phagocytosis, antibody-dependent cytotoxicity, and release of inflammatory mediators.⁶ These functions are mediated through an immunoreceptor tyrosine activation motif within the γ -chain. Fc γ RIII is a low-affinity activatory Fc γ receptor that interacts with IgG1, IgG2a, and IgG2b immune complexes and is widely expressed on circulating leukocytes, especially macrophages and polymorphs.^{7,8} In contrast, Fc γ RI is a high-affinity activatory receptor that interacts predominantly with monomeric IgG2a.⁹ The third receptor class for IgG, Fc γ RII is a single α -chain receptor and contains an immunoreceptor tyrosine inhibitory motif in the cytoplasmic domain. Fc γ RII has been shown to be a negative regulator of activatory Fc γ receptors I and III in immune complex-mediated inflammation.^{10,11}

Mice deficient in the FcR γ chain are protected from several forms of glomerulonephritis, including a spontaneous model of lupus nephritis and an active model of immune complex glomerulonephritis.²⁻⁵ However, the FcR γ chain is the intracellular signaling subunit for both activator murine Fc γ receptors I and III as well as the high-affinity IgE receptor,⁶ a collagen receptor on platelets,¹² the recently described paired immunoglobulin-like receptors,¹³ and the natural killer receptor-P1.¹⁴ Because FcR $\gamma^{-/-}$ mice lack several receptors, reduced glomerulonephritis in FcR $\gamma^{-/-}$ mice suggests, but does not conclusively prove, a role for activator Fc γ receptors in immune-mediated nephritis.

The development of mice lacking the α chain of specific Fc γ receptors has facilitated the study of the individual contributions of these receptors.^{7,15,16} In a passive model of heterologous nephrotoxic nephritis, Fc γ RIII was

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found to be the most important receptor involved in neutrophil infiltration.¹⁷ In this model, rabbit IgG directed against glomerular basement membrane was injected without preimmunization, leading to a glomerular neutrophil influx that resolved within 24 hours. This acute model depends on the interaction of the heterologous deposited glomerular antibody with Fc γ receptors, because the murine immune response does not develop this early in the disease. In contrast, most forms of human glomerulonephritis involve an autologous antibody response to exogenous or endogenous antigen with deposition of host immune complexes. We have therefore studied the model of accelerated nephrotoxic nephritis in which mice were preimmunized with sheep immunoglobulin and then given an injection of sheep anti-mouse glomerular basement membrane globulin. This binds to the glomerulus where it acts as a planted antigen to which mouse IgG becomes bound. This has been shown to be a T-cell-dependent model characterized by leukocyte infiltration, proteinuria, thrombosis of glomerular capillaries, glomerular crescent formation, and renal impairment.¹⁸

We have used this model to elucidate the role of individual activator Fc γ receptors. Specifically we have studied the model in mice lacking Fc γ RIII or lacking both Fc γ RI/III and compared them with matched wild-type (WT) controls and mice lacking the common Fc γ chain.

Materials and Methods

Mice

Fc γ –/– mice were generated in the laboratory of Professor T. Saito, Chiba University, Chiba, Japan.³ Fc γ RIII-deficient mice were generated as previously described.⁷ Fc γ –/– mice were on a pure C57BL/6 background, and Fc γ RIII–/– mice were backcrossed to C57BL/6 for nine generations. Age- and sex-matched C57BL/6 controls were purchased from Harlan Ltd. (Bicester, UK). Fc γ RI/III–/– mice were on a hybrid C57BL/6/129/BALB/c background with a major contribution from C57BL/6 and were compared with a genetically matched control line derived from littermates.¹⁵ All experiments were performed according to institutional guidelines.

Induction of Accelerated Nephrotoxic Nephritis

A sheep was immunized with murine glomerular lysate initially in complete and then incomplete Freund's adjuvant at monthly intervals. A γ -globulin-enriched fraction of the serum was prepared by ammonium sulfate precipitation, after first heat-inactivating complement activity. The precipitate was then extensively dialyzed against phosphate-buffered saline (PBS), and finally sterile filtered before storing in aliquots at –70°C. The endotoxin content of the nephrotoxic serum measured using the BioWhittaker 1000-QCL LPS assay kit was less than 0.1 EU/ml (BioWhittaker, Walkersville, MD).

Mice were immunized intraperitoneally with 0.2 mg of sheep IgG in a 50:50 mix with complete Freund's adjuvant (Sigma Chemical Co., St. Louis, MO). Five days

later, the animals were injected with 5 or 10 mg of nephrotoxic serum via a tail vein. At time points from 2 hours to 26 days after nephrotoxic serum injection, mice were anesthetized with midazolam and fentanyl intraperitoneally and exsanguinated before harvesting the kidneys.

Histological Studies and Quantitative Immunofluorescence

Kidneys were fixed for 2 hours in Bouin's solution, transferred to 70% ethanol, processed to paraffin, and stained with periodic acid-Schiff (PAS) reagent. Samples were assessed by an observer blinded to the experimental group. Glomerular thrombosis was assessed by scoring individual glomeruli for PAS-positive material as follows: grade 0, no PAS-positive material; grade 1, 0 to 25% of glomerular cross-section; grade 2, 25 to 50%; grade 3, 50 to 75%; grade 4, 75 to 100%. Fifty glomeruli were scored per point, and the mean glomerular thrombosis score was calculated for each mouse. Glomerular crescents were defined as glomeruli containing two or more layers of cells in Bowman's space (50 glomeruli counted per point, converted to percentage crescents).

For immunofluorescence, kidneys were embedded in OCT (CellPath, Powys, UK), snap-frozen in isopentane cooled with liquid nitrogen, and stored at –70°C. Sections of 5 μ m were fixed in acetone for 10 minutes. Fluorescein isothiocyanate-conjugated goat anti-mouse IgG (Fc-specific) and fluorescein isothiocyanate-conjugated monoclonal mouse anti-sheep IgG clone GT34 (Sigma Chemical Co.) were used. For quantitative immunofluorescence, blinded sections were examined at $\times 100$ magnification using an Olympus BX4 fluorescence microscope (Olympus Optical, London, UK) and a Photonic Science Color Coolview camera (Photonic Science, Robertsbridge, UK). Samples from each experiment were measured together. The mean intensity of 20 glomeruli for each sample was recorded in arbitrary fluorescence units.

For immunoperoxidase staining for cell markers, kidneys were fixed for 4 hours in periodate lysine paraformaldehyde, washed overnight in 13% sucrose in PBS, and frozen in isopentane cooled with liquid nitrogen. Sections of 5 μ m were stained for macrophages with FA11 (monoclonal rat anti-mouse CD68; Serotec, Oxford, UK) in a three-layer immunoperoxidase technique as previously described.¹⁹

Serum Creatinine, Albumin, and Albuminuria

Serum creatinine and albumin were measured in the Department of Chemical Pathology, Hammersmith Hospital. Serum albumin was analyzed using the bromocresol green method and serum creatinine was measured using an Olympus AU640 autoanalyzer. Individual 24-hour collections of urine were performed using metabolic cages, with free access to food and water. Albuminuria was assessed by radial immunodiffusion against a rabbit anti-mouse albumin as previously described.¹⁹ Hemacombistix (Bayer Diagnostics, Newbury, UK) were used

to assess proteinuria when metabolic cages were not available.

Measurement of the Murine Anti-Sheep IgG Immune Response

Serum mouse anti-sheep IgG levels were measured by enzyme-linked immunosorbent assay.¹⁹ Briefly, enzyme-linked immunosorbent assay plates (Nunc Maxisorb; Fisher Scientific, Loughborough, UK) were coated overnight at 4°C with 100 μ g/ml of sheep IgG (Sigma Chemical Co.). After blocking with 3% bovine serum albumin (ICN Biomedicals Inc., Aurora, OH) diluted serum samples were incubated for 1 hour at 37°C. For each experiment a range of serum dilutions was tested with a standard curve of a known positive sample. Goat anti-mouse IgG (Fc-specific) conjugated to alkaline phosphatase (Sigma Chemical Co.) was used as a secondary and the optical density at 405 nm was measured using *p*-nitrophenyl phosphate as the substrate (Sigma Chemical Co.).

Statistics

Results are expressed as mean $\pm$ SE. Nonparametric tests of significance were applied throughout. For comparing two groups, the Mann-Whitney *U*-test was used. For groups of three or more, the Kruskal-Wallis test was used, with Dunn's multiple comparisons test (*P* values given are from Dunn's test). GraphPad Prism (GraphPad Software, San Diego, CA) was used to analyze the data. Differences were considered significant when *P* was <0.05 .

Results

Fc γ R $^{-/-}$ Mice Were Strongly Protected from Nephritis, but Fc γ RIII $^{-/-}$ Mice Were Only Partially Protected in a Dose-Dependent Manner

Accelerated nephrotoxic nephritis was simultaneously induced in WT (*n* = 8), Fc γ RIII $^{-/-}$ (*n* = 8), and Fc γ R $^{-/-}$ (*n* = 9) female mice, with 10 mg of nephrotoxic serum. All mice were sacrificed on day 7 after nephrotoxic serum injection, except for one WT mouse that was sacrificed on day 5 because of renal disease. WT and Fc γ RIII $^{-/-}$ mice developed glomerular thrombosis, glomerular crescents, and renal impairment whereas Fc γ R $^{-/-}$ mice were completely protected (Figures 1 and 2 and Table 1). At this dose of nephrotoxic serum, there was no statistically significant difference between histological disease, renal impairment, or serum albumin between WT and Fc γ RIII $^{-/-}$ mice, suggesting the involvement of other Fc γ -associated receptors such as Fc γ RI in pathogenesis. Disease was then compared between WT (*n* = 9) and Fc γ RIII $^{-/-}$ (*n* = 12) mice using half the dose of nephrotoxic serum (5 mg) to day 8 after nephrotoxic serum injection. With this lower dose, partial protection of Fc γ RIII $^{-/-}$ mice from glomerular thrombosis and glo-

Figure 1. Glomerular thrombosis score (*n* = 50 glomeruli per point; **A**) and glomerular crescents (*n* = 50 glomeruli per point; **B**), day 7 after 10 mg of nephrotoxic serum (experiment 1) or 5 mg of nephrotoxic serum (experiment 2). Fc γ RIII $^{-/-}$ mice developed substantial disease that was only significantly attenuated compared with WT at the 5 mg dose. Fc γ R $^{-/-}$ mice were strongly protected from disease.

merular crescents was revealed (Figure 1 and Table 1). Despite less evidence of glomerular thrombosis or crescents, Fc γ RIII $^{-/-}$ mice had as many (experiment 1) or more (experiment 2) infiltrating macrophages than WT, indicating a dissociation of inflammatory cell infiltrate and the development of glomerular crescents and thrombosis.

Fc γ RIII $^{-/-}$ Mice Were Not Protected from Early Neutrophil Infiltrate in Accelerated Nephrotoxic Nephritis

Before the development of glomerular thrombosis, there was an influx of neutrophils that peaked in the first 2 hours after nephrotoxic serum injection. The influx of neutrophils was compared between WT, Fc γ RIII $^{-/-}$, and

Figure 2. Representative PAS-stained glomeruli from WT (A), FcγRIII^{-/-} (B), and FcRγ^{-/-} (C) kidneys at day 7 after 10 mg of nephrotoxic serum (NTS) injection to preimmunized mice. A and B show hypercellularity, glomerular crescent formation, and thrombosis; C shows mild hypercellularity only.

FcRγ^{-/-} preimmunized mice 2 hours and 24 hours after 10 mg of nephrotoxic serum. WT and FcγRIII^{-/-} mice both developed neutrophil infiltrates, but FcRγ^{-/-} mice were protected (Figure 3A). WT and FcγRIII^{-/-} mice developed albuminuria in the first 24 hours after disease induction, whereas FcRγ^{-/-} mice were completely protected ($P < 0.05$) (Figure 3B). These results are in contrast to results previously obtained with the heterologous model.¹⁷ To confirm those results using our model, 10 mg of nephrotoxic serum was injected without preimmunization and the glomerular neutrophil influx was quantitated at 2 hours. In this heterologous model, FcγRIII^{-/-} mice were protected ($P < 0.01$) (Figure 3C).

Immune Responses of FcRγ^{-/-} and FcγRIII^{-/-} Mice

The immune response was assessed by quantitative immunofluorescence of deposited glomerular murine immunoglobulins at 2 hours and 24 hours after induction of disease and the end of the experiments (day 7). There was no significant difference between the groups. Data at 24 hours is shown in Figure 4. The circulating mouse anti-sheep IgG antibody response was measured at the end of the experiments. The circulating anti-sheep IgG levels of FcγRIII^{-/-} and FcRγ^{-/-} mice measured by enzyme-linked immunosorbent assay were as strong as

the WT mice (Table 1). In addition, the binding of sheep IgG to the glomerular basement membrane was assessed by quantitative immunofluorescence for each experiment. No significant differences were found (data are shown for the 24-hour time point in Figure 4B).

FcRγ^{-/-} Mice Developed Albuminuria and a Macrophage Influx in a Dose-Dependent Manner

Although FcRγ^{-/-} mice were completely protected from glomerular thrombosis and glomerular crescents in our model, it was noted that after 10 mg of nephrotoxic serum a proportion of the mice developed a macrophage infiltrate and proteinuria at day 7 (Table 1). This phenomenon was studied in more detail by measuring albuminuria at weekly intervals in preimmunized FcRγ^{-/-} mice injected with 10 mg or 5 mg of nephrotoxic serum (Figure 5). FcRγ^{-/-} mice injected with 10 mg of nephrotoxic serum (especially females) had albuminuria at day 7 after nephrotoxic serum that later resolved, whereas those injected with 5 mg of nephrotoxic serum did not develop albuminuria at any time. It is not clear why the females had a greater propensity to develop albuminuria than the males, but it may relate to their smaller size.

Table 1. Accelerated Nephrotoxic Nephritis in WT, FcγRIII^{-/-}, and FcRγ^{-/-} Mice

	WT control Controls	Experiment 1			Experiment 2	
		WT	FcγRIII ^{-/-}	FcRγ ^{-/-}	WT	FcγRIII ^{-/-}
Dose of NTS (mg)	0	10	10	10	5	5
Days after NTS	N/A	7*	7	7	8	8
Sex	m/f	f	f	f	f	f
Number of mice developing disease	0/12	8/8	8/8	0/9	9/9	12/12
% crescents	0 ± 0	31 ± 6	18 ± 3	0 ± 0 [†]	27 ± 4	14 ± 2 [†]
Glomerular thrombosis score	0 ± 0	3.2 ± 0.1	2.7 ± 0.2	0 ± 0 [†]	1.8 ± 0.2	1.1 ± 0.2 [†]
Macrophages/gcs	0.1 ± 0.01	1.2 ± 0.2	1.7 ± 0.2	0.8 ± 0.4 [‡]	1.0 ± 0.1	3.7 ± 0.7 [†]
Serum creatinine (μmol/L)	51 ± 2.0	166 ± 12	118 ± 18	50 ± 3 [†]	84 ± 11	69 ± 6
Albuminuria (mg/24 hour) day 7	0.06 ± 0.001	ND	ND	7.3 ± 4	13 ± 2	14 ± 3
Serum albumin (g/l)	32 ± 1	19 ± 1	13 ± 0.8	30 ± 1 [‡]	16 ± 1	13 ± 1
Serum anti-sheep IgG (relative OD)	0.09	0.88 ± 0.1	0.7 ± 0.1	0.86 ± 0.1	0.7 ± 0.1	1 ± 0.1 [†]

*One WT mouse was sacrificed at day 5.

[†] $P < 0.05$ compared with WT and FcγRIII^{-/-} (experiment 1) or WT (experiment 2).

[‡] $P < 0.05$ compared with FcγRIII^{-/-}, NS compared with WT.

N/A, not applicable; ND, albuminuria was not measured for these mice.

Figure 3. **A** and **B:** Preimmunized mice were injected with 10 mg of nephrotoxic serum (NTS) and sacrificed after 2 or 24 hours. **C:** Naïve mice were injected with 10 mg of NTS and sacrificed after 2 hours. **A:** Glomerular neutrophil infiltration 2 and 24 hours after NTS (50 glomeruli counted per point). gcs, glomerular cross-section. **B:** Albuminuria in the first 24 hours after NTS injection. **C:** Glomerular neutrophil infiltration at 2 hours after NTS to nonimmunized mice. Nonimmunized Fc γ RIII $^{-/-}$ mice were protected from neutrophil infiltration, but preimmunized Fc γ RIII $^{-/-}$ mice were not protected.

Fc γ RI/III Double-Deficient Mice Were Protected from Accelerated Nephrotoxic Nephritis

As stated in the introduction, Fc γ $^{-/-}$ mice lack several receptors that require the Fc γ chain for a signaling subunit. These receptors include both activator Fc γ receptors, as well as several others such as the high-affinity IgE receptor (Fc ϵ RI). To confirm that protection of Fc γ $^{-/-}$ mice in this model was because of the lack of Fc γ RI and III, accelerated nephrotoxic nephritis was induced in male mice lacking both Fc γ RI and III (Fc γ RI/III $^{-/-}$) ($n = 5$) and WT strain-matched controls ($n = 6$) using 10 mg of nephrotoxic serum. Mice were sacrificed at day 8 after nephrotoxic serum injection. Fc γ RI/III $^{-/-}$ mice were substantially protected from glomerular thrombosis (score, 0.5 ± 0.3) compared with WT (score, 2.4 ± 0.2) ($P < 0.005$). Fc γ RI/III $^{-/-}$ mice were also protected from glomerular crescents (percent crescents, $0.4 \pm 0.1\%$) compared with WT (percent crescents, $5 \pm 1.1\%$) ($P < 0.005$; Figure 6, A and B). Representative histology is shown in Figure 7. There was no significant difference in the macrophage infiltrate or proteinuria as assessed by urine dipstick at day 8, but this is consistent with previous findings with Fc γ $^{-/-}$ animals (Figure 6C). Quantitation of deposited glomerular IgG showed no difference in deposited glomerular mouse or sheep IgG between the groups at day 8 after nephrotoxic serum. The enzyme-linked immunosorbent assay for total mouse IgG against sheep IgG showed higher levels in the Fc γ RI/III $^{-/-}$ mice at day 8 than WT animals.

Figure 4. **A:** Representative glomeruli showing immunofluorescence for glomerular sheep and mouse IgG 24 hours after nephrotoxic serum injection. **B:** Quantitative immunofluorescence for glomerular sheep IgG. **C:** Mouse IgG deposition 24 hours after nephrotoxic serum injection. There was no significant difference between the groups.

Discussion

The results presented here confirm that Fc γ $^{-/-}$ mice were protected from accelerated nephrotoxic nephritis, and demonstrate that this was as a result of deficiency of Fc γ RI and Fc γ RIII. Both Fc γ RI and Fc γ RIII appeared to play a role, because both needed to be deficient to protect markedly from disease. Interestingly, in this active model, early neutrophil influx was not entirely dependent on Fc γ RIII, as was the case with the passive model of heterologous glomerulonephritis.¹⁷ These results demonstrate that under different conditions, the relative importance of Fc γ RI and Fc γ RIII can be changed, which is an important consideration if a therapeutic approach of receptor blockade was to be attempted.

To exclude an effect of Fc γ receptors on the priming of the immune response we carefully examined the systemic immune response to sheep IgG and deposition of

Figure 5. Weekly albuminuria measurements in $Fc\gamma RII/III^{-/-}$ preimmunized mice injected with 5 mg or 10 mg of nephrotoxic serum. Mice injected with 10 mg of nephrotoxic serum, especially females, had albuminuria at day 7 that resolved by day 26. **Horizontal line** represents the mean 24-hour albuminuria of a group of normal C57BL/6 mice.

immunoglobulin within glomeruli. There were no differences in either of these parameters in the $Fc\gamma$ receptor-deficient mice, implying that the role of activator $Fc\gamma$ receptors in this model is in the intraglomerular response to deposited IgG.

In a previous study we examined the relative importance of $Fc\gamma R$ chain on intrinsic renal cells and circulating leukocytes by studying mice made chimeric by bone marrow transplantation.¹⁹ Mice lacking the $Fc\gamma R$ chain on bone marrow-derived cells were completely protected from nephrotoxic nephritis whereas absence of $Fc\gamma R$ on intrinsic renal cells had no effect. The results of the present experiments demonstrate that both $Fc\gamma RI$ and $Fc\gamma RIII$ on circulating leukocytes are involved.

There are at least two reasons why $Fc\gamma RI$ may play a role in the accelerated model of glomerulonephritis in addition to $Fc\gamma RIII$. $Fc\gamma RI$, much like the high-affinity IgE receptor of mast cells, circulates loaded with monomeric immunoglobulin.²⁰ Activation occurs on cross-linking of bound antibodies with a target antigen. $Fc\gamma RI$ is more likely to be involved when high titers of specific antibody are present to compete with bound monomeric IgG,

Figure 6. $Fc\gamma RI/III^{-/-}$ mice and WT controls at day 8 after induction of nephritis. **A:** Glomerular thrombosis score ($n = 50$ glomeruli scored per point). **B:** Percent glomerular crescents ($n = 50$ glomeruli). **C:** Macrophages per glomerular cross-section ($n = 50$ glomeruli per point). gcs, glomerular cross-section.

Figure 7. Representative PAS-stained glomeruli from WT control (**A**) and $Fc\gamma RI/III^{-/-}$ (**B**) mice at day 8 after induction of nephritis. The WT mice had thrombosis of glomerular capillaries and occasional crescent formation, whereas the $Fc\gamma RI/III^{-/-}$ mice were protected.

which might occur more often in an active disease model with high levels of circulating specific antibody. Secondly, accelerated nephrotoxic nephritis is dependent on the production of interferon- γ .²¹ Interferon- γ is one of the most potent cytokines involved in the up-regulation of $Fc\gamma RI$ on macrophages.²² Local interferon- γ production may therefore cause an increase in macrophage $Fc\gamma RI$ expression and explain why $Fc\gamma RI$ has a relatively important role in this model.

It is interesting to compare our results with those in other models of inflammation. $Fc\gamma RIII$ alone was shown to be important in several forms of immune complex-mediated inflammation, such as the reverse passive Arthus reaction, autoimmune hemolytic anemia, and heterologous glomerulonephritis.^{7,23,24} However, in other models both $Fc\gamma RI$ and $Fc\gamma RIII$ have been shown to play a role.^{24,25} $Fc\gamma RI$ may play a more important role in models and genetic backgrounds in which T cells are involved and up-regulate expression of $Fc\gamma RI$ on infiltrating leukocytes, whereas $Fc\gamma RIII$ may be relatively more important in passive models.

Macrophages are important effectors of injury in glomerulonephritis. Our results demonstrate that it is possible to uncouple glomerular immunoglobulin deposition and macrophage infiltration from glomerular injury manifested by thrombosis and crescent formation. Indeed, there were more macrophages in the glomeruli of $Fc\gamma RIII^{-/-}$ mice than controls. This implies that the mechanisms of macrophage accumulation in immune complex glomerulonephritis are different from those that prime macrophages for injury and that macrophage activation for injury is critically dependent on $Fc\gamma$ receptors. $Fc\gamma$ receptor-deficient macrophages have markedly impaired antibody-dependent cellular cytotoxicity and phagocytosis.^{6,7,15,16} Interfering with signaling from activator Fc receptors might provide one mechanism to reduce renal injury in established glomerulonephritis and even perhaps allow macrophages to develop a reparative phenotype.

Despite the lack of $Fc\gamma RI$ and $Fc\gamma RIII$, some of the $Fc\gamma R^{-/-}$ mice developed albuminuria, peaking at day 7 after nephrotoxic serum injection, which later resolved. Non- $Fc\gamma R$ -dependent pathways such as complement, direct antibody-mediated injury to podocytes, or T cell-mediated mechanisms may be involved. Angiotensin type 1 receptor blockade ameliorated the macrophage

influx and proteinuria in FcRγ^{-/-} mice in nephrotoxic nephritis.⁴ However, despite mild proteinuria and macrophage influx both FcRγ and FcγRI/III double-deficient mice were almost completely protected from crescents that are the most destructive form of glomerular injury.

In summary, both FcRγ^{-/-} mice and FcγRI/III^{-/-} mice were substantially protected from glomerular thrombosis and crescent formation during murine nephrotoxic nephritis, a model of anti-glomerular basement membrane antibody nephritis. Unlike heterologous glomerulonephritis, in which FcγRIII is responsible for neutrophil infiltration, in this active model of glomerulonephritis both FcγRI and FcγRIII play a role in injury. Blockade of activator Fcγ receptors may therefore be an attractive therapeutic target in established immune complex glomerulonephritis, but our results show that it may be necessary to target both FcγRI and FcγRIII.

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