Resistance of Fc Receptor–deficient Mice to Fatal Glomerulonephritis

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Abstract

Immune complex–mediated inflammation is a common mechanism of various autoimmune diseases. Glomerulonephritis (GN) is one of these diseases, and the main mechanism of the induction of GN has been unclear. We examined the contribution of Fc receptors in the induction of nephrotoxic GN by establishing and analyzing mice deficient in the Fc receptor γ chain (FcRγ). Whereas all wild-type mice died from severe glomerulonephritis with hypernephremia by administration of anti-glomerular basement membrane (GBM) antibodies, all FcRγ-deficient mice survived. Histologically, wild-type mice showed glomerular hypercellularity and thrombotic changes, whereas the renal tissue in FcRγ-deficient mice was almost intact. Deposition of anti-GBM antibody as well as complement components in the GBM were equally observed in both wild-type and knockout mice. These results demonstrate that the triggering of this type of glomerulonephritis is completely dependent on FcRγ cells. (J. Clin. Invest. 1998. 102:1229–1238.) Key words: Fc receptor • knockout mouse • glomerulonephritis • Masugi nephritis • anti-GBM antibody

Introduction

Deposition of autoreactive antibodies to self-organ causes immune complex (IC)–mediated inflammation and autoimmune diseases. Triggering of inflammation has been thought to be mediated mainly by complement and then various tissue-infiltrating cells. However, recent analysis of various Fc receptor (FcR)-deficient (KO) mice has shown that FcR plays crucial roles in IC-triggered inflammations (1–5).

FcRs have important functions in the connection between humoral and cellular responses (6–8). In the mouse system, there are three types of FcγRs: the high-affinity receptor FcγRI, capable of binding to monomeric IgG, and the two low-affinity receptors FcγRII and FcγRIII, which bind only to polymeric IgG. Whereas FcγRI is a single chain receptor, both FcγRI and FcγRIII are composed of multimeric subunits and require a homodimer of the γ subunit (FcγRγ) for their assembly and cell surface expression as well as signal transduction through the FcγR. The FcγR chain is also associated with the high-affinity Fc receptor complex for IgE (FcεRI). FcRs are expressed on hematopoietic cell lineage, but different cell types exhibit different expression patterns of FcRs. All three FcγRs are expressed on myeloid lineage cells including macrophages and neutrophils, whereas natural killer (NK) cells express only FcγRII. By contrast, mast cells express both FcεRI and FcεRI (FcγRII and FcγRIII).

Previous work by Takai et al. (1) demonstrated that FcγR-KO mice showed no expression of FcγRI, FcγRII, and FcγRI, and also that they failed to induce IgG-mediated endocytosis by macrophages and IgE-mediated anaphylaxis by mast cells. More importantly, it was shown that Arthus reaction induced by specific antigen (Ag)-antibody (Ab) IC, as a representative type III inflammation, was severely reduced in both FcγRγ- and FcγRII-KO mice (1, 3). FcR-KO mice also exhibited reduced auto-Ab–dependent experimental hemolytic anemia and thrombocytopenia, representative type II inflammatory responses (2). Since these inflammatory responses have been thought to be mediated primarily by complement, these results on experimental inflammatory type II and III responses suggested the possibility that a wide range of inflammatory diseases and related autoimmune diseases may be initiated by an FcR-dependent, rather than complement-dependent, mechanism. However, it is yet to be uncovered whether the development of long-term organ diseases such as glomerulonephritis (GN) is also mediated by FcR in vivo.

GN is an inflammatory disease of the renal glomeruli characterized by damage to the glomerular basement membrane (GBM) and a wide array of disorders in kidney functions (9). It has been suggested that most cases of GN are induced by immune mechanisms. There are two representative types of immunologically induced experimental GN. One is the deposition of Ag–Ab ICs preformed in circulation or on the wall of glomerular capillaries, and the other is the emergence of auto-Abs against GBM (10) or other renal constituents such as renal tubular Ags. The latter GN can be induced by injecting anti-GBM Ab into rats, rabbits, and mice, and this is known as Masugi nephritis or nephrotoxic serum glomerulonephritis (NTGN) and serves as a model for human Goodpasture ne-
phritis (11–16). On the other hand, there has been accumulating data that the development of GN is independent of Ab and dependent on T cell–mediated responses, particularly the delayed-type hypersensitivity (DTH) mechanism (17–20).

To clarify the function of IC deposition on FcR and FcR-expressing cells in the pathogenesis of GN, we established FcR-deficient mice by means of gene targeting of the FcR gene, and examined the induction and development of NTGN by anti-GBM Ab in these mice. Here we provide definitive evidence that the initial triggering of this type of GN is completely dependent on FcR.

**Methods**

**Cloning of FcRγ gene and construction of targeting vector.** A murine FcRγ genomic gene was isolated from a C57BL/6 genomic library with a mouse FcRγ cDNA probe (21). The 3.65-kb NcoI-MboI genomic DNA fragment encompassing the 5′ upstream of exon 1 and intron 1 was subcloned into pGEM3Z (Stratagene, La Jolla, CA). The NcoI site in intron 1 and the SmaI site in the vector were digested and the fragment was inserted into the HindIII site of pMCinExpA (22). This construct was then digested with SalI and Xhol, and the resulting 4.5-kb fragment was ligated to the Xhol site of pCIC19/MC1-TK (22). The 1-kb short arm sequence spanning exons 3 and 4 was amplified with PCR using FcRγ genomic DNA as a template. Primers used for amplification of intron 3 were 5′-ACCCTGCAGCTCGAGACAGCATCGCTTTG-3′ from exon 4 appended with SalI site and 5′-AAACCATGTTCGTGACAGCATCTTGCTT-3′ from exon 4 appended with ClaI site. The neo cassette was ligated in the opposite transcriptional direction as the FcRγ gene and replaced a 4-kb fragment including exon 2 (containing the starting ATG codon for FcRγ).

**Generation of FcRγ-KO mice.** BL6/III embryonic stem (ES) cells (23) were electroporated with 25 μg of the Clal-linearized target vector (24). Cells were selected in the presence of 150 μg/ml of G418 (GIBCO BRL, Rockville, MD) and 2 mM of gancyclovir and surviving colonies were screened for homologous recombination by PCR with a pair of primers: a sense primer of neo′ gene (5′-GAACCTGGCTGCAATCATTCTGTTCATCAAT-3′) and an antisense primer of the exon 5 sequence of FcRγ gene (5′-AATTCGATGTGGTGTTCATGCTT-3′) from exon 5 appended with ClaI site. The neo′ cassette was ligated in the opposite transcriptional direction as the FcRγ gene and replaced a 4-kb fragment including exon 2 (containing the starting ATG codon for FcRγ).

**Induction of NTGN.** For the induction of NTGN, two sets of 10 female and male C57BL/6 mice (8–12 wk of age; Japan SLC Inc.) were injected intraperitoneally with 250 μg of rabbit IgG (Cappel) in CFA to generate anti–rabbit IgG Ab for induction of facilitating GN (16), followed by intravenous injection with 75 μl of anti-GBM Ab 4 d after the first set of mice served for renal histological examination. Half of the mice were killed 5 d and the other half 8–10 d after the intravenous injection of anti-GBM Ab. The second set was used to observe the natural course of survival for anti-GBM Ab–treated wild-type mice.

**Complement titration.** Since functional titration of mouse complement in the serum has been known to be extremely difficult, the titers of C3 as the representative of complements were measured by sandwich ELISA using two different goat anti–mouse C3 Abs, one of which was coupled with horseseradish peroxidase (Cappel).

**Histological examination and proteinuria measurement.** The serum levels of creatinine, blood urea nitrogen (BUN), and total protein were determined by standard methods for blood chemistry: CrTNTase-POD for creatinine, urease-GLDH for BUN, and Biuret for total protein. Urine was individually collected in a metabolic cage and the protein contents were measured by the Bradford method.
**Immunofluorescent study.** Small blocks of kidney obtained at autopsy were quickly frozen in acetone dry ice and cut into 4-μm sections. The cryostat sections were stained with FITC-coupled IgG of goat anti-mouse IgG (Cappel) and goat anti-mouse complement C3 (Cappel), and FITC-coupled F(ab')2 of goat anti-rabbit IgG (Leinco Technologies, Inc., St. Louis, MO).

**Results**

**Generation of FcRγ-KO mice.** A 15.7-kb genomic gene containing all five exons corresponding to the FcRγ cDNA and the flanking sequences was isolated from a C57BL/6 kidney library (Fig. 1 A) and all exon–intron boundaries were determined (data not shown). The targeting vector was constructed by replacing the fragment containing exon 2 with neomycin-resistance gene (neo') and attaching thymidine kinase gene (TK) from herpes simplex virus (21) at the 5' end, resulting in the targeting vector containing 3.7 kb of 5' long and 1.0 kb of 3' short homologies (Fig. 1 A). The targeting construct was transfected into the BL6/III ES cell line (23). Two independent ES clones with a specific homologous recombination in one allele of the FcRγ gene were obtained and chimeric mice were produced by injecting these two ES clones into BALB/c blastocysts. Germline-transmitted mice were then obtained by mating these chimeras with C57BL/6 mice. These heterozygous mice were intercrossed to generate homozygotes for FcRγ mutation. Using the ES cell line with C57BL/6 origin, FcRγ-deficient mice with a genetic background of pure C57BL/6 were established without any backcross.

The genotypes of wild-type (+/+), heterozygous (+/-), and homozygous (-/-) mice were identified by Southern blotting (Fig. 1 B). The null mutation of FcRγ gene was confirmed by the lack of FcRγ transcript by RT-PCR (Fig. 1 C) and Northern blotting (data not shown). FcRγ-KO mice were

![Figure 1.](image-url)
born normally and were healthy, surviving as long as wild-type mice.

**Lack of FcR expression and phagocytosis function in FcRγ-KO mice.** As described by Takai et al. (1), FcRγ deficiency results in the loss of the surface expression of FcγRI, FcγRII, and FcγRIII, and consistent with in vitro studies (27–29). We confirmed these results for our FcRγ−/− mice by analyzing NK cells first, as these cells lack FcγRII expression and exclusively express FcγRIII which can be stained with anti-FcγRII/III mAb 2.4G2. Since the FcR expression on naive NK cells is relatively low and IL-2-stimulated NK cells express a higher level of FcR on the cell surface, we examined the expression of FcR on these NK cells. As shown in Fig. 2, we confirmed the lack of the FcγR expression on these activated NK cells from FcRγ−/− mice. Similarly, 2.4G2 staining of neutrophils and macrophages, which express FcγRI, FcγRII, and FcγRIII, showed decreases of the FcγR expression on the cell surface of these cells of FcRγ-KO mice although the changes on macrophages were very small (Fig. 2). The remaining staining of these cells, particularly macrophages, was due to FcγRII whose expression is independent of FcRγ.

The lack of functional FcR was confirmed by the failure of phagocytosis by macrophages from these FcRγ-deficient mice. FcR-mediated phagocytosis was assessed by the ability of macrophages to internalize SRBC opsonized with IgG1. While IgG2a-opsonized SRBC were internalized by high-affinity FcγRI, IgG1 and 2b-opsonized SRBC were phagocytosed by low-affinity FcγRII. As shown in Fig. 3, macrophages from wild-type mice internalized the IgG-opsonized SRBC. In contrast, macrophages from FcRγ-deficient mice failed to exhibit any phagocytosis of IgG-opsonized red cells. The observation that these macrophages from FcRγ−/− mice bind opsonized SRBC equally to those from wild-type mice due to the remaining FcγRII (data not shown) indicates that FcγRII/III is responsible for the internalization of opsonized SRBC, and FcRγ-deficient mice lost the ability of phagocytosis due to the lack of cell surface expression of FcγRI/III.

**Fatal nephrotic serum nephritis was induced in wild-type C57BL/6 mice.** Preimmunization of normal rabbit IgG after intravenous injection of anti-GBM Ab induced severe glomerular lesions in wild-type C57BL/6 mice. Female mice showed lesions of greater severity than male mice. Fig. 4A shows a representative renal histology of a female mouse 5 d after the administration of anti-GBM Ab, which demonstrates hypercellular changes of glomeruli and cellular crescent formation. At this stage, the main glomerular alteration was hypercellularity, and mild to moderate cellular crescent formation was observed in ~20% of glomeruli (Fig. 4A). The hypercellularity was probably due to infiltration of monocytes and PMNs, and segmental proliferation of mesangial cells. About 10 d after the intravenous injection of antiserum, the condition of the mice became poor, showing emaciation, ascites, muscle weakness, and little mobility. All of these mice died within 24 h after showing such signs. The typical renal histology at this stage is proliferation of mesangial cells and variable thrombotic lesions of glomeruli. Fig. 4, B and C, shows severe thrombotic glomerular changes characterized by deposition of eosinophilic, amorphous, and fibrillar materials in glomerular tufts and dilatation of tubular lumina, and endothelial cells probably disappeared.

As shown in Fig. 5A, electron microscopy of renal tissue of a female mouse 5 d after the anti-GBM Ab injection revealed that the hypercellular changes were mainly caused by intraglomerular accumulation of monocytes and PMNs. Peripheral interposition of mesangial cells and broad disappearance of epithelial foot processes were also observed. However, no epimembranous deposits, such as those observed in the IC-type GN, could be detected. The renal histological changes had further progressed 10 d after the anti-GBM Ab injection (Fig. 5B). Glomerular endothelial cells had disappeared, and lumina were occluded by electron dense materials. These thrombotic materials were partially positive by fibrin staining method (data not shown). Epimembranous deposits could not be demonstrated at this stage.

Wild-type mice, particularly female mice, started to die as early as 7 d after injection of anti-GBM Ab, and all mice were dead within 12 d (Fig. 6). Since dramatic death from GN occurred in C57BL/6 mice but not in some other strains such as ddY mice in which GN was transient and recovered later (data not shown), the susceptibility to the lethality appears to be linked to their genetic background. It is noteworthy that female mice were more susceptible to this type of GN, demon-
strated by the fact that with a half dose of anti-GBM Ab, only a few male mice died within 14 d, whereas all females died (data not shown).

These results showed that fatal GN was induced in wild-type C57BL/6 by preimmunization with rabbit Ig and subsequent intravenous administration of rabbit anti-GBM Ab.

*Fcγ-deficient mice did not develop NTGN.* To examine the contribution of FcR to the initiation of GN, we compared the

![Image](57x535 to 453x685)

**Figure 3.** Inability of FcγRIII-mediated phagocytosis by macrophages from FcRγ-deficient mice. Surface-biotinylated SRBC were opsonized with antibiotin IgG and added onto the layer of macrophages which were isolated as the adherent cells from the peritoneal exudates of wild-type (+/+) and FcRγ-KO (−/−) mice. After 90 min of incubation at 37°C, non-phagocytosed SRBC were lysed hypotonically with water and micrographs were taken of the remaining cells (×400).

![Image](57x101 to 513x432)

**Figure 4.** Renal histology of representative wild-type C57BL/6 mice 5 d (A) and 10 d (B and C) and FcRγ-deficient mice 14 d (D) after injection of anti-GBM Ab. Mice were immunized intraperitoneally with 250 μg rabbit IgG 4 d before the injection of anti-GBM Ab and killed to examine glomerular changes at early stage (A) and at late stage just before mice died (B–D). Renal sections were subjected to PAS staining. B and C showed the same histology with different magnifications. A, C, and D, ×400; B, ×100.
susceptibility of FcRγ−/− mice to NTGN with that of wild-type mice. The condition of all wild-type mice became poor, showing emaciation, ascites, muscle weakness, and moribundity from 7 to 12 d after the anti-GBM Ab administration as described above. By contrast, all FcRγ−/− mice survived and were quite healthy (Fig. 6).

The kidney function of these mice was examined by hematological analysis of sera. As shown in Table I, whereas wild-type mice showed marked increases in the serum levels of BUN and creatinine, indicating development of renal failure, in FcRγ−/− mice these levels stayed within normal range. Renal histological examination of wild-type mice revealed that all mice developed severe GN with tubular changes (HS = 4.00±1.09), comprising glomerular hypercellularity and variable thrombotic obliteration of glomerular capillary lumina. The histological changes of female mice (HS = 4.70±0.64) were worse than those of male mice (HS = 3.50±1.12). In contrast, as shown in Fig. 4 D, the renal morphology of FcRγ−/− mice was almost normal (HS = 0.45±0.59), and no indication of inflammatory response or tissue damage except weak glomerular hypercellularity was observed. When again analyzed 2–3 wk later, the renal morphology had not changed (data not shown). These results demonstrated that NTGN was not triggered and did not develop in the absence of FcR.

To eliminate the possibility that FcRγ-deficient mice have defective immune responses against rabbit Ig, we examined DTH and the Ab response against rabbit Ig as cellular and humoral immunity, respectively. There were no significant differences between wild-type and FcRγ-deficient mice for both DTH response as measured by footpad swelling and the serum titer of anti–rabbit Ig Ab after immunization with rabbit Ig (data not shown).

Since proteinuria is induced even without renal tissue damage, it was also checked periodically after anti-GBM Ab administration (Table II). While wild-type mice soon developed severe proteinuria, FcRγ−/− mice showed an initial delay, in-
creasing later to finally reach similar levels as the wild-type mice. Wild-type mice exhibited anuria and eventually died, whereas the KO mice recovered from proteinuria. These results demonstrated that acute proteinuria is induced in both wild-type and FcRγ-KO mice but that the development of GN with tissue damage is induced only in wild-type mice.

Deposition of anti-GBM Ab and complement to GBM is not sufficient for initiation of GN. By our protocol with pre-immunization of rabbit Ig, mice may already have raised anti-rabbit Ig Ab when anti-GBM Ab was administered, and may form ICs with anti-GBM Ab in blood, which might cause the failure to react directly on GBM. To exclude this possibility, we analyzed the presence of anti-GBM Ab in the renal area 12 h after the injection of anti-GBM Ab (Fig. 7, A and E). GBMs in both wild-type and FcRγ−/− mice were indistinguishably stained with FITC anti–rabbit Ig Ab, demonstrating that the failure of GN development in FcRγ−/− mice was not due to prevention of specific binding of anti-GBM Ab to GBM by IC formation at the initial stage. In addition, we found that GBM was already stained weakly with FITC anti–mouse Ig Ab which detects mouse anti–rabbit Ig Ab (data not shown), indicating that autologous Ab started to react with rabbit anti-GBM Ab as early as at 12 h. GBM was stained in linear, not granular fashion, demonstrating that anti-GBM Ab reacted along GBM but was not deposited as IC. It is noteworthy that a couple of degranulated PMNs per one slice of glomerulus were observed in wild-type mice but not at all in FcRγ−/− mice in the renal tissues of this stage (data not shown).

To further investigate whether the failure of GN development in FcRγ−/− mice was due to insufficient binding of anti-GBM Ab on GBM at the later stage when wild-type mice developed GN, we performed immunofluorescence staining with FITC anti–rabbit Ig Ab for anti-GBM Ab (Fig. 7, B and F) and with FITC anti–mouse Ig Ab (Fig. 7, C and G). GBMs in both wild-type and FcRγ−/− mice were specifically stained with both Abs in a linear fashion, demonstrating that anti-GBM Ab and mouse anti–rabbit Ig Ab bound to GBM in wild-type and FcRγ−/− mice. This indicated that the failure of GN development in FcRγ−/− mice was not due to insufficient binding of anti-GBM Ab or secondary anti–mouse Ig Ab.

We then investigated whether there was any difference in complement binding in the renal lesion of normal and FcRγ−/− mice after injection of anti-GBM Ab in order to determine the contribution of complements in the induction of this disease. For this purpose, we stained renal sections with anti-C3 Ab. For this purpose, we stained renal sections with anti-C3 Ab. Specific binding of C3 was observed in GBM in a linear fashion in both wild-type and FcRγ−/− mice (Fig. 7, D and H). In addition to the deposition of C3 on the renal tissue, we measured the serum level of C3 by ELISA as the representative of the serum complement titer since functional titration of mouse complement by cytolysis is extremely difficult. The relative amounts of C3 in wild-type and FcRγ-KO mice (n = 5) were

Table I. Blood Chemistry of Mice Injected with Anti-GBM Ab

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Anti-GBM Ab injection</th>
<th>Creatinine</th>
<th>BUN</th>
<th>Total protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>mg/dl</td>
<td>mg/dl</td>
<td>g/dl</td>
</tr>
<tr>
<td>FcRγ+/+</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Female</td>
<td>10</td>
<td>0.529±0.26</td>
<td>259.0±127</td>
<td>3.15±0.76</td>
</tr>
<tr>
<td>Male</td>
<td>10</td>
<td>0.359±0.20</td>
<td>63.6±16.7</td>
<td>3.16±0.63</td>
</tr>
<tr>
<td>Female</td>
<td>10</td>
<td>0.143±0.03</td>
<td>23.4±4.30</td>
<td>4.39±0.26</td>
</tr>
<tr>
<td>Male</td>
<td>10</td>
<td>0.138±0.04</td>
<td>24.8±3.13</td>
<td>4.57±0.22</td>
</tr>
<tr>
<td>FcRγ−/−</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
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<tr>
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<td>4.85±0.33</td>
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<tr>
<td>Female and male</td>
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<td>0.138±0.05</td>
<td>21.0±4.70</td>
<td>5.13±0.72</td>
</tr>
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</table>

Serum was collected from female FcRγ+/+ mice just before death, and 8 d after anti-GBM injection from other mice. Three sets of identical experiments were performed. Each value was measured as described in Methods and expressed as mean±SD. n indicates the number of mice tested.

Table II. Proteinuria Induced by Anti-GBM Ab Administration

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Days after anti-GBM Ab treatment</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>FcRγ+/+</td>
<td>3.7±0.7</td>
</tr>
<tr>
<td>FcRγ−/−</td>
<td>3.1±0.8</td>
</tr>
</tbody>
</table>

Urine was collected from individual mice (n = 5, male) at the indicated times after anti-GBM Ab administration (50 μl in this experiment). The numbers indicate mean±SD of protein level (mg/dl).
47.2 ± 10.6 and 34.0 ± 15.6, respectively, indicating that the complement titer was not significantly different between wild-type and FcRγ-KO mice. The weak staining of C3 in wild-type mice was probably due to the severe damage of glomeruli. These results clearly demonstrated that the deposition of IC and complements per se did not trigger any significant inflammatory response in evoking GN.

Discussion

NTGN induced by anti-GBM Ab represents an experimental model for human Goodpasture’s syndrome (14). Patients suffering from this type of GN show rapidly progressive renal lesions and severe hemorrhagic pulmonary involvement. Although NTGN has been analyzed extensively and the target Ag was found to be the NC1 domain of type IV collagen (30), the molecular basis of this disease has been largely undetermined. The recent progress in gene targeting has provided new approaches for dissecting the complicated mechanisms of the induction of diseases. In this study, we established FcRγ-deficient mice in order to analyze the function of FcR in NTGN. Since FcRγ−/− mice lack the cell surface expression of FcγRI, FcγRIII, and FcεRI on various cells, we used these mice as FcR-deficient mice to investigate the function of FcR in the induction of nephritis. To this end, we provide definitive evidence that the induction of NTGN by anti-GBM Ab is completely dependent on FcR but is independent of complements.

In the classical model of anti-GBM nephritis (11–13, 15, 31), the intravenous injection of heterologous anti-GBM Ab induces glomerular inflammation, which is initiated by binding of anti-GBM Ab to the GBM and followed by activation of complements with accumulation of PMNs and monocytes (32). Deposition of fibrin, necrosis of glomerular capillaries, and crescent formation may be seen in severe cases. The thrombotic change may become accelerated, resulting in diffuse obliteration of glomerular tufts, which has been categorized as a specific type of NTGN (11, 15). This early reaction of the so-called heterologous phase is accompanied by proteinuria. After 5–7 d, the second phase develops as a consequence of the binding of autologous Ab to the heterologous Igs already deposited along the GBM. When rabbit Ig is immunized before
anti-GBM Ab injection, the autologous phase develops much faster and stronger (16). In our experimental model, severe NTGN could be thus induced in C57BL/6 mice. Although a mouse model with anti-GBM disease has been established recently even by immunizing with a single collagen Ag (20), this study is the first case of fatal NTGN in the mouse. It is of particular interest that the lethality from this nephritis is influenced by the genetic background of the mice (20), because other mice such as ddY recovered easily from this acute disease. It was surprising to find that all FcRγ−/− mice remained healthy and survived even after the application of an accelerated type of lethal nephritis. In wild-type C57BL/6 mice, male mice were more resistant to lethal nephritis and females died earlier. Histological analysis as well as blood chemistry confirmed the correlation between the lethality and severity of nephritis; all female mice eventually developed severe thrombotic changes, obliterating glomerular tufts. Therefore, we concluded that the wild-type mice died from GN induced by anti-GBM Ab.

Binding of anti-GBM Ab to GBM was observed equally in wild-type mice and FcRγ−/− mice from the initial phase (several hours) to the subsequent nephritic stage (several days). Autologous Ab (mouse anti-rabbit IgGs) bound to GBM was equally confirmed in both groups during the course of the experiments. Furthermore, we showed that not only anti-GBM Ab but also complements (C3) bound to GBM in a linear fashion equally in wild-type and FcRγ−/− mice. The fact that FcRγ−/− mice survived and did not develop any significant signs of nephritis, in spite of the equivalent binding of heterologous and autologous Abs as well as complements to wild-type mice, clearly indicates that the glomerular deposition of these Abs and complements was not sufficient to trigger GN. In contrast, proteinuria was induced in both wild-type and KO mice, suggesting that the deposition of IC induced transient proteinuria independently of further induction of GN as suggested previously (33). Collectively, these results indicate that FcRγ+ cells are required to initiate inflammatory processes and tissue damage. The complement-independent and PMN-dependent induction of nephritis has been demonstrated by using technically limited systems in which complements were depleted by cobra venom factor treatment (34) and PMNs were depleted by irradiation (28, 34), or by the use of beige mice (35). Our results are consistent with these earlier reports and provide more unequivocal evidence for the use of FcR-deficient mice. Recent studies suggest that anti-GBM GN is induced in T cell–dependent and Ab-independent fashion (17–20). Our present data demonstrate that IC and FcR are required for GN induction, and hence T cell–mediated response may not necessarily be required for the induction, but rather may play a role in the later phase.

By using similar FcR-deficient mice, Sylvestre et al. (4) demonstrated recently that Arthus reaction induced by specific Ag–Ab complex was complement-independent but FcR-dependent. The same authors also showed, by transferring mast cells from FcR−− mice into mast cell–deficient w/w mutant mice, that mast cells are primarily responsible for inducing Arthus reaction (34). Provided that the FcR-dependent mechanism for the induction of nephritis would be similar to that for the induction of Arthus reaction, FcRγ+ cells inducing anti-GBM nephritis might also be mast cells. However, there have been several reports with indirect evidence to demonstrate that PMNs were crucial for inducing anti-GBM nephritis (28, 32, 35). Together with our finding that PMNs appeared in glomeruli at the early stage of our model, the FcRγ+ cells used to trigger GN may be PMNs. Currently, this question is being addressed by experiments using our system, in which various FcRγ+ cells will be transferred into anti-GBM Ab–injected FcRγ−− mice.

There are several different types of GN including NTGN and Heymann nephritis, as well as IC-induced nephritis including serum sickness and SLE. The former has now been found to be completely FcR-dependent and complement-independent in this study. A recent study by Clynes et al. demonstrated that spontaneously occurring GN in NZB/NZW mice was also diminished in FcR-deficient mice (36). It still needs to be determined whether FcR-dependent induction can also be extended to other types of GN.

Both Arthus reaction and NTGN were induced in an FcR-dependent fashion, but the FcRγ+ cells inducing the diseases might be different. It is known that PMNs induce reactive oxygen metabolites (ROMs) upon stimulation through Fcy receptors (36, 37). In addition, ROMs have been identified as mediators in the pathogenesis of GN and have been shown to play a role in the cellular injury (36–39). Therefore, it may be speculated that soluble Ag–Ab complex can trigger mast cells to secrete various mediators for the induction of Arthus reaction, whereas auto-Ab–like anti-GBM Ab activates PMNs to produce ROMs to initiate inflammatory diseases.

It becomes more likely that this mechanism observed in NTGN, the FcR-dependent induction of diseases, can be widely applied to many other auto-Ab–dependent inflammatory and autoimmune diseases.

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