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FcR-Bearing Myeloid Cells Are Responsible for Triggering Murine Lupus Nephritis

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Lupus glomerulonephritis is initiated by deposition of IgG-containing immune complexes in renal glomeruli. FcR engagement by immune complexes (IC) is crucial to disease development as uncoupling this pathway in FcRγ−/− abrogates inflammatory responses in (NZB × NZW)F1 mice. To define the roles of FcR-bearing hematopoietic cells and of kidney resident mesangial cells in pathogenesis, (NZB × NZW)F1 bone marrow chimeras were generated. Nephritis developed in (NZB × NZW)F1 mice expressing activating FcRs in hemopoietic cells. Conversely, recipients of FcRγ−/− bone marrow were protected from disease development despite persistent expression of FcRγ in mesangial cell populations. Thus, activating FcRs on circulating hemopoietic cells, rather than on mesangial cells, are required for IC-mediated pathogenesis in (NZB × NZW)F1. Transgenic FcRγ−/− mice expressing FcRγ limited to the CD11b+ monocyte/macrophage compartment developed glomerulonephritis in the anti-glomerular basement disease model, whereas nontransgenic FcRγ−/− mice were completely protected. Thus, direct activation of circulating FcR-bearing myeloid cells, including monocytes/macrophages, by glomerular IC deposits is sufficient to initiate inflammatory responses. The Journal of Immunology, 2006, 177: 7287–7295.

Immunecomplex (IC) deposition in tissue contributes to many autoimmune disease states including systemic vasculitis, arthritis, blistering skin diseases, and glomerulonephritis. Studies of acute murine models of Ab-mediated inflammation in the skin (1–4), joints (5–12), lungs (13), kidneys (14–19), and peritoneum (20, 21) in gene-deficient mice permit the general conclusion that the coordinate expression of activating and inhibitory FcRs on effector cells regulates inflammatory responses. Complement components including C5a contribute directly as chemoattractants and as inducers of preferential up-regulation of activating FcRs on effector cells (20, 22–24).

The initial events following IC deposition in the tissues include the local activation of complement and the triggering of tissue-resident cells through their Fc and complement receptors. The resultant collective action of locally produced chemokines, cytokines, and small molecule mediators of inflammation activates endothelial cells and promotes the adhesion and diapedesis of activated bloodborne effectors, including monocytes and neutrophils, into the tissue. In this scenario, the recruitment of circulating cellular effectors is expected to occur as a consequence of local activation of resident tissue cells. The importance of resident cells including tissue macrophages and mast cells in the initiation of the inflammatory cascade and subsequent recruitment of circulating neutrophils has been demonstrated in the joints (25–27) and in Arthus reactions in the lungs (13, 22, 28), peritoneum (21, 29, 30), and skin (3).

In the kidney, the relevant resident cell that would be expected to initiate the inflammatory response to ICs deposited in glomeruli is the mesangial cell (MC). MC activation contributes directly to glomerular pathogenesis through proliferation and collagen deposition and indirectly through the production of the inflammatory mediators (31, 32) cytokines and chemokines (31, 33). Indeed, FcRs are expressed on cultured rodent and human MC (34–36), and FcγR cross-linking on cultured MC induces matrix deposition (34) and the production of inflammatory mediators including chemokines (37, 38) and cytokines (39). Numerous studies have implicated FcγR cross-linking on MC as a proximal and key step in IC-mediated nephritis, yet few studies have directly demonstrated mesangial expression of FcγR in vivo. Low-level expression of the inhibitory FcγRIIB on glomerular cells was detectable by immunohistochemistry (17), but other studies have failed to detect FcγR at the RNA level (40). FcγR−/− mice are protected from the development of nephritis despite IC mesangial deposition (18, 19). However, a requisite inflammatory role of FcRs on MC in vivo remains unclear.

Recent work in the anti-glomerular basement membrane (anti-GBM) model suggests instead that circulating hemopoietic cells directly engage immune deposits in the mesangium, initiating the inflammatory response without prior recruitment by FcR-engaged MC. Transferred wild-type (WT) neutrophils become activated in FcRγ−/− hosts bearing IC mesangial deposits, arguing that acute injury can be initiated by FcR cross-linking-circulating neutrophils (41). In bone marrow (BM) chimeras (42) using FcRγ−/− and FcRγ−/− donors and recipients, anti-GBM nephritis required FcR-bearing cells in the hemopoietic compartment, suggesting that MC FcR engagement is not necessary for the induction of the IC-mediated inflammatory responses (42). Although these short-term acute models provide important mechanistic insights, the spontaneous nephritis model in (NZB × NZW)F1 mice most closely approximates pathogenetic mechanisms mediating human lupus nephritis. We have addressed the role of FcRγ in intrinsic renal...
cells or hemopoietic cells in the spontaneous NZB/NZW lupus nephritis model and find that mesangial FcR expression is not required for disease development. Furthermore, we have partially constituted anti-GBM nephritis in FcRγ⁻/⁻ by transgenic expression of FcRγ in the monocyte/macrophage compartment, implicating direct activation of this FcR-bearing cellular subset in the initiation of the inflammatory phase of IC-mediated nephritis. Thus, direct activation of FcR-bearing monocyte/macrophages is sufficient to induce inflammatory responses in response to glomerular IC deposition.

Materials and Methods

Mice

(NZB × NZW)F1, FcRγ⁻/⁻ mice were generated from an intercross of NZB FcRγ⁺/⁺ male and NZW FcRγ⁺/⁺ female mice (18). To generate BM chimeras, 10 × 10⁶ BM cells obtained from 3- to 4-week-old (NZB × NZW)F1, FcRγ⁻/⁻ and FcRγ⁺/⁺ mice (The Jackson Laboratory) were injected i.v. into the tail vein of lethally irradiated recipients (1000 rad × 1 dose). Chimeric mice were given oral ciprofloxacin in the water ad libitum for 14 days after reconstitution and followed for the development of proteinuria weekly for 9 mo. Proteinuria was read using Urostix for the NZB/NZW mice and scored positive if 2⁺ measurements (>250 mg/dl) were recorded for two successive readings. A subset of mice was sacrificed at 6 mo for histopathological analysis of the kidney. These studies were reviewed and approved by the Institution Animal Care and Use Committee of Columbia University.

CD11b⁻/γ⁺ Tg⁺ mice were generated after injection of oocytes obtained from FcRγ⁻/⁻ mice. The transgenic construct was generated by insertion of the murine FcRγ cDNA (550-bp fragment) as an EcoRI fragment (43) into pBluescript (203) (a gift from D. G. Tenen, Harvard Medical School, Boston, MA; see Ref 44) containing the 1.7-kb 5'-flanking sequences of the mouse CD11b promoter and the 3'-flanking region from the human growth hormone gene. A NotI/HindIII fragment (containing 5'-CD1b promoter-FcRγ cDNA-HGH-3') was injected into the oocytes and three founder lines harboring the transgene were further analyzed for expression. Of these three founders, only one (line 14) expressed the FcRγ chain in peritoneal macrophages.

Accelerated anti-GBM nephritis

Mice were immunized with 100 μg of sheep IgG in CFA 3 days before i.v. injection of 150 μl of specific sheep anti-mouse GBM serum. Urine was obtained daily and blood obtained on the day before injection with anti-GBM sera and then at the time of sacrifice 7 days later. Urine samples were diluted in PBS and protein quantified by the Bradford method (Bio-Rad) using an ELISA plate reader at OD₅₇₀.

Anti-dsDNA and soluble immune complex ELISAs

Diluted serum (1/100) from 6- to 7-mo-old NZB/NZW-γ⁻/⁻ and NZB/ NZW-γ⁺/⁺ mice were added to ELISA plates coated with C1q (Sigma-Aldrich) for detection of ICs (45, 46) and dsDNA-coated plates (United Biotech) for detection of Abs to chromatin. After washing away unbound serum, rat anti-mouse IgG (BD Pharmingen) was added. Alkaline phosphatase-conjugated AKP polyvalent anti-rat IgG (BD Pharmingen) was used as secondary Ab. After incubation with p-nitrophenyl phosphate substrate, the samples were read spectrophotometrically at 405 nm with an ELISA reader (Molecular Devices).

Immunofluorescence and immunohistochemistry

For histological analysis, formalin-fixed sections were stained with H&E or periodic acid-Schiff (PAS). To detect IC deposition, paraffin-embedded sections were stained with 1/1000 diluted FITC goat anti-mouse C3 and IgG (Valeant Pharmaceuticals). To detect FcγRI, a polyclonal anti-FcγRI rabbit IgG (gift from Dr. J. Ravetch, The Rockefeller University) or rat anti-Mac-1 (clone 7D4/16; BD Pharmingen) followed by rabbit anti-rat IgG Alexa594 (Molecular Probes). Biotinylated goat anti-rabbit IgG, followed by either streptavidin-FITC or streptavidin-HRP was used for detection. A Nikon Eclipse 600 microscope equipped with a RT Spot digital camera was used for imaging.

Renal pathological assessment

PAS sections were prepared from WT, FcRγ⁻/⁻, and FcRγ⁺/⁺ CD11b-γ Tg⁺ kidneys on day 7 after induction of accelerated glomerulonephritis (five per group). Slides were examined in a blinded fashion by one of us (V. D’A.). Severity of the following seven categories of histological activity were semiquantitatively graded as follows: glomerular fibrinoid necrosis 0–4, endocapillary hypercellularity 0–4, glomerular leukocyte infiltration 0–4, crescents 0–4, tubular degeneration 0–4, casts 0–4, and interstitial inflammation 0–4. The cumulative pathological score is the sum of all seven categories and has a possible range of 0–28.

MC and NK culture

Glomeruli were isolated with successive sieving (47). Kidneys were minced with scissors and tissue fragments were passed through a no. 60 mesh size (Fisher Scientific) and then sequentially passed through no. 100 and no. 200 sieves. Glomeruli were digested with 0.1% collagenase type IV (Sigma-Aldrich) and 0.1% trypsin (Invitrogen Life Technologies) for 30 min at 37°C before plating in 24 wells in DMEM/10% FCS. Cells were passaged in n-valine-substituted medium to eliminate fibroblasts. After 2 wk in culture, cells exhibited a stellate morphology and were replated. Immunostains were smooth muscle actin-positive, weakly 2G2 (BD Pharmingen) and Mac-1+ , confirming their MC origin. RNA was prepared from MC using TRIzol and cDNA was generated using the cloned avian myeloblastosis virus first-strand synthesis kit according to the manufacturer’s protocol (Invitrogen Life Technologies). Primer sequences for RT-PCR amplification (30 cycles) of FcγRI were as follows: 5'-CCAGGATGACGGCG-3' and 5'-ACAGTAGAGTGGTAGTGAAG-3'. These primers amplify a 137-bp band corresponding to exons 1 and 2 of the γ-subunit. The band is not amplified in genomic DNA due to interfering intrinsic sequences. The housekeeping gene, HPRT, was amplified from cDNA using the following primer sequences: 5'-AGCTACTGTGAATGTACGCTA-3' and 5'-AGAGGTCTTTTCTACCCAGCA-3'. For assessing MC chimerism, genomic DNA was subjected to PCR analysis using the following primer sequences: neo, CTCTGCTTCTAGCCTATCOC; y-1, ACCTACTTCTACTGCAAGACTGCA; y-2, and TACGCGCTGGC TATAGCTGCTT. Annealing temperature was 62°C. Knockout and WT-amplified products were 260 and 224 bp, respectively.

Hemopoietic chimerism was assessed in cultured NK cells obtained after isolation of the adherent cell population from a 14-day culture of nylon wool nonadherent splenocytes grown in IL-2 (10,000 U/ml). Flow cytometric analysis used anti-NK1.1 PE and 2G2-FITC (BD Pharmingen). Murine NK cells do not express FcRIII (48) and thus the anti-FcRII/III mAb (2A2G) recognizes only FcRII on these cells.

Western blot analysis of FcγRI expression

Protein extracts were obtained from B cells, T cells, NK cells, and neutrophils were immunoblotted with polyclonal rabbit anti-mouse FcγRI chain IgG and anti-f-actin Abs. Neutrophils were obtained from thioglycolate-elicted peritoneal exudates (4 h after i.p. injection of thioglycolate) after GR-1⁺ bead selection (Miltenyi Biotec). Adherent peritoneal macrophages were obtained from thioglycolate-elicted exudates (72 h after i.p. injection of thioglycolate) and then sequentially passed from CD43⁺ and CD14⁺ splenocyte populations, respectively. All cell populations were lysed in TBS buffer that contained 1% Triton X-100, 2 mM EDTA, and complete mini-protease inhibitors (Roche).

Phagocytosis assays

Rabbit IgG-opsonized SBRs were prepared with subagglutinating quantities of rabbit anti-sheep RBC IgG (MP Biomedicals). After washing away free Ab, IgG-opsonized RBCs were added to adherent macrophages for 1 h at 37°C. Unopagocytosed RBCs were removed by osmotic lysis, and phagocytosis plates were fixed with PBS/0.25% glutaraldehyde before microscopic examination.

Blood albumin and urea nitrogen measurements

Blood samples were read by the Clinical Chemistry Laboratory at the Irving Clinical Research Center at Columbia-Presbyterian Hospital.

Results

BM chimeric NZB/NZW mice reveal a requirement for FcγR-expressing hemopoietic cells for nephritis development

(NZB × NZW)F₁ female mice develop a uniformly fatal rapidly progressive IC nephritis heralded by the serological appearance of anti-chromatin IgG autoantibodies at 4–6 mo of age. Disease progression is swift, with a median survival of 180 days. However, in (NZB × NZW)F₁, FcγR⁻/⁻ female mice, IgG autoantibodies occur with equivalent titers and are deposited similarly in the kidney.

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Anti-chromatin IgG, IgMs, and circulating IC were similar in all groups (ANOVA, BM transfer. Intrinsic renal cells remained predominantly recipient-derived 4 mo after transplant. As previously seen in nontransplanted NZB/NZW, FcRγ deficiency has little impact on the development of autoantibodies and their glomerular deposition when assessed 6 mo after transplant. All experimental groups developed anti-chromatin autoantibodies and anti-C1q-binding activity (indicative of the presence of circulating ICs and/or anti-C1q autoantibodies (45, 46)) regardless of the FcRγ genotype status of the host or recipient (Fig. 2, A and B). Circulating ICs were deposited in the glomeruli in a similar fashion as assessed by immunofluorescence studies demonstrating equivalent IgG and complement glomerular deposition in all experimental groups (Fig. 2C).

Although the afferent limb of autoimmunity was intact regardless of the FcRγ genotype, the effector response required activating FcR-expressing hemopoietic cells. Proteinuria occurred in 90–100% of the NZB/NZW recipients of WT BM regardless of the host genotype, whereas proteinuria occurred in only 10–20% of recipients of NZB/NZW FcRγ−/− marrow (Fig. 3A). Thus, WT hemopoietic cells can transfer disease susceptibility to FcRγ−/− hosts and conversely FcRγ−/− BM-derived cells limit disease development in WT NZB/NZW hosts, indicating that hemopoietic expression of activating FcRs is both necessary and sufficient for the development of nephritis.
Histological analysis revealed fulminant glomerulonephritis in recipients of WT BM with glomerular hypertension, mesangial and endocapillary hypercellularity, neutrophilic and monocytic infiltration, necrosis, crescent formation, and sclerosis (Fig. 3B). In contrast, disease protection was seen in γ−/− BM recipients with histological changes limited to mesangial expansion and mild endocapillary and mesangial hypercellularity.

These data suggest that FcR expression by resident cells of the kidney does not critically contribute to the initiation of the effector response in IC-triggered nephritis. The immunohistochemical analysis of FcRγ expression in Fig. 1 was performed on mice 4 mo posttransplant and before disease onset. To confirm that MC remained genotypically host derived at 6–9 mo, the time point when proteinuria became evident, MC were isolated and additional immunostains were performed (Fig. 3C). In nephritic FcRγ+/−→FcRγ−/− chimeric mice at 6 mo posttransplant, many dual-positive FcRγ+ Mac-1+ cells were present, indicative of infiltrating myeloid lineage cells. In healthy FcRγ−/−→FcRγ+/+ chimeric mice sacrificed 9 mo posttransplant, immunostaining demonstrated persistent glomerular γ expression, presumably in Mac-1+ MC. In an additional experimental approach to establish the donor vs host FcRγ status of MC, MC populations were enriched from disrupted glomeruli of chimeric mice at 6 mo posttransplant in nephritic FcRγ−/−→FcRγ−/− mice and at 9 mo from non-nephritic FcRγ−/−→FcRγ−/− mice and assessed for the presence of WT and knockout FcRγ alleles by genomic PCR. Enriched MC populations exhibited typical stellate morphology and were smooth-muscle actin positive (data not shown). PCR analysis of genomic DNA of these MC populations (Fig. 3, C, insets, and D) at 6 mo indicated that genotypically these populations continued to include predominantly host-derived MC. Notably, however, in glomerular cultures obtained from FcRγ−/−→FcRγ−/+ mice at 9 mo posttransplant, there was also PCR evidence for donor-derived contributions consistent with replacement of some MC with BM-derived precursors, although this was not evident by immunohistochemistry. One possible explanation for the discrepancy between the immunohistochemical data and the PCR data are that the
enriched MC populations might have included other cells, including contaminating leukocytes that were detectable by these sensitive PCR assays. Taken together, these data indicate that FcR expression of MC is neither necessary nor sufficient to initiate an inflammatory nephritogenic response to IC. Rather, inflammation occurs in NZB/NZW as a direct consequence of FcR engagement on circulatory cells.

**Lineage-specific transgenic reconstitution of FcRγ in monocytes/macrophages partially restores the ability to develop nephritis in FcRγ−/− mice**

To genetically determine the role of myeloid cells by transgenic manipulation of FcRγ−/− mice, we turned to the anti-GBM nephritis model in the C57BL/6 FcRγ−/− background. In previous studies in the autologous anti-GBM disease model, activating FcRs expressed on hematopoietic cells were found to be required for disease development (42). To assess the specific contributions of FcR-bearing myeloid cells, transgenic mice expressing FcRγ driven by the CD11b promoter (Fig. 4A) were generated in FcRγ−/− C57BL/6 mice (CD11b−γ Tg−). Three Tg+ founder mice were analyzed for functional expression of FcRγ in peritoneal macrophages. One of these transgenic founder lines (line 14) exhibited FcRγ expression in peritoneal macrophages, but not in B cells, T cells, NK cells, or neutrophils (Fig. 4B). Functional expression in peritoneal macrophages was shown by restored FcR-mediated phagocytosis in CD11b−γ Tg+ (Fig. 4C). Lack of expression of FcRγ in MC of CD11b−γ Tg+ was demonstrated by RT-PCR analysis of RNA obtained from cultured MC. CD11b−γ Tg+ MC did not express detectable FcRγ at the RNA level neither in the resting state nor after stimulation with IC or IFN-γ for either 6 h (data not shown) or 24 h (Fig. 4D).

The transgenic CD11b−γ Tg+ mice provided a unique opportunity to address the singular role of FcR-bearing monocytes/macrophages to the development of nephritis. Mice were immunized with sheep IgG in CFA 3 days before i.v. administration of specific sheep anti-GBM sera (Fig. 5). Severe proteinuria, hyperalbuminemia, and uremia developed in all WT C57BL/6 mice by day 7 whereas FcRγ−/− mice, as expected, were completely protected from disease development (Fig. 5). In contrast, CD11b−γ Tg+ mice developed moderate proteinuria and consequent hyperalbuminemia. Histopathological assessment of H&E-stained renal sections was consistent with the induction of mild glomerulonephritis in CD11b−γ Tg+, with increased glomerular cellularity noted (Fig. 6, A and B). This likely reflects myeloid cell expression of FcRγ rather than IC-induced activation of the CD11b−γ promoter in MC as cultured CD11b−γ Tg+. MC did not demonstrate IFN-γ- or IC-induced FcRγ expression (Fig. 4D). Severity of histological activity was semiquantitatively graded using seven criteria (glomerular fibrinoid necrosis, 0–4; endocapillary hypercellularity, 0–4; glomerular leukocyte infiltration, 0–4; crescents, 0–4; tubular degeneration, 0–4; casts, 0–4; and interstitial inflammation, 0–4). Average scores for the groups for each of the seven categories were, respectively: WT (2.8, 3.6, 3.6, 0.6, 4.0, 4.0, 1.0); CD11b−γ Tg+ (0, 2.1, 1.5, 0, 2.1, 1.9, 0); and FcRγ−/− (0, 0, 0, 0, 0, 0, 0). Cumulative pathological scores were 20 ± 1.2 (mean ± SD), 2.6 ± 1.3, and 6.6 ± 1.5, for WT, FcRγ−/−, and CD11b−γ Tg+ animals, respectively. Thus, CD11b−γ Tg+ animals developed an intermediate level of glomerulonephritis manifested as increased proteinuria and histological evidence of mildly increased glomerular endocapillary cellularity and leukocyte infiltration.

To determine whether the increased cellularity and leukocyte infiltration were due to the glomerular recruitment of Mac-1+ circulating monocytes/macrophages, immunostaining of renal sections was performed (Fig. 6, C and D). All three groups of mice showed similar levels of glomerular mouse anti-sheep IgG deposition, confirming that the failure to develop fulminant nephritis in FcRγ−/− was not due to differences in the production and deposition of anti-sheep IgG in the kidney. In the absence of activating FcR in FcRγ−/−, there was no evidence of infiltrating Mac-1+ macrophages despite deposition of ICs. In WT mice, Mac-1+ infiltrating cells were prominent. Macrophage influx was evident as well in CD11b−γ Tg+ animals, indicating that reconstitution of activating FcR expression in CD11b-Mac-1+ cells was sufficient to restore their direct recruitment and activation in glomeruli, with injurious consequences manifested by proteinuria.

**Discussion**

These studies provide the rationale for the systemic delivery of FcR-targeted therapeutics in lupus. Previous work has shown that activating FcRs are required for nephritis pathogenesis in the autologous and heterologous anti-GBM models and in spontaneous
FIGURE 5. Lineage-restricted expression of FcRγ in macrophages is sufficient for induction of accelerated glomerulonephritis. A, Proteinuria, urinary protein content was quantified daily and mean values of five animals per group are shown. Proteinuria differed significantly among the groups (ANOVA, p = 0.009). By day 7, significantly elevated proteinuria was seen in CD11b-γ Tg mice but not in FcRγ−/−. *, p = 0.016. CD11b-γ Tg vs FcRγ−/−, two-sample t test (two-tailed). B, Serum albumin levels, serum obtained at day 7 was analyzed for serum albumin content. Serum albumin was significantly different between groups (ANOVA, p = 0.004). Relative hypoalbuminemia occurs in CD11b-γ Tg mice but not in FcRγ−/−. The t test p values (two-sample, two-tailed) are shown. Normal mouse albumin is 1.6 mg/ml. Blood urea nitrogen levels, serum obtained at day 7 was analyzed for serum urea nitrogen content. Uremia occurs in WT mice but not in CD11b-γ Tg mice or in FcRγ−/−. Uremia differed significantly between groups (ANOVA, p < 0.0001). The t test p values (two-sample, two-tailed) are shown. Normal mouse blood urea nitrogen levels were 17.3.

disease in NZB/NZW. FcRγ−/− animals fail to develop proteinuria and inflammatory responses despite persistent glomerular IgG and C3 deposition (18, 19, 49, 50). In this study, we have determined the FcR-mediated contributions of intrinsic renal cells vs circulating hematopoietic cells in disease pathogenesis. NZB/NZW mice harboring either FcRγ−/− or FcRγ+/+ BM populations developed comparable serological levels of antichromatin IgGs and IgG/complement glomerular deposition. However inflammatory responses and disease development were abrogated in mice containing FcRγ−/− BM, suggesting that blockade of FcR activation on circulating leukocytes is sufficient to limit effector responses in lupus nephritis despite the persistence of mesangial IC deposition. The absence of FcRγ expression in recipient cells, including renal resident cells, did not limit the incidence or severity of nephritis development in mice bearing WT FcRγ BM populations. Thus, development of nephritis in NZB/NZW required FcRγ expression on hematopoietic cells, establishing these cells as therapeutic targets, whereas FcRγ in MC was dispensable.

To confirm that MC remained recipient derived at the time of disease onset and progression, two experimental approaches were used. Immunohistochemical staining of renal sections obtained at 5 and 9 mo posttransplant demonstrated persistent expression of recipient FcRγ genes and a lack of expression of donor FcRγ in MC populations. Using sensitive PCR genomic DNA assays of short-term, enriched MC cultures obtained from mice 6 mo posttransplant also showed that genotypically MC remained predominantly of recipient origin. By 9 mo posttransplant, however, genetic PCR-based evidence for replacement of some MC by BM precursors was seen. Recent reports using GFP-expressing BM chimeras have suggested that mesangial cell populations are replaced by hematopoietic precursors. However, one of these studies involved an injury model using Thy1 Abs and in both studies only a small fraction of MC was replaced during the observation periods (51, 52). In our studies, it is unclear whether the PCR detection of donor FcRγ alleles of enriched MC cultures resulted from replacement of recipient MC with hematopoietic precursors between 6 and 9 mo or resulted instead from contaminating leukocytes in these enriched glomerular cultures. MC populations remained mostly, if not completely, of recipient origin throughout the observation period, implying that FcRs on MC do not contribute dominantly to the initiation of NZB/NZW lupus nephritis.

Our data are consistent with the notion that ICs deposited in glomeruli may be directly accessible to circulating cells (15, 53, 54). In the skin and lung, by contrast, adoptive transfer studies have demonstrated that FcR-mediated activation of tissue-resident leukocytes in these tissues (3, 22) was sufficient to initiate inflammatory responses and to recruit FcR-deficient neutrophils. In the kidney, however, the specialized endothelium in the renal glomeruli is fenestrated, enabling transit of plasma out of the vascular space (55, 56). This same property also provides glomeruli the anatomic distinction of permitting circulatory cells direct access to tissue ICs deposited in the GBM. Thus, unlike the situation in the skin and lung, this may permit direct initiation of the glomerular inflammatory response by bloodborne leukocytes without a requirement for resident cell-derived recruitment signals.

To determine the singular importance of activating FcR expression in monocyte/macrophage lineage cells as opposed to other BM-derived cells in the induction of nephritis, we targeted FcRγ expression to the CD11b+ compartment in FcRγ−/− animals. FcRγ expression by monocytes/macrophages partially reconstituted the ability to develop nephritis in the anti-GBM model, such that significant levels of proteinuria occurred in CD11b-γ Tg mice. The presence of activating FcRs on macrophages in CD11b-γ Tg mice was sufficient to induce their accumulation in renal glomeruli, presumably as a result of direct FcR activation by glomerular ICs. Histological inflammatory changes were significantly more intense in CD11b-γ Tg− mice than those seen in FcRγ−/−. Thus, activating FcR expression on circulating macrophage CD11b+ subsets is sufficient to induce their direct recruitment into renal glomeruli with injurious consequences manifested by proteinuria. Because the inflammatory response remained of mild
Increased macrophage infiltration was seen in WT and CD11b- mice. Our data with nephritis. Depletion studies have demonstrated that macrophages FcR-bearing effectors is central to the induction of IC-triggered inflammatory cascade. However, FcR engagement is likely a key proximal step in this cascade.

Our studies underscore other recent studies in the anti-GBM model (41, 42), which suggest that direct activation of hemopoietic FcR-bearing effectors is central to the IC-mediated inflammatory nphritis, including granulocytes and CD11b- subsets of monocytes/macrophages cell types not specifically targeted by the CD11b- transgene. Taken together, these studies show that among possible FcR-bearing cell types, expression on myeloid effector cells is sufficient to convey disease susceptibility. Other proinflammatory mediators contribute ultimately to disease (cytokines, chemokines, reactive oxygen, and nitrogen species, etc.); however, FcR engagement is likely a key proximal step in this inflammatory cascade.

Our studies underscore other recent studies in the anti-GBM model (41, 42), which suggest that direct activation of hemopoietic FcR-bearing effectors is central to the IC-mediated inflammatory nphritis, including granulocytes and CD11b- subsets of monocytes/macrophages cell types not specifically targeted by the CD11b- transgene. Our data with CD11b- mice also support the notion that activating FcRs, specifically on monocyte/macrophages, are pivotal to the development of nephritis. Thus, the relative expression/function of activating and inhibitory FcRs (16, 17, 19, 58) on monocyte lineage cells likely modulates IC-triggered glomerulonephritis. FcR-bearing monocytes and macrophages may contribute directly to injury as effectors and also indirectly by modulating IC-mediated Ag presentation and/or by facilitating recruitment and activation of lymphocyte effectors to the tissue site.

Systemic lupus erythematosus (SLE) is characterized by the activation of polyclonal B and T cell self-reactive populations that promote tissue destruction through the recruitment and activation of inflammatory cells. In many regards, the NZB/NZW lupus nephritis model shares pathogenetic features with human SLE, including the hallmark of anti-chromatin IgG autoantibodies, female predominance, and shared genetic disease susceptibility loci, including the FcyR region on chromosome 1q23, which is syntenic in mouse and humans.

Our studies suggest that down-modulation of activating FcR function on bloodborne leukocytes would be predicted to abrogate the inflammatory response in human SLE potentially providing an adjunct therapy or replacement for lymphocyte-targeted immunosuppression. Interestingly however, the ambiguous results of some population studies in human SLE (reviewed in Ref. 59) suggest discordantly that disease is associated with polymorphisms conveying reduced functionality (FcRIIB-232Thr (62)). However, the concept that SLE is associated paradoxically with enhanced FcR signaling is not supported by all studies, including that of Blank et al. (63), which noted an association between SLE and an inhibitory FcRIIB promoter polymorphism (−343 C/C promoter) with reduced expression. It is unclear whether these polymorphic alleles are merely markers of other closely linked genetic contributors on chromosome 1 or rather indicative of hidden challenges of FcγR-targeted therapy that might have pleiotropic modulatory effects on FcR function in Ag presentation, IC catabolism, B cell regulation, as well as myeloid effector cell-triggered inflammation.
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Disclosures

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