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Strain Distribution Pattern of Susceptibility to Immune-Mediated Nephritis

Chun Xie,* Ruchi Sharma,* Hongwei Wang,* Xin J. Zhou,† and Chandra Mohan2*  

The genetic basis of immune-mediated nephritis is poorly understood. Recent studies have demonstrated that the NZW mouse strain is more prone to immune-mediated nephritis compared with C57BL/6 and BALB/c strains. The present study extends these findings by challenging 12 additional inbred strains of mice with rabbit anti-mouse glomerular basement membrane (GBM) reactive sera. Compared with control sera-injected mice and anti-GBM-injected A/J, AKR/J, C3H/HeJ, DBA/2J, MRL/MpJ, NOD/LtJ, P/J, SJL/J, and SWR/J mice, the anti-GBM-injected BUB/BnJ, DBA/1J, and 129/svJ mice developed severe proteinuria and azotemia. Their kidneys exhibited pronounced glomerulonephritis, with crescent formation, as well as tubulointerstitial disease, with these phenotypes being particularly profound in 129/svJ mice. However, these strains did not appear to differ in the nature of their xenogeneic immune response to the administered rabbit sera, either quantitatively or qualitatively. Collectively, these findings allude to the presence of genetic elements in the BUB/BnJ, DBA/1J, and 129/svJ genomes that may potentially confer susceptibility to immune-mediated nephritis. Detailed studies to dissect out the immunological and genetic basis of renal disease in these three strains are clearly warranted.  


The pathogenic events leading to immune-mediated nephritis can perhaps be parsed into two categories: 1) spontaneous events that lead to the formation of pathogenic autoantibodies, and 2) kidney-intrinsic events that lead to renal disease as a consequence of the immunological insult. Although gene-mapping studies in murine lupus have been useful in identifying loci facilitating autoantibody formation, very few of the identified loci appear to have the potential to impact renal disease directly (1–4). However, there is accumulating evidence supporting the notion that genetic factors may also dictate the renal-intrinsic processes that lead to immune-mediated nephritis. A particularly useful approach for studying end-organ susceptibility to immune-mediated damage is the experimentally induced anti-glomerular basement membrane (GBM)3 model, first described by Masugi in 1934 (5). In this model, preformed rabbit anti-GBM serum is deliberately injected into recipient mice. Such an abrupt exposure to nephrophilic (or nephrotoxic) Abs has been documented to compromise renal function in a brisk and reproducible fashion. In this model, one essentially short-circuits the chain of pathogenic events that is otherwise required for the formation of pathogenic autoantibodies, so that one may directly assess how kidneys of different genotypes may handle the same immunological insult. Indeed, this model has been very useful in demonstrating the respective roles played by several different molecules, including complement, FcR, cytokines/chemokines, and adhesion molecules, in mediating nephritis (6–14).

Our recent studies focusing on NZW mice have indicated that the above experimental model also has the power to identify inbred genomes that may potentially exhibit enhanced susceptibility to immune-mediated nephritis (15). Although the NZW genome is known to contribute to disease when bred to other strains (e.g., NZB, BXSB), NZW mice themselves are relatively healthy and free of autoantibodies. Surprisingly, however, we noted the NZW strain to be more susceptible to anti-GBM-induced disease, compared with C57BL/6 (B6) and BALB/c controls. Ongoing studies are aimed at elucidating the specific genetic contributions of NZW to renal diseases, using B6-based congenic strains bearing individual lupus susceptibility loci of NZW origin. Hence, an apparently normal strain may possess latent genes that have the potential to facilitate renal disease. This raises the possibility that other normal strains may also harbor similar genetic elements; these strains may be free of spontaneous renal disease, simply because they do not generate autoantibodies spontaneously. To test this hypothesis, we have challenged 12 additional inbred strains of mice of diverse genealogical origin (16, 17), with potentially nephriogenic anti-GBM Abs, to identify any differential disease susceptibility to immune-mediated nephritis.

Materials and Methods

Nephrotoxic rabbit sera

GBM-reactive nephrotoxic serum (NTS) was generated by Lampire Laboratories (Pipersville, PA). Essentially, renal cortices of B6 kidneys were minced and then pressed through a series of sieves of decreasing pore size (230-, 150-, and 75-μm mesh). The glomeruli were collected on the finest sieve, washed with cold PBS, and sonicated for 7 min. The glomerular sonicates were then used to immunize rabbits (2 mg per rabbit; three injections administered 21 days apart). Sera obtained from these rabbits 50 days following the primary immunization stained the glomeruli and the GBM strongly, but not the tubules, as determined by immunofluorescence, and is referred to as NTS (15). Two independent batches of NTS were used in this study. In addition, preimmune rabbit serum was used as a negative control (placebo).
Mice and NTS nephritis

A/J, AKR/J (AKR), BUB/BaJ (BUB), C3H/HeJ (C3H), DBA/1 (DBA/1), DBA/2J (DBA/2), MRL/MpJ (MRL), NOD/LtJ (NOD), P/J, SJL/J (SJL), SWR/J (SWR), and 129sv/J (129) mice were purchased from The Jackson Laboratory (Bar Harbor, ME). These different strains were selected based on their disparate genealogical origins (16, 17). All mice were maintained in a specific pathogen-free colony. Two- to 3-mo-old females were used for all studies. NTS nephritis was induced using one of two protocols. In the high-dose protocol, mice were first sensitized on day 0 with rabbit IgG (250 μg/mouse, i.p.), in adjuvant. On days 4–6, the mice received either NTS or placebo (preimmune rabbit serum). In both cases, 200 μg of total Ig (per 25 g of body weight) was administered i.v. per mouse, in a 300-μl volume. The same dose (per unit body weight) was used for all strains. Twenty-four-hour urine samples were collected from all mice on days 0, 7, 14, and 21 using metabolic cages, with free access to drinking water. Urinary protein concentration was determined using the Coomassie Plus protein assay kit (Pierce, Rockford, IL). Blood was collected on days 0, 7, 14, and 21, for measuring blood urea nitrogen (BUN), using a urea nitrogen kit (Sigma-Aldrich, St. Louis, MO). All animals were sacrificed on day 21, and the kidneys were processed for light microscopy, as described below.

Strains that appeared to be more susceptible to anti-GBM-induced nephritis were subjected to a second, low-dose protocol, where mice were sensitized with rabbit IgG on day 0, as in the above protocol. However, these mice received a single dose (150 μg per 25 g of body weight) of anti-GBM NTS (drawn from an independent batch) on day 5, and were monitored for evidence of disease through day 14.

Histopathology

Three-micrometer sections of formalin-fixed, paraffin-embedded kidney tissues were cut and stained with H&E and periodic acid-Schiff. These sections were examined in a blinded fashion, for any evidence of pathology, in the glomeruli, tubules, or interstitial areas, as described before (15). The same sections were examined in a blinded fashion, for any evidence of pathology in the glomeruli, tubules, or interstitial areas, as described before (15). Three-micrometer sections of formalin-fixed, paraffin-embedded kidney tissues were cut and stained with H&E and periodic acid-Schiff. These sections were examined in a blinded fashion, for any evidence of pathology, in the glomeruli, tubules, or interstitial areas, as described before (15). Three-micrometer sections of formalin-fixed, paraffin-embedded kidney tissues were cut and stained with H&E and periodic acid-Schiff. These sections were examined in a blinded fashion, for any evidence of pathology, in the glomeruli, tubules, or interstitial areas, as described before (15). Three-micrometer sections of formalin-fixed, paraffin-embedded kidney tissues were cut and stained with H&E and periodic acid-Schiff. These sections were examined in a blinded fashion, for any evidence of pathology, in the glomeruli, tubules, or interstitial areas, as described before (15).

ELISA

Mouse Abs to rabbit Ig were assayed by ELISA, as described before (15). Briefly, purified rabbit Ig (Sigma-Aldrich) was coated onto Immulon I plates (Dynatech, Chantilly, VA), and then blocked. Serially diluted mouse sera were added to these plates, and any bound mouse anti-rabbit Ig was detected using alkaline phosphatase-conjugated goat anti-mouse IgG (that was not cross-reactive with rabbit Ig) (Roche, Indianapolis, IN) and p-nitrophenyl phosphate substrate (Sigma-Aldrich). For the measurement of isotype-specific antibodies, enzyme-conjugated anti-mouse IgG1, or IgG2a (Zymed Laboratories, San Francisco, CA; or BD Pharmingen, San Diego, CA) was used instead. To facilitate interstrain comparisons, sera from the different mice were all assayed within the same ELISA plate. Serum from an NTS-injected mouse with high levels of anti-rabbit Ig Abs was used as standard in all plates; the reactivity of this serum (at 1/200 dilution) was arbitrarily set to 100 U/ml, and the other results were expressed relative to this standard. For the experiments shown in Fig. 7C, serum Ig was purified using affinity chromatography with protein G-Sepharose columns (Pierce, Rockford, IL); Ab concentration was determined spectrophotometrically.

Cytokine determination

129 mice, and two control strains, A/J and SWR, were injected i.p. with rabbit IgG (250 μg per mouse) in adjuvant. All mice were sacrificed 6 days later, and spleens were removed to prepare single-cell suspensions. Splenocytes were seeded to 96-well plates (1 × 10^6 cells/well) and cultured with varying doses of NTS, or anti-CD3 (2 μg/ml) and anti-CD28 (2 μg/ml), in RPMI 1640/5% FCS for 48 h. The cells were then stimulated with 10 ng/ml PMA and 250 ng/ml ionomycin (Sigma-Aldrich) for the last 5 h in the presence of 2.0 μM monensin (BD Biosciences, San Diego, CA). The supernatants were collected for ELISA. The cells were first stained with anti-CD4 and anti-CD8 (BD Biosciences), then fixed and permeabilized, and finally stained with fluorochrome-labeled anti-IFN-γ and anti-IL-4 (BD Biosciences). The cells were analyzed using a FACSScan (BD Biosciences). IFN-γ and IL-4 levels in culture supernatant were determined using IFN-γ and IL-4 ELISA kits (R&D Systems, Minneapolis, MN), respectively, according to the manufacturer’s protocol.

Statistics

For intergroup comparisons, the data were first tested for normality. Where normality tests passed, Student’s t test was applied. Otherwise, a nonparametric Mann-Whitney rank sum test was used. Statistical analyses were performed using SigmaStat (Jandel Scientific, San Rafael, CA). All results are expressed as mean ± SEM.

Results

Proteinuria and azotemia in anti-GBM-challenged mice

To ascertain the impact of different inbred genomes on susceptibility to immune-mediated nephritis, 12 different inbred strains were challenged with GBM-reactive NTS, and monitored over a period of 21 days for evidence of renal disease. On day 0, before
the beginning of the experiment, almost all strains exhibited proteinuria levels of <1.2 mg per 24 h (Fig. 1), consistent with the levels noted earlier in other normal strains (15). There were three exceptions: one of the eight C3H mice studied exhibited day 0 urinary protein levels of >5 mg/24 h; four of the six DBA/1 mice studied displayed day 0 urine protein levels of 1.4–1.5 mg/24 h; all MRL mice studied showed proteinuria levels of 1.3–2.0 mg/24 h, on day 0. However, upon challenge with NTS, three strains distinguished themselves from the rest of the pack. The 129, BUB, and DBA/1 strains demonstrated significantly elevated proteinuria levels (p < 0.001), beginning as early as day 14, as diagrammed in Fig. 1.

As diagrammed in Fig. 2, the strain differences in proteinuria described above were mirrored by similar differences in BUN. Thus, whereas the other strains typically exhibited BUN levels of <35 mg/dl, 129 and BUB mice revealed significantly increased BUN levels (p < 0.001), well in excess of 40 mg/dl. In these mice, elevated BUN was maximal on day 21, whereas elevated proteinuria levels became apparent as early as day 14. Importantly, the mice with the highest proteinuria levels also tended to have higher BUN, among the 129 (r = 0.61; Fig. 3A) and BUB strains (r = 0.89; B). Clearly, glomerular autopspecificity of the injected sera was absolutely necessary for the observed phenotypes, because these strains did not develop elevated proteinuria or BUN when challenged with just the placebo (i.e., preimmune rabbit sera) (Fig. 3). In contrast to the 129 and BUB mice, elevated BUN was not noted in NTS-injected DBA/1 mice (Figs. 2 and 3C).

**Renal pathology in anti-GBM-challenged mice**

To get a better understanding of the underlying renal disease, we next examined the kidneys of these mice. As expected, the same three strains that exhibited proteinuria also disclosed more severe histological GN (Figs. 4A and 5). BUB and DBA/1 mice showed GN scores in the range of 3–4, whereas the 129 mice exhibited the most severe glomerular disease (typically grade 4); in contrast, the remaining nonsusceptible strains typically revealed GN scores in the range of 0–2, on day 21 (Fig. 4A). The marked GN in the three susceptible strains was also accompanied by significant glomerular crescent formation. This was particularly prominent among the NTS-injected 129 mice, which exhibited crescents in 60–80% of their glomeruli (Figs. 4B and 5). Finally, the three susceptible strains also displayed significant TIN, with the 129 strain being the most profoundly affected (Fig. 4C). Despite these pathological changes, mortality was not common over the 21-day period of

![FIGURE 2.](http://www.jimmunol.org/) Elevated BUN levels in NTS-injected 129 and BUB mice. Twelve different strains of mice (n = 4–8 each) were challenged with NTS (nephrotoxic anti-GBM sera), and monitored for BUN levels (y-axis, in mg/dl), over a period of 21 days (x-axis). Depicted p values refer to the differences observed between the NTS-injected BUB or 129 mice, and the remaining NTS-injected strains, at matched time points, using Student’s t test or the Mann-Whitney rank sum test, respectively. Each dot represents a single mouse. D, Day.

![FIGURE 3.](http://www.jimmunol.org/) Correlation between proteinuria and BUN in the NTS- and placebo-injected mice. The corresponding proteinuria and BUN levels (as measured on day 21) are coplotted for all NTS-injected (●) or placebo-injected (○) 129 (A), BUB (B), and DBA/1 mice (C). Each circle represents a single mouse. Indicated also are the correlation coefficients relating these two phenotypes, for each strain.
study (data not shown). It was also interesting to note that histological GN of intermediate grade was observed in AKR, C3H, DBA/2, and P mice, although crescent formation, TIN, and clinical nephritis were clearly absent.

Xenogeneic immune response to rabbit anti-GBM sera

Given that all tested strains had received an identical immune insult, we reasoned that the more prominent disease seen in 129, BUB, and DBA/1 mice may be the consequence of two nonmutually exclusive factors: 1) a stronger xenogeneic immune response to the injected rabbit Ig, or 2) intrinsic, local (i.e., renal) differences in susceptibility to Ab-mediated nephritis. To test the first possibility, we compared the serum levels of mouse anti-rabbit Abs in the 12 different strains, following challenge with rabbit NTS. As demonstrated in Fig. 6A, NTS-injected 129, BUB, and DBA/1 mice did not show higher levels of IgG anti-rabbit Abs in their sera, compared with the other strains. Conversely, SJL mice, which exhibited the strongest xenogeneic response, were clearly free of renal disease (Figs. 1, 2, 4, and 6). This remained true over a range of serum dilutions; the serum dilution pro

A potential caveat in interpreting the above results is that the second step Ab used to measure IgG, IgG1, and IgG2a anti-rabbit Abs in these ELISAs may not have reacted equally well with the Ig from all the different strains studied. This may potentially be a concern, because the 12 strains studied possessed very different Igh allotypes (21). When comparing only a couple of different strains, it is relatively straightforward to standardize or control for this variable; however, this becomes almost intractable if one attempts to standardize this variable for all 12 strains (bearing at least eight different Igh allotypes). We adopted a couple of alternative approaches to address this issue. First, the proteinuria levels in the NTS-injected 129, BUB, and DBA/1 mice did not correlate with the serum levels of IgG anti-rabbit Abs (Pearson’s product-moment coefficient, −0.40) (Fig. 8A), or with the IgG2a:IgG1 anti-rabbit response ratios (Pearson’s product-moment coefficient, −0.29) (B). These observations suggested that the accentuated renal disease seen in these strains was not simply the result of an exaggerated or altered (i.e., Th1-skewed) anti-rabbit, xenogeneic immune response against the administered rabbit NTS.

Second, we tested whether the second-step reagent used for the ELISA depicted in Figs. 6 and 7 was equally good at recognizing IgG Abs from the different strains. For this purpose, we isolated Ig from a disease-prone strain (i.e., DBA/1), and two less susceptible strains (SWR and SJL), using protein G columns. Purified serum IgG from these three strains (five mice each) was quantitated spectrophotometrically, and graded amounts were plated serially onto ELISA plates. As depicted in Fig. 8C, the second-step reagent, enzyme-coupled goat anti-mouse IgG, reacted equally well with the IgG from all three strains. These findings further support the notion that the serological data reported in Figs. 6 and 7 was not confounded by differences in the ability of the ELISA to measure Abs of different allotypes equally well. Finally, kidneys from the different strains studied did not differ

FIGURE 4. GN and TIN in NTS-injected mice. Kidney specimens from all NTS-injected mice (on day 21) were examined by light microscopy for evidence and grade of GN (A), glomerular crescent formation (B) (expressed as a percentage of 100 glomeruli examined per mouse kidney), and the grade of TIN (C), as detailed in Materials and Methods. Each circle represents a single mouse. Data pertaining to the 129, DBA/1, and BUB strains were compared with the data pooled from all of the other strains, using the Mann-Whitney rank sum test (because the test for normality failed); the corresponding p values are depicted. Placebo-injected 129, BUB, and DBA/1 mice exhibited average GN scores of 0, 0.7, and 0.1, respectively, and average TIN scores of 0, 0.3, and 0.1, respectively.
significantly in the extent of rabbit Ig, mouse Ig, or C3 deposits they exhibited on their glomeruli, as gauged by indirect immunofluorescence; thus, all strains typically exhibited rabbit IgG immunofluorescence of 3.0 U, mouse IgG immunofluorescence of 2.5–3.0 U, and C3 deposits of 1.0–1.5 U, on a 0–3 semiquantitative fluorescence scale (data not plotted). Collectively, the above findings indicated that the significant glomerular disease seen in 129, BUB, and DBA/1 mice was not the consequence of a stronger anti-glomerular insult.

To confirm the findings gleaned from measuring isotype-specific responses (i.e., IgG2a and IgG1 Abs, as depicted in Fig. 7), we proceeded to directly test Th1 and Th2 cytokine production by the different strains in response to challenge with rabbit Ig. For this purpose, we selected the most severely affected strain, 129, and two unaffected strains, SWR and A/J. Six days postsensitization with the rabbit Ig (in adjuvant), splenocytes from the three strains were tested for IFN-γ and IL-4 production, with or without stimulation with NTS; anti-CD3 stimulation served as a positive control. As is evident from Fig. 8, D and E, the 129 strain did not exhibit a more robust Th1-skewed xenogeneic immune response, compared with the two control strains. Hence, the enhanced disease seen in these mice was not related to a stronger anti-glomerular insult.

Confirmation of results with an abbreviated anti-GBM protocol

In view of the elaborate nature of the 21-day anti-GBM protocol, and the numbers of mice one typically has to study in such genetic studies, we experimented with alternative disease induction protocols that were more abbreviated, and used lower amounts of NTS. At the same time, this exercise offered us the opportunity to confirm the above-reported strain differences. For these confirmatory studies, the three susceptible strains, 129, BUB, and DBA/1, as well as an independent control strain, B6, which has previously been shown to be significantly less prone to anti-GBM disease (15), were sensitized on day 0 with rabbit Ig, and administered an alternative batch of NTS on day 5, as a single low-dose (150 μg of Ig per 25 g of body weight). All mice were monitored for proteinuria and BUN on days 0 and 14. As depicted in Fig. 9, 129 and DBA/1 mice were clearly susceptible to low-dose NTS as well; indeed two of the five 129 mice tested died prematurely. In comparison, the B6 controls were relatively disease free. Surprisingly, BUB mice were not susceptible to low-dose anti-GBM-induced nephritis.

Discussion

Taken together with the earlier report (15), the present findings help classify the different inbred strains into two broad categories: 1) strains that are particularly susceptible to immune-mediated nephritis, including 129, BUB, DBA/1, and NZW, and 2) strains that are less susceptible to nephritis, including A/J, AKR, BALB/c, C3H, C57BL/6, DBA/2, MRL, NOD, P/J, SJL, and SWR. Collectively, these two studies suggest that renal susceptibility to immune (and perhaps other forms of) damage may be genetically programmed, and that the four susceptible genomes identified thus far may hold important clues concerning the molecular pathways that mediate immune nephritis. Each of the additional susceptible strains identified in the present study is of unique interest, based on what is already known about these strains.
The 129 inbred mouse strain is perhaps the most commonly used strain for targeted mutagenesis studies. Indeed, an accumulating number of genes when knocked out (on the 129 genetic background) are associated with phenotypes resembling lupus nephritis, as reviewed (3, 4). However, several of these phenotypes vanished when some of the gene knockouts (e.g., $C1q^{-/-}$ and $C1r^{-/-}$) were knocked out.

**FIGURE 6.** Anti-rabbit Ig response in the NTS-injected mice. A. Depicted are the serum levels of IgG mouse anti-rabbit Ig in the different NTS-injected strains, measured on day 21 ($n = 4–8$ each). All ELISA were performed in parallel, and the results were standardized using the same reference standard. Each circle represents a single mouse. The xenogeneic immune response observed in the NTS-injected 129, BUB, and DBA1 mice was not significantly higher than that noted in the remaining strains, pooled. Depicted in B–D are the IgG Ab reactivities to rabbit Ig assayed in serial dilutions (1/50, 1/200, 1/800, 1/3,200, 1/12,800, 1/51,200, and 1/204,800) of the SJL, 129, and A/J sera, respectively, drawn on day 21 ($n = 4–5$ each). The vertical dotted lines indicate the serum dilutions that yielded half-maximal anti-rabbit seroreactivity.

**FIGURE 7.** Anti-rabbit IgG1 and IgG2A response in the NTS-injected mice. Depicted are the serum levels of IgG2a (A) and IgG1 (B) mouse anti-rabbit Ig in the different NTS-injected strains, as measured on day 21 ($n = 4–8$ each). Importantly, all ELISA were performed in parallel, and the results were standardized using the same reference standard. Each circle represents a single mouse. The IgG2a:IgG1 ratios for the xenogeneic anti-rabbit response were also computed (C), using the data shown in A and B. In all of the plots, the xenogeneic immune response observed in the NTS-injected 129, BUB, and DBA1 mice was not significantly higher than that noted in the remaining strains, pooled.
were backcrossed onto the C57BL/6 background (22–24). These observations raise the possibility that the renal disease observed in an unknown fraction of the 129-based gene knockout studies reported thus far may simply have been the product of yet-to-be-defined 129-derived disease genes. Our demonstration of the 129 strain to be one of the most nephritis-prone of all of the strains experimentally challenged, adds weight to this notion. Hence, it is important to consider potential 129-derived genetic contributions in interpreting nephritis-related phenotypes observed in 129-based knockout studies. Moreover, it is clearly necessary to map and identify the 129-derived genetic elements responsible for the increased renal disease.

The identification of BUB and DBA/1 mice as being particularly nephritis-prone strikes yet another chord. Both of these strains, particularly DBA/1, have long been noted to be susceptible to collagen-induced arthritis (25, 26). Indeed, the DBA/1 strain is perhaps the most commonly used strain for the study of collagen-induced arthritis. This raises the interesting possibility that the BUB and DBA/1 genomes may harbor genetic loci modulating multiple end-organ diatheses. This may be not be a surprise, because several of the molecules known to be important in modulating nephritis (e.g., adhesion molecules, FcR and complement, cytokines and chemokines, free radicals, etc.) have also been documented to impact arthritis. More recently, DBA/1 loci conferring arthritis susceptibility have been identified (27, 28). Mapping the responsible DBA/1 nephritis susceptibility loci may help identify shared genetic elements that subserve both types of end-organ disease in this strain. In contrast to the DBA/1 (and 129) mice, BUB mice may be more susceptible to immune nephritis only when challenged with larger doses of NTS, as suggested by the findings of the replication studies presented in Fig. 8.

With respect to the less susceptible strains, whereas the C3H, DBA/2, MRL, P/J (and C57BL/6) strains developed modest degrees of proteinuria following NTS challenge, the remaining strains showed minimal clinical or morphological evidence of the immunological assault. However, it should be kept in mind that these less susceptible strains may not be absolutely resistant to spontaneously arising nephritis, in view of the observation that C57BL/6-based lupus congenics and MRL/lpr mice clearly have the potential to develop florid lupus nephritis (29–32). The development of end-organ disease in these less susceptible strains may be contingent upon the presence of additional genetic aberrations (e.g., Sle3, or the FAS<sup>pr</sup> mutation). Additionally, we have observed that some of these strains (e.g., C57BL/6) may be susceptible to immune nephritis if the dosage of the administered anti-GBM sera is increased (data not shown). Hence, the spontaneous
development of nephritis in MRL/lpr mice (despite the relative resistance of the MRL strain to anti-GBM-induced disease in this report) may be attributed to FAS<sup>+</sup> mutation; it may be contingent upon chronic exposure to nephrophilic autoantibodies. In this context, it remains to be established whether the FAS<sup>+</sup> mutation itself may promote renal disease in this experimental model.

It is somewhat surprising that the SJL strain did not exhibit prominent renal disease given that this strain had previously been reported as being particularly susceptible to an alternate protocol of experimental nephritis (20). As we evaluate the very different disease outcomes in this strain (which appear to be contingent upon the experimental protocol used), several factors ought to be weighed in. Whereas Kalluri et al. (20) had repeatedly immunized their strains with bovine collagen NC1, the protocol detailed in this paper entails a single sensitization against rabbit Ig; it is certainly conceivable that the resulting spectrum and evolution kinetics of autoantibodies in these two protocols may be very different. Moreover, whereas disease peaked in our susceptible strains (but not SJL) as rapidly as 2–3 wk following immunization, Kalluri et al. (20) noted disease in the SJL strain 12 wk following collagen NC1 immunization. Clearly, in both studies, the SJL strain does not appear to be acutely susceptible to immune-mediated nephritis. In future studies, it would also be important to study how the 129, BUB, and DBA/1 strains might respond to immunization with collagen NC1.

One potential explanation for the more severe renal disease seen in the 129, BUB, and DBA/1 strains may relate to an exaggerated systemic immune response to the administered rabbit Ig, compared with the xenogeneic immune response observed in the other strains. Several observations argue against this notion. First, previous work with the NTS-induced nephritis model had indicated that the host’s humoral immune response did not contribute significantly to the observed pathology, because host mice devoid of B cells developed a similar disease (33). Second, the 129, BUB, and DBA/1 mice receiving preimmune rabbit sera (i.e., the placebo) did not develop any pathology or disease, suggesting that the observed end-organ pathology was absolutely dependent upon the presence of a nephrophilic specificity (i.e., NTS was absolutely required). Third, the levels of mouse anti-rabbit Ig (i.e., the xenogeneic immune response) seen in the NTS-injected 129, BUB, and DBA/1 mice were not significantly different from that noted in the remaining strains, quantitatively or qualitatively (Figs. 6 and 7). Finally, the most diseased mice were clearly not the ones that had also developed the most exuberant (or the most Th1-skewed) xenogeneic immune responses (Fig. 8).

An important alternative scenario to entertain is that the glomerular targeting of NTS to the kidneys of these three susceptible strains may have fired up local pathogenic cascades that may have been more profound or elaborate, compared with the molecular and cellular events triggered in the less susceptible strains. Such local differences may arise in two different ways. First, the 129, BUB, and DBA/1 genomes may encode resident renal cells (e.g., mesangial cells, podocytes, etc.) that may be intrinsically aberrant, i.e., they may be hypersensitive to the immune insult. Alternatively, or additionally, these disease-susceptible genomes may have the potential to impact the function of infiltrating systemic immunocytes (e.g., macrophages, T cells, etc.). The present findings do not distinguish between renal-intrinsic factors in the respective genomes vs potential systemic contributions affecting the renal infiltrates. Transplant-based approaches as well as detailed studies of intrinsic renal cells cultured from these strains are clearly warranted to distinguish between these possibilities. An added task at hand is to identify the genetic elements responsible for the increased renal disease seen in 129, BUB, and DBA/1 mice. Collectively, these future studies are likely to elucidate the panoply of molecules (in systemic and/or renal-intrinsic cells) that may potentially modulate the severity of immune-mediated nephritis.

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