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Terminal Deoxynucleotidyl Transferase Deficiency Reduces the Incidence of Autoimmune Nephritis in (New Zealand Black × New Zealand White)F₁ Mice¹

Carmen Conde,* Sandra Weller,* Susan Gilfillan,‡ Luc Marcellin,† Thierry Martin,2* and Jean-Louis Pasquali*

Terminal deoxynucleotidyl transferase (TdT) enzyme activity in lymphocytes generates diversity in the Ag receptor repertoires by adding template-independent N nucleotides and disrupting homology-directed rearrangements. The importance of this diversity in vivo and the significance of the suppression of TdT during fetal life remain uncertain. Previous studies have shown that in TdT knockout mice (TdT°), 1 the T cell repertoire is less peptide oriented; and 2) natural autoantibody, particularly anti-DNA autoantibodies, are less polyreactive, and their mean affinities are reduced. Consequently, the suppression of TdT during early T/B cell ontogeny may participate in controlling autoimmunity. To study the impact of TdT suppression in autoimmune-prone mice, we introduced the TdT null mutation into the (NZB × NZW)F₁ mouse strain. We show that TdT deficiency significantly reduces the incidence of autoimmune nephritis and prolongs survival compared with those in control mice. Surprisingly, the long-term survivor TdT° mice produced amounts of anti-ADN and anti-histone autoantibodies similar to those of their TdT⁺ littermates. However, these TdT° mice showed no evidence of renal inflammation, and the immune deposits were restricted to the mesangium, whereas basal membrane deposits were clearly correlated with overt renal disease. The present study supports the idea that the absence of TdT enzyme activity in lymphocytes protects mice against autoimmunity and could offer a therapeutic approach to autoimmune diseases. Moreover, our results may help to unravel the mechanisms of lupus nephritis. The Journal of Immunology, 1998, 161: 7023–7030.

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(NZB×NZW)F_{1} (designated B/W) mouse strain for the additional following reasons. B/W mice spontaneously develop an autoimmune disease that resembles human systemic lupus erythematosus (SLE), including the occurrence of severe lethal glomerulonephritis (21, 22). IgG anti-dsDNA autoantibodies are the principal feature in both B/W mice and human SLE, and their presence correlates with nephritis (21–23). The relationship between pathological anti-DNA Abs and natural autoantibodies remains questionable. It is not yet clear whether pathological anti-DNA Abs originate from and are regulated by the pool of natural autoantibodies. Experiments using the B/W mouse strain suggest that during the initial stage of the disease, low affinity anti-ssDNA IgM autoantibodies arise as a by-product of polyclonal B cell activation, possibly involving an intrinsic B cell abnormality (24, 26, 27; reviewed in Ref. 25). During disease progression, pathogenic high affinity IgG anti-dsDNA autoantibodies may arise from this pool as a result of an Ag-driven expansion and maturation of a few B cell clones (24, 28, 29; reviewed in Refs. 25 and 30). The mechanisms underlying this breakdown of tolerance are still unclear; in particular, the respective roles of an abnormal B cell preimmune repertoire or T cell regulation have not been established. Given the potential impact of the suppression of TdT on the preimmune anti-DNA B cell pool, the study of TdT° B/W mice may contribute to resolving these issues.

Anti-dsDNA autoantibodies appear to play a major role in glomerulonephritis; however, the conclusions derived from many studies are that only a subset of these autoantibodies is pathogenic and that the structural/molecular properties of the nephritogenicity are still unclear. The presence of cationic residues, namely Arg, within the CDR regions, especially CDR3, of autoantibody heavy chains may be essential for their high affinity for dsDNA and their nephritogenic properties (29, 31; reviewed in Refs. 25 and 30). Arg residues are rare in CDR3 regions of Abs that do not bind DNA but can be generated by somatic mutations, N addition, or unusual V-(D)-J rearrangements (28, 29). The respective roles of these mechanisms remain to be elucidated, but the last two clearly depend on the action of TdT in the B cell population, whereas the first may be indirectly affected by the expression of this enzyme in the T cell pool.

In this study we show that introduction of the TdT° null mutation into B/W mice partially protects them from nephritis even though such mice produce equivalent amounts of anti-DNA and anti-histone autoantibodies as their TdT° littermates. However, healthy TdT° B/W mice do not show evidence of renal inflammation, and although they have Ig glomerular deposits, the pattern is different from that observed in littermates with nephritis. These results may help to unravel the mechanisms of lupus nephritis and the molecular basis of nephritogenic autoantibodies.

Materials and Methods

Mice

TdT° C57BL/6 mice carrying a mutation in the TdT gene and therefore deficient in this enzyme were provided by C. Benoist and D. Mathis (Institut de Genetique et de Biologie Moleculaire et Cellulaire, Strasbourg, France) (1). The mice were maintained in a conventional mouse facility. NZB and NZW mice were purchased from The Jackson Laboratory (Bar Harbor, ME). The TdT° allele was backcrossed separately onto the NZB and NZW inbred strains. Mice bearing the TdT° allele were identified by PCR analysis of tail DNA using a mixture of primers as described previously (19). The NZB and NZW mice of the fourth and sixth backcrosses (N4 and N6) heterozygous for the TdT° allele were intercrossed, and the N4F1 and N6F1 progeny was analyzed. The TdT°/° and TdT°/+ littermates designated as a whole TdT° were used as controls. Only female animals were used in the present study.

Microsatellite mapping using single sequence length polymorphisms

Since both NZB and NZW MHC (H2) loci have been shown to be linked with lupus traits (reviewed in Refs. 32 and 33), we have selected the NZB and NZW TdT°/+ mice with H-2^{b} and H-2^{e}, respectively, in the second backcross generation for the subsequent crosses. This was performed using Southern blot analysis with an Apo cDNA probe.

Oligonucleotides flanking simple sequence repeats were synthesized at the Institut de Genetique et de Biologie Moleculaire et Cellulaire and electronically labeled with [γ-32P]ATP. The relative positions of the markers and the sequences of the primers were obtained from The Mouse Genome Database of The Jackson Laboratory via internet at http://www.informat-ics.jax.org/medi.html). When the markers that have been described to map the susceptibility loci in NZB/W mice were not polymorphic between the C57BL6–129 and the NZB/NZW alleles, other neighboring markers were used. Amplification of the simple sequence repeats was achieved by PCR using tail DNA in a PTC-100 thermal cycler (MJ Research, Watertown, MA). The program for PCR reactions (total volume, 20 μl) was generally 30 cycles of 30 s at 94°C, 30 s at 54°C, and 1 min at 72°C. PCR products were run on 8% polyacrylamide gels at 12 V/cm for 2–3 h. The autoradiograms obtained were scored accurately.

Segregants from the fourth backcross were genotyped with the following linkage markers (loci contributing to SLE susceptibility are indicated in bold): D1 Mit33 (34), D1 Mit15 (35), D1 Mit36 (Lbw7) (35), D1 Mit21 (36), D4Ndc2 (Lbw2) (35), D4 Mit12 (36), D4 Mit48 (37), D5 Mit10 (Lbw3) (35), D6 Mit25 (Lbw4) (35), D7 Mit70 (34), D18 Mit8 (Lbw6) (35), and D18 Mit142. At the N4 backcross generation, het- erozygotes for NZB/NZW alleles at most of the linkage markers were used for the intercrosses and for backcrosses. BW N4F1 mice were geno- typed using the same markers.

Evaluation of renal disease

Mice were evaluated weekly for proteinuria using Usistix (Bayer Diagnostics, Puteaux, France). Urine samples were graded 0 to 4+, corresponding to the following approximate protein concentrations: 0 = negative or trace; 1+ = 30 mg/dl; 2+ = 100 mg/dl; 3+ = 300 mg/dl; 4+ = >2000 mg/dl. Mice with negative determinations of proteinuria and no evidence of disease up to 12 mo of age were identified as not expressing lupus-like disease and were allocated a negative disease phenotype; the disease is only expressed in designated long-term survivors. Mice with significant proteinuria (≥3+) on two or more consecutive examinations before 12 mo of age were designated positive for renal disease.

Histological analysis

The severely ill and wasted mice with evidence of renal disease were killed for kidney collection when they appeared moribund. Mice that were killed were necropsied as deaths at the time they were killed in Fig. 1B. The mice with no evidence of renal disease were killed at the end of the study at 12 mo.

Kidneys were immediately collected from sacrificed mice, fixed in Bouin’s solution, and embedded in paraffin or frozen at −80°C in OCT (Miles, Elkhart, IN) for immunofluorescence assays. Sections were cut and stained with hematoxylin-eosin or Masson trichrome using standard meth- odology. Glomerular disease was graded by a pathologist blinded to the group of origin of the kidney sections. Scores were determined using a grading system (0 to 4+) based on the intensity and extent of histopathological changes (proliferation, crescent formation, necrosis, and tubular alteration) as previously described (38, 39).

IgM, IgG, and IgG subclass deposits were assessed by immunofluorescence on kidney cryocut section using fluorescein-conjugated rat anti- mouse IgG, IgM, IgG1, IgG2a, IgG2b, or IgG3 (PharMingen, San Diego, CA). All Abs were used at a 1:5 dilution and were incubated for 20 min at room temperature. After washing with PBS, pH 7.2, for 6 min, the sections were counterstained with Evans blue. The sections were scored on a 0 to 3+ scale.

ELISA and serological assays

The mice were bled by retro-orbital puncture under anesthesia at monthly intervals from the age of 4 mo. The sera were stored at −20°C until analysis for Ab levels.

Total levels of IgM, IgG, IgG1, IgG2a, IgG2b, and IgG3 were deter- mined by ELISA. For IgG subclasses, microtiter plates (Nunc, Roskilde, Denmark) were coated with goat anti-mouse IgG/M(H+L) Abs (Jackson ImmunoResearch Laboratories, West Grove, PA) at 5 μg/ml in PBS overnight at 4°C. After washing and saturation for 30 min at 37°C with PBS and 1% BSA, serum samples were titrated in PBS containing 0.1%
Tert deficiency reduces the development of significant proteinuria and prolongs survival in B/W F1 mice

To follow the progression of renal disease, B/W F1 N4 female mice were evaluated weekly from 4–12 mo of age for the presence of proteinuria (Fig. 1A). The mice with a dipstick reading of 3+ (proteinuria ≥300 mg/dl) or higher on at least two consecutive examinations were considered positive for renal disease. Fewer TdT− mice than TdT+ littermates developed significant proteinuria by 6 mo of age and throughout the remainder of the study. By the end of the study, only 31% of the TdT− mice (4 of 13) vs 100% of the TdT+ mice (7 of 7) had developed evidence of significant proteinuria (p < 0.02, by Mann-Whitney U test).

Consistent with the partial protection against renal disease, TdT deficiency significantly enhanced survival in B/W F1 mice (Fig. 1B). This effect was already apparent at 7 mo of age, at which stage 90% of the TdT+ mice were alive vs 40% of the control group. By the end of study all the TdT+ mice had presented evidence of severe nephritis and were dead, whereas 9 of 13 TdT− mice were alive and had no proteinuria (p < 0.02, by Mann-Whitney U test). For clarity, we refer to these nine mice as long-term survivors.

We also studied a group of B/W F1 female mice from the sixth backcross generation (15 TdT+ and 7 TdT−). At 6 mo of age, 9 of 15 TdT+ vs 0 of 7 TdT− mice had developed nephritis (p < 0.02, by Mann-Whitney U test). During the further follow-up, all the TdT+ mice remained healthy, without proteinuria, and four (aged 6–9 mo) were sacrificed for the generation of hybridomas. At 1 yr of age, the three remaining TdT− animals were still healthy, whereas 14 of 15 TdT+ mice had developed severe nephritis before reaching this age. Fig. 1C depicts the cumulative percent survival curve of all the TdT+ (n = 22) and TdT− (n = 20) B/W N4 and N6 female mice studied (Kaplan-Meier analysis). The difference was significant (p < 0.0001, by log-rank test).

Results

Genetic analysis

We studied 20 female mice B/W F1 from the N4 backcross generation. Thirteen were homozygous for the TdT− mutation. All mice were genotyped by PCR for the 12 microsatellite markers described in Materials and Methods that were previously shown to be closely linked to nephritis, death, and anti-chromatin autoantibody production in the B/W F1 model (33–37). The results indicate that all the mice were B/W heterozygous at the Sle1, Lbw5, Lbw6, and Lbw7 loci (not shown). The presence of 129 or B6 alleles at the other loci was occasionally observed in some TdT+ and TdT− mice, with no significant differences between the two groups.

Tert of IgG and IgM anti-histone autoantibodies were assessed by ELISA. Microtiter plates were coated overnight at 37°C with 10 μg/ml of histone H1, H2A, H2B, H3, or H4 in PBS buffer. The histones were purchased from Boehringer Mannheim (Mannheim, Germany). After washing and saturation with PBS/BSA, serum samples were diluted in 0.5% PBS containing 0.1% Tween 20 and 0.5% gelatin and incubated overnight at 4°C. After washing, plates were incubated with the relevant peroxidase-conjugated goat anti-mouse Ig (Jackson Immunoresearch Laboratories). The titers are the highest dilutions still giving a positive signal (log2 titers) in the ELISA in which twofold serum dilutions were tested starting from a 1/100 dilution.

All ELISA assays were performed in triplicate. In each test, sera from MRL lpr/lpr and BALB/c mice were included as positive and negative controls, respectively.
TABLE I. Renal histology and immunofluorescence in TdT° and TdT° mice with nephritis and from TdT° long-term survivor mice

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<td>Mice</td>
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* The severely ill TdT° and TdT° mice with nephritis were sacrificed prior to death for kidney collection. The TdT° mice with no evidence of renal disease were killed at 12 mo and were designated as long-term survivors.

1 Results from individual mice are represented.

2 Glomerular and tubular disease were scored based on the intensity and extent of pathological changes as follows: +++, very severe abnormalities; +++, severe abnormalities; +, moderate abnormalities; -, mild abnormalities; and −, no abnormalities.

3 Intensity of staining was scored as follows: +, detectable; ++, mild; and +++, severe.

4 Percentage of glomeruli with Ig deposits. +, 25%; ++, 50–75%; and +++, >75%.

Therefore, the data from B/W N6 mice confirmed that TdT deficiency induced significant protection against nephritis and enhanced survival in B/W mice. Since N4 and N6 mice had similar clinical phenotypes, the results to be presented below concern only B/W N4 female mice.

TdT deficiency significantly reduces the development of proliferative glomerulonephritis in B/W F1 mice

Kidney specimens from all mice were examined by light and immunofluorescence microscopy for the presence of glomerulonephritis and Ig deposits. The animals showing no evidence of renal disease were sacrificed at the end of the study at 12 mo. The results are presented in Table I and Fig. 2. All the TdT° mice and all the TdT° animals with proteinuria developed lesions typical of severe proliferative glomerulonephritis, including reduced numbers of discernible glomeruli with fibrinoid necrosis and sclerosis, increased mesangial and glomerular cellularity with loss of normal architecture, crescent formations, and tubular alterations such as dilatation and cast formation (Fig. 2, B and C). In contrast, histologic examination of kidneys from clinically healthy TdT° mice killed at 12 mo revealed no significant glomerular or tubular disease (Fig. 2A). Indeed, the sections were difficult to distinguish from the BALB/c controls.

Surprisingly, the immunofluorescence staining revealed IgG and IgM deposits in all TdT° and TdT° mice (Table I and Fig. 2, D–F). However, the patterns were clearly different. Moderate to intense mesangial and capillary wall granular deposits were observed in TdT° and TdT° mice with nephritis with no significant differences between the two groups. In contrast, capillary wall staining could not be detected in long-term survivor TdT° animals. We also examined the IgG subclass deposits in two mice randomly selected from TdT° mice and from TdT° long-term survivor mice a

TdT deficiency does not prevent the production of hypergammaglobulinemia and autoantibodies

Because of the potential role of certain autoantibodies in the pathogenesis of renal disease in B/W F1 mice, we examined whether the beneficial effect of TdT deficiency could be related to a disability to generate humoral autoimmunity. For this, the mice were bled monthly to measure serum levels of Ig isotypes and autoantibodies against ssDNA, dsDNA, and individual histones. Serum Ig isotypes were measured at 4 and 9 mo of age (or, in the case of mice that died before reaching this age, i.e., four TdT° and one TdT°, in the last sample). At 4 mo of age all mice produced significantly increased amounts of IgM and all IgG subclasses compared with normal BALB/c mice housed in the same environment. The high IgG serum levels were mainly attributable to an increased production of IgG2a and IgG3. It is worth noting that there were no substantial differences among the three groups of mice. At 9 mo of age, similar observations were made for IgM, IgG2a, and IgG3; however, only TdT° long-term survivors still presented increased levels of IgG2b (data not shown).

Titters of IgG and IgM autoantibodies against dsDNA, H2A, and H2B were evaluated monthly. Results of these analyses at 5, 6, and 8 mo of age are presented in Figs. 3 and 4. Surprisingly, each type of autoantibody developed at levels comparable among the three groups of mice, albeit variable within each group. Similar results were obtained for IgG and IgM against ssDNA, H1, H3, and H4 (not shown). Considering that the C. luciliae assay may provide a more stable and specific substrate than ELISA for the determination of anti-dsDNA activity (40), we also examined serum binding to Crithidia kinetoplast. Again, there were no significant differences in anti-dsDNA titers among the three groups of mice (data not shown). In B/W F1 mice anti-dsDNA autoantibodies are predominantly of the IgG2a isotype (reviewed in Refs. 21 and 41); to determine whether TdT deficiency could alter the subclass profiles of IgG anti-dsDNA, we examined the serum from mice at 8 mo of age (IgG anti-dsDNA titers increased over time, generally reaching their maximum level at this age; for mice that were already dead, we used the last sample before death). As shown in Fig. 5, the levels of IgG1, IgG2a, and IgG3 anti-dsDNA were comparable among the three groups of animals; in fact, the only significant difference consisted of higher IgG2b titers in TdT° long-term survivors compared with those in TdT° littermates (p < 0.01, by Mann-Whitney U test).

Thus, TdT deficiency does not inhibit autoantibody production in B/W F1 mice, and the absence of nephritis in TdT° animals is not correlated with a particular autoantibody profile.
Discussion

We previously demonstrated that TdT deficiency reduces the pool of anti-DNA B cells in the preimmune repertoire in otherwise normal B6 mice and the mean affinity of the expressed anti-DNA Abs (20). In this study we show that TdT deficiency in B/W F1 mice reduces the development of autoimmune nephritis.

Since most of the data presented in this study concern the N4 backcross generation, it may be argued that the disease resistance in the B/W F1, TdT° stock could be ascribed to the presence of 129- or B6-derived resistance alleles. We think this very unlikely for the following reasons. 1) All the studied mice were heterozygotes \( H2^{d/z} \); B/W MHC heterozygosity represents the most important contribution to disease from each strain (\( H2^{d/z} \) vs \( H2^{d/d} \) for NZB and \( H2^{w/w} \) for NZW). 2) Our genotypic analysis did not reveal any bias for the presence of 129 or B6 allele in the long-term survivor group. 3) The TdT° segregants developed the disease at the expected rate. 4) Preliminary data from the N6 backcross generation confirm our results; at about 6 mo of age, 9 of 15 TdT° vs 0 of 7 TdT° mice had developed nephritis (\( p < 0.02 \)).

While numerous SLE susceptibility loci have been described, none linked with nephritis has been mapped to chromosome 19 where TdT is located.

The mechanism(s) by which TdT deficiency reduces the development of autoimmune nephritis remains to be elucidated. Although the exact mechanisms of nephritis in B/W mice are still under debate, the following statements are best supported by the
existing data (reviewed in Refs. 41 and 42). 1) Nephritogenic autoantibodies represent the proximal cause of the glomerulonephritis. 2) They probably comprise a family of autoantibodies that binds to epitopes on chromatin (including but not limited to dsDNA). 3) Immune complex (formed either in the circulation or in situ) deposits in the kidney induce disease by activating the complement. 4) These deposits may relate to the propensity of the immune complexes to bind via the autoantigen to components of the glomerular basal membrane, such as type IV collagen, heparan-sulfated glycosaminoglycans or anionic phospholipids. In our study nephritis is closely correlated with the subendothelial deposition of IgG and mainly of IgG2a and IgG3 in both TdT° and TdT°° animals. In contrast, mesangial immune deposits per se do not seem to induce renal lesions; this is reminiscent of the fact that in human SLE, mesangial Ig deposits are a common finding and are not indicative of nephritis. Our results are also in accord with adoptive or passive transfer experiments with either anti-DNA hybridomas or anti-DNA mAbs, which usually resulted in mesangial Ig deposits with a subsequent mild mesangial hypercellularity (43–45). Very few anti-DNA mAb (mAb H147 (45), 6-19 (46), and 11F8 (47)) have been shown to induce severe proliferative glomerulonephritis upon adoptive transfer; in these cases, immunofluorescence revealed intense mesangial and subendothelial immune deposits. We also show in this study that TdT does not alter the subclass profiles of the immune deposits. Taken together, our

FIGURE 3. Effect of TdT deficiency on the spontaneous anti-DNA autoantibody production in B/W mice. Serum levels of IgM anti-dsDNA (A) and IgG anti-dsDNA (B) Abs in TdT°°, TdT°, and TdT°° long-term survivor B/W mice, 5, 6, and 8 mo of age. Each symbol represents the value from an individual animal. Twofold serum dilutions were tested starting with a 1/100 dilution. The titers are the highest dilutions still giving a positive signal in the ELISA.

FIGURE 4. Effect of TdT deficiency on spontaneous anti-H2A/H2B autoantibody production in B/W mice. Serum levels of IgM anti-H2A (A), IgG anti-H2A (B), IgM anti-H2B (C), and IgG anti-H2B (D) autoantibodies in TdT°°, TdT°, and TdT°° long-term survivor B/W mice, 5, 6, and 8 mo of age. These are the same mice as those in Fig. 3.

FIGURE 5. IgG1, IgG2a, IgG2b, and IgG3 subclass distribution of anti-dsDNA Abs in the sera of TdT°°, TdT°, and TdT°° long-term survivors B/W mice. Sera at 8 mo of age or from the last sample before death were used. Twofold serum dilutions were tested starting with a 1/100 dilution. The titers are the highest dilutions still giving a positive signal in the ELISA. Each symbol represents the value from an individual animal.
findings indicate that the absence of nephritis in a large proportion of TdT° mice is not explained by a different subclass composition of the immune deposits and consequently by their failure to activate the complement; rather, it may be related to the variation in their location, which, in turn, is probably influenced by the antigenic specificities of the deposited IgG.

Therefore, the most immediate conclusion is that TdT deficiency hampers the production of nephritogenic Abs. Such Abs induce nephritis and/or immune deposits presumably because they are somatically mutated and class switched by αβ T cell-dependent mechanisms. The molecular basis of nephritogenicity is, however, still controversial; this is due in part to technical problems (for instance, several monoclonal anti-dsDNA capable of inducing nephritis when injected into nonautoimmune mice may actually be antinucleosome Abs). The lack of consensus regarding the definition of nephritis, as mentioned above, further muddies the water. Keeping in mind these restrictions, the autoantibodies most closely associated with nephritis are anti-DNA Abs. To be precise, only a subset of anti-DNA Abs is probably nephritogenic; they are predominantly of the IgG isotype with high affinity binding preference for dsDNA; their V regions and in particular the H-CDR3 are frequently enriched with charged residues, namely arginines and aspartic acids (reviewed in Refs. 25 and 30). One notable feature is that many of these residues have been generated by N addition and/or specific V-D rearrangements, both of which are TdT-dependent mechanisms. In B/W mice, low affinity anti-DNA IgM autoantibodies arise as a by-product of polyclonal B cell activation, possibly involving an intrinsic B cell abnormality (24, 26, 27; reviewed in Ref. 25). In the course of the disease, pathogenic high affinity anti-dsDNA seem to arise from this pool as a result of an Ag-driven expansion and maturation of a few B cell clones (24, 28, 29). Since TdT deficiency significantly reduces both the number of anti-DNA B cells and the mean affinity of anti-DNA IgM in adult normal mice (20), the probability of such B cells being efficiently activated to undergo an Ag-driven maturation may be significantly reduced in TdT° mice, resulting in the partial protection against nephritis observed in our study.

However, in this view it is surprising that long-term survivor B/W F1 TdT° mice develop similar serum levels of anti-dsDNA, particularly of the IgG2a and IgG3 isotypes, as their TdT° and TdT+ littermates with nephritis. Several hypotheses can be considered.

First, nephritogenicity and high affinity for dsDNA without proteins may be separate properties as suggested by recent publications (48–51). In support of this statement, Rubin has provided evidence that Abs to other epitopes on chromatin (especially H2A/H2B) are more highly associated with nephritis than are Abs to dsDNA in humans with lupus (52). However, in our study anti-H2A/H2B IgG serum levels were not significantly different in the three groups of mice. Other epitopes may be important, as mentioned below.

Second, the development of renal disease in B/W mice is certainly a multistep process. SCID mice populated with pre-B cells from B/W mice produce hyper gammaglobulinemia of IgM, IgG2a, and IgG3 and high titers of antinuclear autoantibodies, suggesting that these features are due to intrinsic B cell abnormalities (26, 27). It is therefore not surprising that TdT+ mice share these properties. TdT deficiency may protect against nephritis by a direct effect on the fine specificities and/or affinities of these autoantibodies or by altering the T cell repertoire. It has been suggested that polyreactivity may be a distinguishing feature of nephritogenic autoantibodies (53–56). The experimental support for this hypothesis was derived from kidney eluate Ig characterizations and from mAb studies that suggested that anti-DNA Abs could bind directly to components of the glomerular basal membrane (44, 53–54). In this respect it is noteworthy that TdT deficiency seems to particularly hamper the generation of polyreactive anti-DNA Abs (20).

Third, the reduced incidence of autoimmune nephritis in TdT° mice may be due to a lack of expression of TdT in T cells. In B/W F1 mice, the production of somatically mutated anti-DNA and antinucleosome IgG and the development of nephritis are clearly dependent on T cell help (57–59). It has been proposed these Th cells may be specific either for DNA binding proteins such as histones or for peptides derived from the variable regions of the anti-DNA Abs themselves. Particularly interesting is that their TCRs seem enriched with anionic residues generated by N addition (60, 61). Presumably, these negatively charged amino acids are critical for the binding of cationic peptides derived from DNA binding proteins or from anti-DNA Abs V regions. Consequently, we may speculate that TdT deficiency could significantly impair the generation of such TCR. Alternatively, in view of our serologic data, T cells may play a role in the development of nephritis (i.e., in an autoantibody-independent manner); this possibility is supported by data from Craft’s group showing that MRL mice deficient in αβ T cells develop immune deposits in kidney without overt glomerulonephritis (62). The identity of the disease-relevant cells remains a mystery.

The data reported here indicate that although murine lupus is a complex genetic trait with contributions from the MHC and multiple other genes, the suppression of TdT and the resulting restriction of the B and TCR diversity are sufficient to significantly protect against autoimmune nephritis. The most plausible reason is that the absence of N nucleotides considerably hampers the generation of nephritogenic autoantibodies and/or pathogenic T cells. The respective role of the T and B cells will be evaluated by adoptive transfer experiments. Interestingly, these results are almost certainly not restricted to the B/W model, since the TdT° mutation also appears to protect against insulitis when backcrossed onto the nonobese diabetic background (S. Gilfillan, unpublished observations). They suggest that TdT suppression may offer a new therapeutic strategy to prevent or control autoimmune diseases. Finally, these findings support the hypothesis that the controlled expression of TdT during the fetal and neonatal periods may be part an insurance against autoimmunity. Introducing extensive self-multireactivity and recognizing self with too much avidity at a period when the Ag receptor repertoires are mainly self-directed and when autoreactive T cells are less efficiently deleted could be deleterious.

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References


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