Biomarkers, Genomics, Proteomics, and Gene Regulation

Acquired Loss of Renal Nuclease Activity Is Restricted to DNaseI and Is an Organ-Selective Feature in Murine Lupus Nephritis

Natalya Seredkina and Ole P. Rekvig

From the Molecular Pathology Research Group, Institute of Medical Biology, Faculty of Health Sciences, University of Tromsø, Tromsø, Norway

An acquired loss of renal DNaseI promotes transformation of mild mesangial lupus nephritis into membranoproliferative end-stage organ disease. In this study, we analyzed expression profiles of DNaseI in other organs of lupus-prone (NZB×NZW)F1 mice during disease progression to determine whether silencing of the renal *DNaseI* gene is an organ-specific feature or whether loss of DNaseI reflects a systemic error in mice with sever lupus nephritis. The present results demonstrate normal or elevated levels of DNaseI mRNA and enzyme activity in liver, spleen, and serum samples from (NZB×NZW)F1 mice throughout all the stages of lupus nephritis. DNaseI activity was dramatically reduced only in kidneys of mice with sever nephritis and was the only nuclease that was down-regulated, whereas six other nucleases (DNaseII1 to 3, caspase-activated DNase, Dnase2a, and endonuclease G) were approximately normally expressed in kidneys, liver, and spleen. Loss of renal DNaseI was not accompanied by changes in serum DNaseI activity, suggesting independent mechanisms of DNaseI regulation in circulation and in kidneys and an absence of compensatory up-regulation of serum DNaseI activity in the case of renal DNaseI deficiency. Thus, silencing of renal DNaseI is a unique renal feature in membranoproliferative lupus nephritis. Determining the mechanism(s) responsible for DNaseI down-regulation might lead to the generation of new therapeutic targets to treat and prevent progressive lupus nephritis. *(Am J Pathol 2011, 179:1120–1128; DOI: 10.1016/j.ajpath.2011.05.011)*

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by production of a variety of auto-antibodies to nuclear antigens.[1] The formation and se-quential deposition of immune complexes (ICs) in visceral organs represents a basic pathogenic mechanism of the disease.[2] The potentially most severe clinical manifestation of SLE is lupus nephritis,[3] which is initiated through IC deposition in glomeruli, leading to kidney dysfunction and, finally, renal failure.[4,5] However, progression of the disease varies in intensity. Some patients experience progression from the mild mesangial form to full-blown membranoproliferative nephritis, whereas others remain with a benign mesangial pattern throughout life.[6,7]

In a recent study, we observed that lupus nephritis in female (NZB×NZW)F1 (BW) mice is a principally two-step organ disease.[8] The early phase correlates with deposition of complexes of chromatin fragments and IgG in the mesangial matrix. Progression of the disease, which is characterized by deposition of large chromatin fragments in glomerular basement membranes (GBMs) and severe proteinuria, correlates with an acquired loss of renal DNaseI mRNA and enzyme activity. Loss of DNaseI, a dominant renal nuclease,[9] correlates with reduced chromatin degradation during regular apoptosis in kidneys.[10,11] In the case of impaired clearance of apoptotic cells,[12] loss of DNaseI may explain the increased presence of chromatin fragments in membranes and matrices of affected glomeruli. Binding of such fragments to GBMs is probably most facilitated by increased expression of matrix metalloproteases 2 and 9, observed in nephritic kidneys.[13]

Expression profiles of DNaseI in other organs in BW mice during kidney disease progression have not been assayed. In sera, DNaseI is reduced in mice with lupus

nephritis.[14,15] The reason for this, however, is unclear. Either DNaseI is down-regulated in organs responsible for secreting DNaseI or serum DNaseI is reduced just because of consumption during nephritis. Therefore, it is important to verify whether silencing of the renal *DNaseI* gene is an organ-specific feature or whether it reflects a systemic error in BW mice with sever lupus nephritis.

The aim of the present study was, therefore, to determine i) whether loss of renal DNaseI is unique to the kidney or occurs simultaneously in other organs as well, ii) whether silencing of renal DNaseI is a selective process not involving other nucleases in defined stages of the disease, and iii) whether there is any compensatory up-regulation of DNaseI or other nucleases in kidneys and other organs.

In this study, we examined DNaseI expression in kidneys, livers, and spleens of BW mice at different stages of lupus nephritis. This allowed us to analyze for deviations in DNaseI levels in one strongly producing organ (liver) and in one marginally producing organ (spleen). Published data have demonstrated that the liver is the main source of serum DNaseI,[16] whereas in the spleen, the amount of DNaseI is very low.[17,18] Both organs are also responsible for clearance of circulating ICs[19,20] and participate in the pathogenesis of lupus nephritis in the case of defective chromatin degradation. Therefore, the data described in this study are important in informing whether the processes observed in the kidney are unique to DNaseI or represent global systemic errors in the body.

The present results demonstrate that the amount of renal DNaseI is reduced strictly in membranoproliferative (end-stage) nephritis and that DNaseI is the only nuclease that is down-regulated of seven analyzed nucleases [DNaseI, DNaseI1 to 3, caspase-activated DNase (CAD), Dnase2a, and endonuclease G (EndoG)]. Although dramatically reduced in the kidneys, DNaseI is not reduced in the spleen or in the liver. Expression of the other six nucleases remained largely, but not absolutely, stable in spleen and liver throughout the observation period. These data may point to mechanisms responsible for silencing of the DNaseI gene as a future causal therapeutic target to treat and prevent progressive lupus nephritis.

Materials and Methods

Animals

Female BW and BALB/c mice were purchased from Harlan Laboratories UK Ltd. (Blackthorn, UK). Ttreatment and care of the animals were in accordance with the guidelines of the Norwegian Ethical and Welfare Board for Research Animals, and the study was approved by the Institutional Review Board at the University of Tromsø.

Collection of Samples from Groups of Mice

Serum samples from BW mice were collected from pre-nephritic mice (group 1, *n* = 3), mice with mesangial nephritis (group 2, *n* = 3), and mice with membranoproliferative nephritis (group 3, *n* = 6). Sera were stored at −20°C. Degree of proteinuria was monitored weekly using sticks from Bayer Diagnostics (Bridgend, UK). Ani-

mals were euthanized by CO_2 suffocation. Kidneys, livers, and spleens were extirpated, cut, snap frozen, and i) fixed in RNAlater (Qiagen Nordic, Oslo, Norway) for further analysis of gene transcription, ii) fixed in 4% buffered depolymerized paraformaldehyde and embedded in paraffin for immunohistochemical (IHC) analysis, or iii) fixed in 8% buffered depolymerized paraformaldehyde for immune electron microscopy (IEM) (kidneys and livers only).[21] The same samples were collected from sex- and age-matched BALB/c control mice.

Double-Stranded DNA–Specific ELISA

Murine double-stranded DNA (dsDNA)–specific antibodies were detected and titrated by standard indirect enzyme-linked immunosorbent assay (ELISA), as described in detail elsewhere.[22]

IEM

IEM was performed on murine kidney and liver sections as described previously.[21] In short, sections were incubated with rabbit anti-mouse IgG (RaM IgG; Cappel, ICN Pharmaceuticals Inc., Aurora, OH), followed by protein A conjugated with 5-nm gold particles (PAG-5 nm, University of Utrecht, Utrecht, The Netherlands) to detect *in vivo*–bound IgG autoantibodies. Micrographs were taken using a Jeol JEM-1010 transmission electron microscope (Jeol Ltd., Tokyo, Japan).

IHC Analysis

Endonucleases in kidneys and livers were detected using a Polink-2 Plus horseradish peroxidase detection kit (Golden Bridge International Inc., Mukilteo, WA), as described in detail elsewhere.[10] DNaseI was detected by DNaseI-specific antibody (Santa Cruz Biotechnology Inc., Santa Cruz, CA) diluted 1:50 for kidney sections and 1:30 for liver sections. CAD and EndoG were detected by CAD-specific antibody (diluted 1:150 for kidney and 1:70 for liver) and by EndoG-specific antibody (diluted 1:100 for kidney and 1:70 for liver) from BioSite (San Diego, CA). Secondary antibodies and diaminobenzidine chromogen solution were used according to instructions provided in the kit. Sections were counterstained with hematoxylin and Scott's solution and were analyzed using an Olympus BX51 microscope (Olympus, Center Valley, PA).

Immunoexpression Score

To evaluate the degree of protein expression, we blindly quantified the intensity of staining in 10 view fields per each kidney and liver section. The immunoexpression score (IEXP-SC) was adapted from Richardsen et al[23]: IEXP-SC = AEX × ISI, where AEX is the percentage cellular area of expression and ISI is the immunostaining intensity (grades 1 to 3). The product gives a score from 0 to 300, expressed as follows: negative, IEXP-SC = 0; weakly positive, IEXP-SC = 1 to 100; moderately positive, IEXP-SC = 101 to 200; and strongly positive, IEXP-SC = 201 to 300.

Gene Expression Analysis

Total RNA was isolated using the EZ1 RNA tissue mini kit (Qiagen). The quality of RNA was analyzed using the Agilent bioanalyzer and the RNA 6000 assay kit (Agilent Technologies Inc., Santa Clara, CA). cDNA was transcribed from 2 μg of RNA using the cDNA archive kit (Applied Biosystems, Carlsbad, CA). For real-time PCR, we used the following predesigned TaqMan gene expression assays (Applied Biosystems): Dnase1, Mm01342389_g1; Dnase1l1, Mm00510102_m1; Dnase1l2, Mm00481868_g1; Dnase1l3, Mm00432865_m1; CAD, Mm00432822_m1; Dnase2a, Mm00438463_m1; EndoG, Mm00468248_m1; endogenous control–mouse actin β, 4352933E; and TATA binding protein, Mm00446973_m1. The assays were performed using the ABI Prism 7900HT sequence detection system (Applied Biosystems). Expression levels were calculated using the $\Delta\Delta C_T$ method. Data are given as fold change compared with transcription in 12-week-old mice. The protocol for analysis of DNaseI expression in spleens was modified owing to low expression levels. To detect mRNAs, we had to use a two times higher concentration of cDNA than we used for kidney and liver samples.

DNase Radial Diffusion Assay to Determine Activity of Neutral pH–, Ca^{2+}-, and/or Mg^{2+}-Dependent Endonucleases

To evaluate DNase activity in kidneys, livers, spleens, and sera, a DNase radial diffusion assay was performed as described elsewhere.[24] Ten milligrams of tissue was homogenized in DNase reaction buffer (40 mmol/L Tris, pH 7.6, 2 mmol/L $CaCl_2$, and 2 mmol/L $MgCl_2$) with 0.1% Triton X-100 (Roche Diagnostics GmbH, Mannheim, Germany). Cell debris was removed by centrifugation at 13,000 × g, and supernatant was stored at −80°C. Total protein amount was measured using BCA assay (Pierce Biotechnology, Rockford, IL). For analysis, tissue samples were thawed on ice, protein concentration was normalized for every tissue to be equal in all samples, and 2.5-μL aliquots of tissue lysates were loaded into 1-mm wells in a 1% agarose gel containing 50 μg/mL (for livers and spleens) or 100 μg/mL (for kidneys and sera) of calf thymus DNA (Sigma-Aldrich, St. Louis, MO) and 1 μg/mL of ethidium bromide in DNase reaction buffer. Serum samples were loaded directly to the gel after defrosting on ice. Recombinant human DNaseI from Amersham Biosciences (Piscataway, NJ) was used as a standard. The gel was incubated in a humidified chamber at 37°C for 17 hours and was photographed under UV illumination.

Denaturing SDS-PAGE Zymography

DNaseI activity in the kidneys, livers, and sera was determined after protein separation in a 10% SDS–polyacrylamide gel containing 40 μg/mL of heat-denatured salmon sperm DNA (Invitrogen, Carlsbad, CA) as described elsewhere.[25]

Native PAGE Zymography

To analyze DNaseI in spleens, we also performed the more sensitive native PAGE zymography instead of SDS-PAGE.[16] DNaseI activity was determined after protein separation in 7.5% polyacrylamide gels containing 30 μg/mL of heat-denatured salmon sperm DNA (Invitrogen) as described in detail elsewhere.[16,17] Liver samples were analyzed by SDS-PAGE and native PAGE zymographies, and both techniques demonstrated similar results in liver tissue.

Statistical Analysis

One-way analysis of variance with Dunnett's post hoc test was used in this study. Differences were considered significant at $P < 0.05$.

Results

Analyses of Serum dsDNA-Specific Antibodies and Renal and Liver Morphologic Characteristics in BW Mice

To investigate the DNaseI mRNA level and enzyme activity in different organs of BW mice, we divided female BW mice into three groups according to age, DNA-specific antibody titer, and disease severity (Table 1). Group 1 represents young, nonproteinuric, dsDNA-specific antibody–negative BW mice. IEM analysis of corresponding kidney sections did not demonstrate any morphologic changes (Figure 1 and Table 1). Group 2 is composed of nonproteinuric animals with variable titers of serum ds-DNA-specific antibody and deposits of ICs, seen as electron-dense structures (EDSs) containing IgG in the glomerular mesangial matrix (Figure 1 and Table 1). Nephritic BW mice with severe proteinuria, circulating dsDNA-specific antibodies, and EDSs with IgG in the mesangial matrix and in GBMs represent group 3 (Figure 1 and Table 1). Liver tissue of animals from each group was also analyzed by IEM. We did not find any immune deposits or EDSs in liver capillary and arterial walls in any of the mice included in this study (Table 1 and Figure 1). Sex- and age-matched BALB/c mice were used as control mice. They were all negative for DNA-specific antibodies and did not have any immune deposits in kidneys or livers (data not shown).

DNaseI Expression in Kidneys, Livers, Spleens, and Sera of BW Mice

Relative DNaseI gene expression levels in different organs from mice in groups 1 to 3 were analyzed by real-time PCR, corresponding enzyme activity, and protein expression. mRNA levels of DNaseI (columns) and enzyme activity (gels) in individual mice were determined in kidneys, livers, and spleens (Figure 2, A–C).

DNaseI mRNA is normally expressed in prenephritic kidneys (group 1; Figure 2A) and in kidneys from mice with mesangial nephritis (group 2; Figure 2A); however, DNaseI mRNA is dramatically reduced in kidneys from

Table 1. Laboratory Variables and Morphologic Characteristics of BW Mice Included in This Study

Mouse group no.	Mouse no.	Age (weeks)	Anti-DNA antibody titer	Proteinuria*	EDSs		
					MM	GBM	Liver
1	1	12	0	−	−	−	−
	2	12	0	−	−	−	−
	3	12	0	−	−	−	−
2	4	30	100	1+	+	−	−
	5	30	1400	−	+	−	−
	6	30	3000	−	+	−	−
3	7	32	800	4+	+	+	−
	8	26	200	4+	+	+	−
	9	32	200	4+	+	+	−
	10	27	500	4+	+	+	−
	11	24	400	4+	+	+	−
	12	24	400	4+	+	+	−

*Proteinuria was determined by urine stix: 0 to 1+, ≥0.3 g/L (regarded as physiologic proteinuria); 2+, ≥1 g/L; 3+, ≥3 g/L; and 4+, ≥20 g/L.
GBM, glomerular basement membrane; MM, mesangial matrix.

group 3 BW mice (Figure 2A). The low DNaseI mRNA level in group 3 mice is also reflected by low enzyme activity as determined by zymography (Figure 2A) and by weak protein expression in kidneys as determined by IHC analysis (Figure 2E). Low DNaseI expression was presented uniformly in glomeruli and tubuli in kidney sections from group 3 mice.

To analyze whether silencing of the DNaseI gene in nephritic BW mice is an organ-specific feature, mRNA levels were determined in livers and spleens of the same BW mice. The data demonstrate that DNaseI mRNA expression is not reduced in livers (Figure 2B) or in spleens (Figure 2C) from all groups of mice. These results compare well with assays on DNaseI enzyme activity as analyzed by gel zymography (Figure 2, B and C, for livers and spleens, respectively) and IHC analysis of livers for DNaseI protein expression *in situ* (Figure 2E). DNaseI mRNA level and enzyme activity in livers in group 2 and in spleens in groups 2 and 3 tended to be higher compared with group 1 results (Figure 2, B and C, for livers and spleens, respectively). This finding was statistically significant, however, only for DNaseI mRNA levels in spleens from group 2 mice. IHC analysis of DNaseI on liver sections reflects the results of DNaseI mRNA levels (Figure 2E). Quantification of the staining intensity of DNaseI in kidney and liver sections of BW mice demonstrates loss of DNase I in kidneys but not in livers (see Supplemental Figure S1 at *http://ajp.amjpathol.org*).

The data in Figure 2D demonstrate no reduction in serum DNaseI enzyme activity, as determined in group 3 compared with group 1 and 2 mice. Zymograms of serum samples exhibit a pattern similar to that seen in zymograms of liver lysates, in agreement with a report showing that liver is a main source of serum DNaseI.[16]
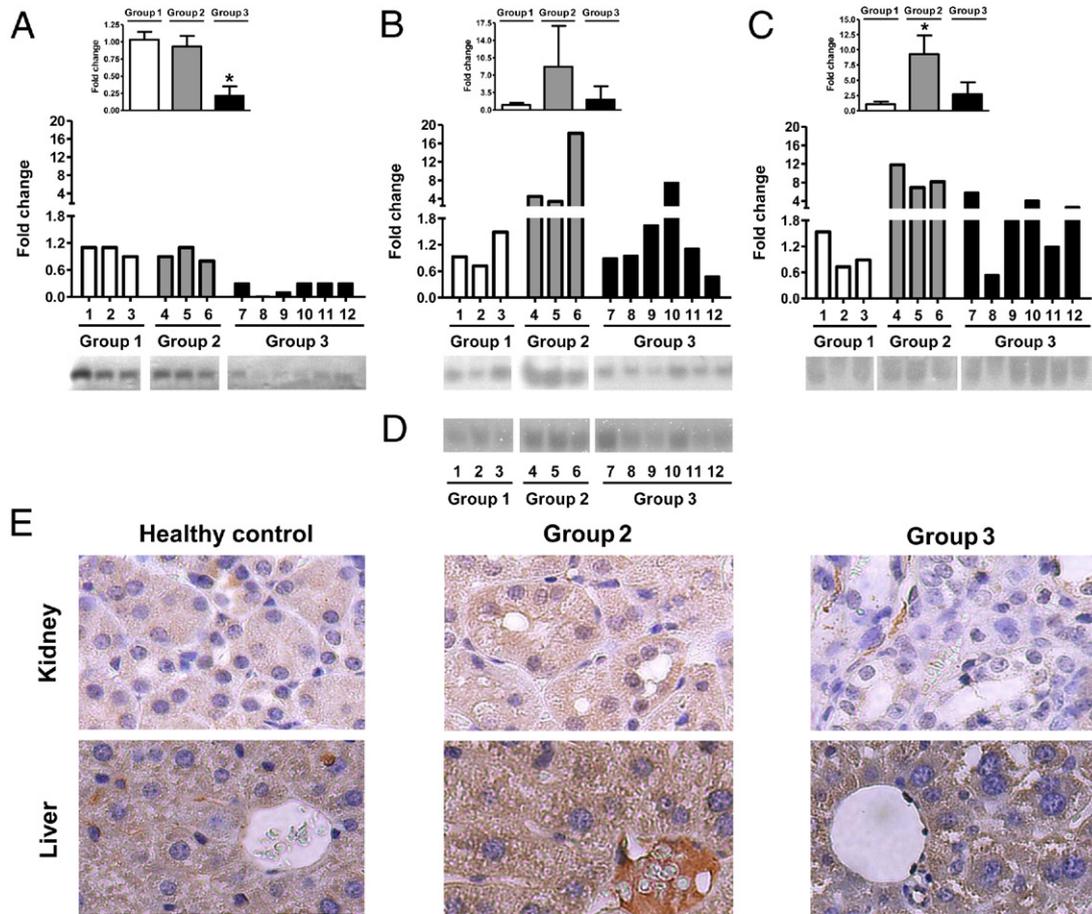
The mRNA level and enzyme activity of renal DNaseI in age- and sex-matched BALB/c mice are presented in Supplemental Figure S2A (available at *http://ajp. amjpathol.org*). As demonstrated, DNaseI is expressed at stable levels in all groups of control animals. Delta C_T values and calculated fold changes of renal DNaseI mRNA levels in examined groups of BW and BALB/c mice are shown in Supplemental Table S1 (available at *http://ajp.amjpathol.org*).

Total Nuclease Activity in Kidneys, Livers, Spleens, and Sera of BW Mice

Results of DNaseI zymography led us to analyze whether DNaseI levels affect total nuclease activity in kidneys, livers, spleens, and sera of BW mice during progression of lupus nephritis. We observed nuclease activity, as determine by a single radial diffusion nuclease assay, in kidneys, livers, spleens, and sera at different stages of the disease (Figure 3). Calculated group mean ± SD values are given as insets in each
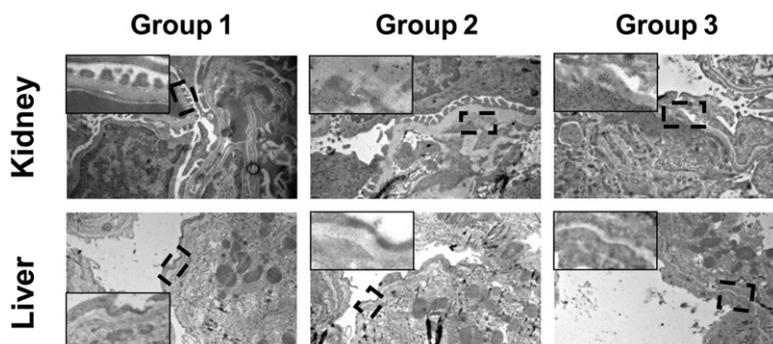

Figure 1. IEM analyses of EDSs in kidneys and livers from BW mice at different stages of lupus nephritis. BW mice were divided into three groups according to kidney morphologic features (**top panels**): group 1, mice without EDSs in glomeruli; group 2, animals with EDSs in mesangium; and group 3, mice with EDSs in the mesangial matrix and in GBMs. *In vivo*–bound IgGs are traced by 5-nm gold particles. No EDSs were found, however, in arterial or capillary walls in corresponding liver sections in BW mice in groups 1 to 3 (**bottom panels**). **Insets:** Magnified areas of basement membranes included in the dashed boxes.

Figure 2. DNaseI expression in organs and sera of BW mice at different stages of lupus nephritis. The mRNA levels (columns) and enzyme activity (gels) of DNaseI are determined in kidneys (**A**), livers (**B**), and spleens (**C**) of individual BW mice in prenephritic group 1 mice and in mice with mesangial nephritis (group 2) or with membranoproliferative nephritis (group 3). **Insets:** Mean ± SD DNaseI mRNA levels in each group for each organ. *$P < 0.05$ versus group 1. The mRNA levels of DNaseI in livers and spleens of BW mice with severe nephritis (group 3) are not reduced, whereas they are dramatically low in kidneys of the same animals. The enzyme activity of DNaseI measured by SDS-PAGE zymography for kidneys and livers and by native PAGE zymography for spleens correlates well with results of mRNA levels. **D:** The DNaseI activity in sera of individual BW mice in the different groups of lupus nephritis, as measured by SDS-PAGE zymography, is similar to the expression patterns of DNaseI activity in livers and is not reduced in group 3 BW mice. **E:** IHC analyses of DNaseI expression in kidney (**top panels**) and liver (**bottom panels**) sections in group 2 and 3 BW mice is demonstrated compared with expression in age-matched controls. Expression of DNaseI in tubular cells of BW mice with severe nephritis (group 3) is nearly undetectable, whereas it is normally present in liver sections in the same animals. Original magnification, ×200.

panel. The results demonstrate that there is no decrease in total nuclease activity among groups 1, 2, and 3 in livers or spleens of BW mice, whereas total nuclease activity in kidneys of animals with severe nephritis (group 3) is markedly reduced compared with that in mice from groups 1 and 2. In serum samples (Figure 3D), total nuclease activity was not reduced during lupus nephritis progression, similar to the re-
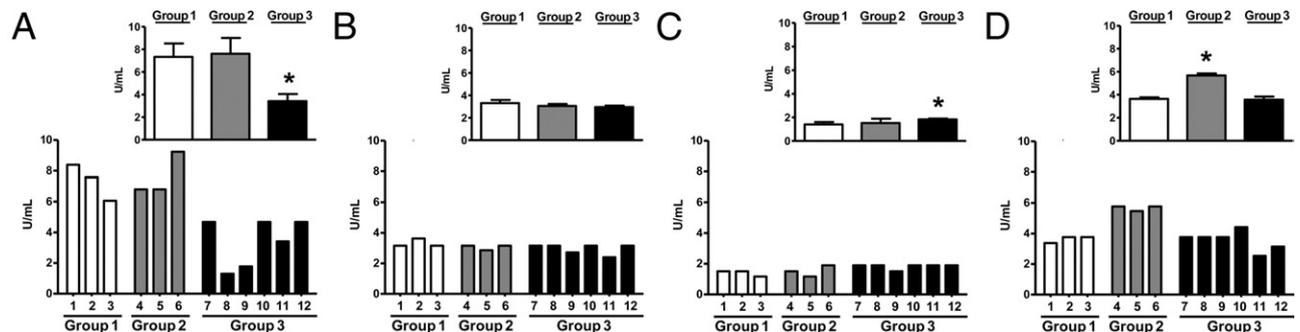
Figure 3. Total nuclease activity in organs and sera of BW mice at different stages of lupus nephritis. Nuclease activity in kidney (**A**), liver (**B**), spleen (**C**), and serum (**D**) samples of individual BW mice (**bottom row**) is measured by a single radial diffusion nuclease assay. **Top row:** Mean ± SD of total nuclease activity in each group for each organ. *$P < 0.05$ versus group 1. In kidneys only, nuclease activity is markedly reduced in group 3 BW mice. Data are given as DNaseI unit equivalents.
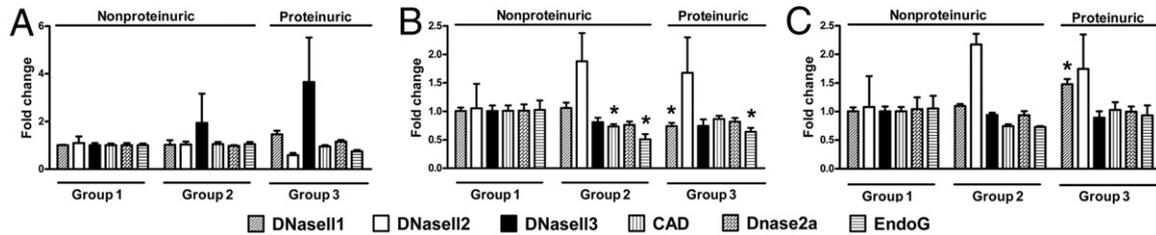
Figure 4. Expression profiles of DNaseII1 to 3, CAD, Dnase2a, and EndoG in BW mice during progression of lupus nephritis. The mRNA levels of different nucleases are determined in kidney (**A**), liver (**B**), and spleen (**C**) samples in prenephritic group 1 mice, in mice with mesangial nephritis (group 2), and in mice with membranoproliferative lupus nephritis (group 3). Values are given as mean ± SD. *$P < 0.05$ versus group 1. No significant changes in transcription levels of DNaseII1 to 3, CAD, Dnase2a, and EndoG were observed in kidneys in BW mice at different stages of lupus nephritis.

sults of DNaseI zymography on serum samples shown in Figure 2D.

Expression Profiles of Other Nucleases in Kidneys, Livers, and Spleens from Group 1 to 3 BW Mice

We measured gene expression levels of other endonucleases by real-time PCR applied to kidneys, livers, and spleens to analyze for two possible phenomena: selectivity of DNaseI down-regulation compared with the other nucleases and possible compensatory changes of other nucleases in all three organs in situations where the important renal *DNaseI* gene is shut down. Figure 4 demonstrates mRNA levels of DNaseII1 to 3, CAD, Dnase2a, and EndoG in kidneys, livers, and spleens of BW mice in groups 1 to 3. The mean of each nuclease mRNA level in each group of mice is presented for each organ. No significant difference is found in the transcriptional levels of any of the nucleases in kidneys, livers, or spleens, except for those indicated by an asterisk in Figure 4, B and C, compared with prenephritic (group 1) mice. IHC analysis performed on kidney (Figure 5A) and liver (Figure 5B) sections confirmed the results of real-time PCR on CAD and EndoG, as the staining intensity was consistently similar in all groups of mice. Quantitative scoring analysis of staining intensity of CAD and EndoG on kidney and liver sections showed stable expression throughout the observation period (see Supplemental Figure S1, A and B, respectively, at *http://ajp.amjpathol.org*). Expression profiles of renal DNaseII1 to 3, CAD, Dnase2a, and EndoG in age- and sex-matched BALB/c mice showed largely stable values for all endonucleases studied throughout the observation period (see Supplemental Figure S2B at *http://ajp.amjpathol.org*).

Discussion

The role of decreased DNaseI in the pathogenesis of SLE has been discussed for decades. DNaseI was seen as a promising target for substitutive therapy to prevent autoimmunity,[26] but it has been discredited after insufficient results of the trial treatment.[27] In a single study by Macanovic et al,[26] injection of DNaseI in prenephritic BW mice postponed the disease, whereas treating nephritic animals decreased levels of proteinuria and serum cre-

atinine. However, these observations were not followed by further penetrating studies that would have provided insight into the therapeutic effect of DNaseI treatment. In another study, administration of DNaseI in BW mice did not affect development or progression of lupus nephritis.[28] Similarly, intravenous or subcutaneous administration of recombinant human DNaseI in patients with lupus nephritis classes III to V did not show any effect on kidney function or activity of the disease.[27] This finding may signify that serum DNaseI has little or no influence on degradation of chromatin in dying renal cells.[29] Only intracellular renal DNaseI initiates and ensures a safe and effective degradation of chromatin in dying cells in the kidneys.[29]

Tubular cells dominate among renal cells that express DNaseI (Figure 2E),[9,15,30] but DNaseI is also expressed in mesangial cells (N. Seredkina, unpublished data).[15] Detection of apoptotic cells in kidney sections demonstrated that tubular and glomerular cells undergo apoptosis.[10,11,21] Reduced DNaseI expression is furthermore
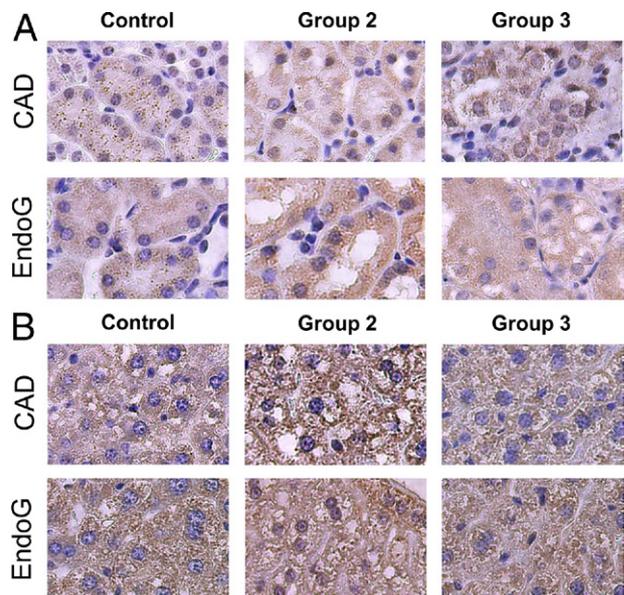
Figure 5. IHC analyses of CAD and EndoG in kidneys (**A**) and livers (**B**) from BW mice. Expression of CAD (**top panels**) and EndoG (**bottom panels**) proteins was determined in kidney (**A**) and liver (**B**) sections of BW mice in groups 2 and 3 and compared with data obtained in BALB/c control mice. No difference in nuclease expression was observed in organs of BW mice with severe nephritis compared with control animals. Original magnification, ×200.

observed uniformly in glomerular and tubular cells in BW mice with severe nephritis.[15] An acquired loss of renal DNaseI in lupus nephritis may, therefore, be the event that results in reduced chromatin fragmentation in a dominant proportion of renal cells, resulting in exposure of large chromatin fragments in the kidneys. Such fragments participate in the formation of ICs in GBM and, thus, account for progression of lupus nephritis into end-stage organ disease.

To better understand the role of DNaseI expression in progressive lupus nephritis, it became important to determine three aspects of this phenomenon: i) Is shutdown of renal DNaseI unique to DNaseI, or do other renal nucleases behave in a similar manner? ii) Is renal DNaseI shutdown unique to kidneys, or is DNaseI down-regulated in other organs as well? iii) Are other nucleases up-regulated in compensation in situations where DNaseI is lost in the kidneys?

Data in the present study provide answers that explain all these aspects. For the first, data confirm that renal DNaseI is lost when lupus nephritis transforms mild into severe membranoproliferative organ disease.[8,15] In expression profiles of seven nucleases analyzed in kidneys, livers, and spleens, highly reduced nuclease expression is restricted to DNaseI, in agreement with a recent preliminary observation.[15] Furthermore, the DNaseI shutdown was restricted to the kidneys of BW mice with severe nephritis because expression seemed normal in livers and spleens of the same individual animals. No clear tendency for compensatory up-regulation of other nucleases involved in chromatin degradation was observed in livers and kidneys, whereas DNaseII1 was slightly up-regulated in spleens of group 3 BW mice.

Serum DNaseI activity in the different groups of mice correlated with DNaseI activity in the liver but not in the kidney, in accordance with other results.[16] There are several reports about reduced serum DNaseI activity in patients with SLE [31–34] and in lupus-prone mice.[14,15] All of those analyses are based on measurement of nuclease activity in sera by radial diffusion assay, which is a sensitive but not a DNaseI-specific method. Published data indicating the correlation of serum DNaseI activity with activity of SLE or with renal involvement in SLE remain, however, controversial. The first study, by Chitrabamrung et al,[31] of 36 patients with SLE demonstrated that individuals with active lupus (including active lupus nephritis) had the lowest levels of enzymatic activity compared with those in healthy volunteers or in patients with rheumatoid arthritis and scleroderma. This observation was challenged by Sallai et al,[33] who demonstrated that serum DNaseI did not correlate with disease activity in 113 patients with SLE with or without lupus nephritis. Moreover, activity of serum DNaseI in patients with SLE was not reduced compared with enzymatic activity in patients with undifferentiated connective tissue disease.[33] Data in the present study demonstrate that total serum nuclease activity, and selective serum DNaseI activity, was not reduced in BW mice during progression of lupus nephritis. Thus, serum DNaseI may not be important for the kidneys in a situation where renal DNaseI is lost. This may also explain why injection of DNaseI does not affect the activity of lupus nephritis in patients.[27] This leaves us with the perception that renal intracellular DNaseI is required for safe degradation and elimination of chromatin from dying renal cells. Without this enzyme, chromatin degradation is impaired and large chromatin fragments accumulate *in situ,* where they are released from dying cells. This may, indeed, be one of the factors that results in impaired clearance of apoptotic secondary necrotic cell debris.[35–38]

We found increased DNaseI mRNA expression and enzyme activity in liver and spleen in the early and late stages of lupus nephritis. The observed increase in DNaseI expression in those organs was associated with high titers of dsDNA-specific antibodies in serum. Because one common function of liver and spleen is uptake of circulating ICs, we assume that DNaseI participates in chromatin degradation of cleared ICs. This may eventually explain increased DNaseI expression in these organs.

Selective loss of renal DNaseI creates a basis for *in situ* deposition of chromatin, a phenomenon independently demonstrated in different experimental nuclease deficiencies. In several such studies, it is demonstrated that chromatin fragments accumulate *in situ,* where they are released from dead cells,[39] instead of entering the circulation.

The present results add weight to the assumption that DNaseI shutdown is secondary to early dsDNA-specific antibody–mediated deposition of ICs in the mesangial matrix.[8] This may mean that inflammation linked to (silent or mild) mesangial nephritis in fact imposes the loss of renal DNaseI, with the consequences for renal function as described herein. This proposed mechanism is in agreement with results of high-resolution techniques to determine the nature of target structures in the glomeruli. These structures appear as EDSs by transmission electron microscopy and are composed of chromatin fragments and IgG molecules, as determined by different forms of IEM and by co-localization TUNEL IEM assay.[40–42]

Comparative studies of components and localization of EDSs in skin and glomeruli in SLE demonstrated that they are composed of similar chromatin-related structures.[43,44] Deposition of ICs in glomeruli did not, however, predict deposition in skin.[43,44] Examination of DNaseI expression in skin of MRL-lpr/lpr and BW mice revealed stable DNaseI activity during the development of lupus nephritis.[44] Those results indicate that chromatin in chromatin-IgG complexes that deposit in skin have another origin than from skin itself. In harmony with this, published data demonstrate the presence of polyomovirus large T antigen in extracellular chromatin associated with glomerular and skin membranes,[43] suggesting that chromatin fragments in kidneys and skin might derive from polyomavirus-infected renal cells because renal polyomavirus infection is a frequent finding in patients with lupus nephritis.[22] Further investigations are needed to confirm whether chromatin fragments found in skin EDSs of patients with SLE may have a renal origin.

The precise mechanism by which the expression of renal DNaseI shuts down is unknown but is currently being analyzed in the Molecular Pathology Laboratory at the University of Tromsø. Contemporarily, we are following three lines of analyses: i) transcriptional interference

with a convergently encoded gene (*Trap1*) that overlaps with the *DNaseI* gene in their 3′ untranslated region and ii) regulation by microRNAs or iii) by DNA methylation. The initial event accounting for DNaseI shutdown may, however, represent a response to inflammatory signals provided by the early mesangial nephritis. This would be in favor of transcriptional interference with the *Trap1* gene because *Trap1* is up-regulated during stress and inflammation.[45,46] Therefore, we currently perform *in vitro* studies using cultures of primary mesangial and tubular cells exposed to autoantibodies, nucleosomes, ICs, and proinflammatory cytokines to determine the impact of the inflammatory mesangial nephritis environment on DNaseI expression in renal cells. We also analyze the impact of albumin overload on the ability of tubular cells to express DNaseI because its contribution to renal proximal cell apoptosis has been reported.[47,48] Furthermore, microRNAs that target DNaseI mRNA have been identified in the mesangial nephritis and in end-stage organ disease in the Molecular Pathology Laboratory at the University of Tromsø (studies in progress).

From the data presented herein, adding to previous results,[8,10,11,15] we may conclude that the processes accounting for transformation of mild mesangial nephritis into severe end-stage organ disease is linked to events in the kidneys and is selective for the kidneys. This event is regulated by renal DNaseI shutdown.

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