Prominent Renal Expression of a Murine Leukemia Retrovirus in Experimental Systemic Lupus Erythematosus

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Abstract. A role for retroviruses in human systemic lupus erythematosus (SLE) and in mouse lupus models such as the New Zealand Black and White mice (NZB/W) strain has been postulated. This study compared the gene profile of nephritic NZB/W kidney with nondiseased NZW controls. The most highly upregulated gene (5.5-fold) hybridized with an expressed sequence tag on a cDNA microarray, which was sequenced and found to correspond with an endogenous murine retrovirus related to the Duplan strain (EDV, L08395). NZB/W kidney contained the full-length 4.2-kb EDV transcript. By 4 wk of age in NZB/W mice, an age preceding renal histologic disease, the EDV transcript was more than threefold increased relative to NZB or NZW control strains. This upregulated expression tended to fall with progression of renal histologic disease. By in situ hybridization, the EDV transcript was highly expressed in tubules of NZB/W mice. There was also upregulated expression of EDV transcript in NZB/W lung and brain, sites of inflammation in this strain, but not in spleen or liver. Thus, using microarrays, the most highly expressed gene in mouse lupus nephritis corresponded to an endogenous retrovirus. This retroviral transcript was highly expressed in the kidneys of lupus mice and tended to decline with advancement of disease. The remarkable upregulation of the EDV transcript only in the setting of active disease suggests this transcript is involved in inflammatory disease.

Human systemic lupus erythematosus (SLE) is a multiorgan autoimmune disease characterized by the loss of tolerance to self antigens and the production of autoantibodies (1). Several spontaneous murine models of human SLE have been developed, beginning with the identification in 1959 of New Zealand Black mice (2). The most extensively studied of these models are the female F1 cross between New Zealand Black and White mice (NZB/W) and MRL/Mp-Fas−/− (MRL/lpr) mice (2,3). Studies of these mice have contributed greatly to elucidation of SLE pathogenesis. NZB/W mice exhibit several autoimmune features and mild glomerulonephritis (GN) late in life. NZW mice exhibit only minimal autoimmunity, but they contribute several genes that are critical to the profound SLE-like disease phenotype found in NZB/W mice (4,5). MRL/lpr mice differ from the lupus-prone congenic MRL/+ strain by the nearly complete absence of the membrane Fas protein, necessary for apoptosis, which is due to a retroviral insertion in the Fas gene (6). As in human SLE, mice of these strains also develop elevated levels of IgG autoantibodies to nuclear antigens, including anti-double stranded DNA antibodies (Abs) (2).

NZB/W mice develop severe proliferative GN (2). The earliest changes occur before 20 wk of age and include accumulation of immune complexes and proliferation within the mesangial region. Later in the course of disease, immune complexes localize in the peripheral capillary loops, basement membrane thickening occurs, and there is proliferation of intrinsic glomerular cells leading to obliteration of capillary lumina (7). With the severe glomerular injury, crescent formation occurs. Ultimately, glomerulosclerosis occurs in the terminal phase of disease. NZB/W mice also have tubular basement membrane immune deposits and develop tubulointerstitial nephritis as in human lupus nephritis (2,8).

Although animals of the various lupus strains are thought to die from renal failure (7), this has come into question from recent studies investigating the effects of intercellular adhesion molecule-1 deficiency in MRL/lpr mice. This prevented mortality apparently by protecting against pulmonary inflammation while not affecting the renal disease (9). In addition to pulmonary involvement (9,10), central nervous system pathology also occurs in lupus mice, as is true in human SLE (11,12).

A considerable amount of evidence has been accumulated linking viruses and particularly retroviruses to the pathogenesis of SLE. Postulated roles for retroviruses in SLE include as intrinsic or mimicry antigens and/or as stimulants of autoimmunity (13–15). Among the best-characterized retroviruses and their products are the inserted retrotransposon in the Fas gene (6) and the endogenous retroviral envelope glycoprotein, gp70, to which intrinsic Abs are directed. Immune complexes composed of gp70-anti-gp70 deposit in glomeruli of mice with SLE (16–19). Murine leukemia viruses (MuLV) have also
been implicated in human and murine autoimmune diseases (13,20).

Given the completion of the first draft of the human genome and the progress made with mouse genes (21,22), the ability to determine expression of a diversity of genes in different tissues at various times and under normal and abnormal conditions is now possible. Traditional approaches, such as Northern analyses, RT-PCR, and RNase protection assays, are limited in their capacity. Thus, the technique of massively parallel DNA analysis has been developed over the past decade (23). Here, we used this technique to screen for relevant genes in murine lupus nephritis. With this approach, we identified a MuLV that was highly expressed in murine lupus and was characterized in detail.

Materials and Methods

Mice

NZB, NZW, and NZB/W mice were obtained from Jackson Laboratories (Bar Harbor, ME). In initial studies, 20 female NZB/W and 15 female NZW mice were obtained at 8 wk of age and followed longitudinally. Blood was obtained monthly for determination of blood urea nitrogen (BUN) levels as measured by a Beckman Analyzer (Fullerton, CA). Groups of three animals were sacrificed every 8 wk starting at 16 wk of age to harvest tissue for RNA isolation and histologic studies. In subsequent studies, animals along with strain controls were sacrificed at the indicated times.

Histologic Studies

Kidneys were removed and divided into sections snap frozen for immunofluorescence (IF) microscopy and fixed in 10% buffered formalin for light microscopic evaluation. Four-micrometer cryostat sections were processed for direct IF microscopy using FITC-conjugated Abs to mouse C3 and IgG (Cappel Laboratories, Durham, NC). For light microscopy, 4-μm sections stained with periodic acid Schiff were provided as coded slides to a renal pathologist (MH) who was blinded to the origin of each section. For each slide, the extent of GN was graded semiquantitatively on a scale from 0 to 4 according to the schema of Passwell et al. (24).

RNA Isolation

Total RNA was isolated from various tissues by a single-step guanidinium isothiocyanate-phenol-chloroform extraction (Trizol Reagent; Life Technologies, Gaithersburg, MD) (25). For microarray studies, poly(A+) RNA was purified by oligo-dT affinity chromatography (26).

Microarray Studies

One-microgram poly(A+) RNA from NZW and NZB/W renal cortices were fluorescently labeled with Cy3 and Cy5, respectively, during the reverse transcription reaction to produce cDNA. Labeled targets were then hybridized with 7854 mouse genes and expressed sequence tags (EST) incorporated into Mouse GEM 1 microarrays (Incyte Genomics, Palo Alto, CA). Expression data were normalized to internal controls to account for variable intensity of the two fluorophores and a balanced differential expression ratio provided for each gene. In 50 genes that are reasonable candidates as “housekeeping” genes, the balanced differential expression ratio was 1.0 to 1.3, validating this approach.

Reverse Transcriptase–PCR

cDNA was produced from 5 μg of total RNA by RT using oligo-dT primers (Superscript Preamplification System, Life Technologies). Subsequent PCR was performed in tubes containing 1/200 of the generated cDNA, 50 mM KCl, 20 mM Tris-HCl, 2.5 mM MgCl2, 100 μM of deoxynucleotide triphosphate, 0.1 μM of each primer, and 2.5 U of Taq polymerase. The primers used were: 5′-AGAGAGAGGCG-CGAAACGTC-3′ and 5′-GGCAATGCAGACCTCTCAA-3′; spanning bases 2447 to 2750 of the nucleotide sequence for L08395 (discussed further below). Twenty-five cycles of a 1-min denaturation at 94°C, 1-min annealing at 60°C, and 1-min extension at 72°C were performed. In pilot studies, this input of cDNA and number of cycles was limiting in all instances. To quantify the PCR product synthesized and to control for the integrity of RNA, PCR was simultaneously performed using primers for glyceraldehyde-3-phosphate dehydrogenase (G3PDH) (27). For these reactions, 1/50 of the cDNA generated by RT from 5 μg of RNA input was subjected to 30 cycles of PCR, which was also in the linear range of product accumulation. In all cases, negative controls in which RT was omitted but samples were otherwise handled identically (i.e., subjected to PCR) were included.

Products generated by RT-PCR were electrophoresed through agarose gels and stained with ethidium bromide. Gels were photographed under UV light illumination. Photographs were scanned into TIFF, and band intensity was analyzed using NIH IMAGE software (Scion Corp., Frederick, MD). In each instance where comparisons were made, RT-PCR was performed contemporaneously and products were electrophoresed within the same gel.

Kinetic Real-Time PCR

One μg of total RNA from renal cortex was subjected to RT as described above. Subsequently, kinetic PCR was performed with the following primers: 5′-ACTGGGAGCGCTGATACGATG-3′ and 5′-GATAGCTGAGGGTGGAGC-3′, spanning bases 2548 to 2669 of L08395; the labeled probe was 5′-[FAM]CCTGCAAGGAAAAACCT-GT AA-[TAMRA]′ (bases 2587 to 2606 of L08395). Included in the same tube were primers and probe for G3PDH: 5′-GGCAATAT-TCACGGCCACGT-3′, 5′-AGATGTGATGGCTTCC-3′, and 5′-[FAM]AAAGGCGAAATGGGAAAGCGTC-3′-[TAMRA]′. Reactions were performed using a Cepheid Smart Cycler System (Sunnyvale, CA). Concentration curves were determined for both L08395 and G3PDH using serially diluted NZB/W renal cortical RNA. Concentrations of L08395 and G3PDH were calculated from the respective curves. The relative concentration of L08395 was obtained by normalizing with G3PDH for each sample.

Northern Analyses

Twenty-five micrograms of total RNA from renal cortex was subjected to electrophoresis through a denaturing agarose gel. RNA was transferred to a nylon membrane by capillary action and crosslinked to the membrane by UV irradiation. As probe, the 303-bp IMAGE EST clone 522713 in kidney. This clone in pBluescript SK– vector was linearized with EcoRI, and labeled riboprobes were syn-
thesized with T7 (antisense probe) or T3 RNA polymerase (sense probe) using digoxigenin uridine-triphosphate as substrate according to manufacturer’s instructions (Genius RNA labeling kit; Boehringer Mannheim, Mannheim, Germany). Four-micrometer cryostat sections from NZB/W and NZW mice were fixed in 4% paraformaldehyde in PBS. Subsequently, sections were treated with protease K (10 μg/ml) for 4 min at 37°C. This reaction was stopped with 0.1 M glycine in PBS for 10 min at room temperature. Sections were dehydrated sequentially with 70%, 95%, and 100% ethanol, air-dried, and then prehybridized in a humidified chamber for 1 h in hybridization buffer (50% deionized formamide, 4× SSC, 1× Denhardt’s solution, 500 μg/ml heat-denatured herring sperm DNA, 250 μg/ml yeast tRNA, and 10% dextran sulfate; all from Sigma Aldrich, St. Louis, MO). Sections were rinsed in 2× SSC and incubated overnight at 42°C with 100 μl of hybridization buffer containing 300 ng/ml labeled cRNA probe. The following washes were then performed: 2× SSC for 1 h at room temperature, 1× SSC for 30 min at room temperature (done twice), 0.5× SSC for 30 min at 42°C, and 0.5× SSC for 30 min at room temperature. Colorimetric detection of the bound labeled cRNA probe was performed using the Genius Nonradioactive Nucleic Acid Detection Kit (Boehringer Mannheim). Slides were washed in buffer 1 (100 mM Tris-HCl, 150 mM NaCl, pH 7.5), blocked with 2% normal sheep serum and 0.3% Triton X-100 at room temperature and incubated overnight at 4°C with anti-digoxigenin Ab conjugated with alkaline phosphatase (1:500 dilution). Slides were washed sequentially in buffer 1 and buffer 2 (100 mM Tris-HCl, 100 mM NaCl, 50 mM MgCl₂, pH 9.5) and then incubated at room temperature with developing solution (45 μl nitroblue tetrazoilum salt (5-bromo-4-chloro-3-indolyl phosphate, mono-(p-toluidium) salt, 50 mg/ml in dimethylformamide], 35 μl X-phosphate solution [5-bromo-4-chloro-3-indolyl phosphate, mono-(p-toluidium) salt, 50 mg/ml in dimethylformamide], and 2.4 mg of levamisole [Sigma] in 10 ml of buffer 2). The color reaction was stopped with buffer 3 (10 mM Tris-HCl, 1 mM EDTA, pH 8). After dehybridization in a series of graded ethanols, slides were mounted with coverslips and observed under the microscope. Serial sections hybridized with sense probes served as controls.

**Sequencing**

The nucleotide sequence of IMAGE EST clone 522713 in pBluescript SK™ was determined on an ABI 373A DNA Sequencer using the Big Dye Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, CA). The initial round of sequencing was done with T3 and T7 primers flanking the insert and thereafter with primers designed from the derived sequence. Sequence comparisons were made with Genetics Computer Group software (Madison, WI).

**Statistics Analyses**

Statistical analyses were performed with Minitab software (State College, PA). All data are expressed as mean ± SEM. Two-sample t testing and one-way ANOVA followed by Fisher’s pairwise comparisons were used. P values < 0.05 were taken as indicating statistical significance.

**Results**

**Longitudinal Analysis of Renal Disease in NZB/W Mice**

To examine the course of renal disease occurring over time in NZB/W mice, we followed a cohort of NZB/W mice. At times ranging from 16 to 52 wk of age, animals were sacrificed for detailed histologic analyses. An age-matched group of NZW mice was followed contemporaneously as a control. As expected, animals developed renal disease starting with mesangial deposition of immune complexes and complement, along with proliferation of cells in the mesangial region (Figure 1, A through C). With advancing age, immune complex and complement deposits were present in peripheral capillary loops of glomeruli, which were associated with endocapillary cell proliferation (Figure 1, D through F). In some animals, extra-capillary proliferation (glomerular crescents) and foci of tubulointerstitial inflammation were also present. Animals that died spontaneously had renal failure (BUN, 166.2 ± 54.6 mg/dl; \( n = 5 \)) as did one that was studied at 52 wk of age (BUN, 198 mg/dl at sacrifice). In this animal, extensive glomerulosclerosis, fibrocellular and fibrous crescents, and tubulointerstitial inflammation were present (Figure 1G).

Consistent with their autoimmune background, NZW mice had a modest extent of mesangial IgG and C3 deposition and cellular proliferation at the later ages studied. At all times points, renal function was normal in these mice (BUN, <30 mg/dl).

**Identification of a MuLV Transcript in NZB/W Kidney**

To examine gene expression changes that might be relevant to lupus nephritis, our initial studies concentrated on active renal disease. At 32 wk of age, one of three NZB/W animals had prominent GN (score = 2), while all three control NZW animals had normal renal morphology. Renal cortical RNA obtained from these two groups were used in a single microarray study in which the relative transcript abundance of 7854 mouse genes and EST was compared. In this type of analysis, balanced differential expression ratios greater than 2 are generally accepted as indicating significant differences in gene expression levels (29). From this experiment, some genes shown to be altered were expected, such as the heightened expression of MHC and complement mRNA in NZB/W kidney (24). The balanced differential expression ratio for the commonly used “housekeeping” gene, G3PDH, was 1.0; as such, we used this as control for the remainder of the experiments here.

The gene that was most upregulated in NZB/W renal cortex compared with NZW controls was IMAGE EST clone 522713 (AA087673, derives from C57BL/6 mouse skin) with homology to “mouse DNA with endogenous MuLV” sequence. This gene was 5.5-fold upregulated in the NZB/W mouse renal cortex compared with the NZW controls. In addition, the absolute expression was among the highest of all genes (signal intensity, 20,554 expression “units”). As the sequence data from this clone only contained 174 bases, the entire 1665-bp insert was sequenced and found to correspond to bases 2394 to 4058 of a MuLV known as EDV (endogenous sequence related to the Duplan strain of murine retrovirus; L08395) (30). This transcript was further studied.

**Expression of EDV Transcript in Lupus Nephritis**

A PCR-based approach was used to amplify a 304-base stretch in open reading frame (ORF) A of the EDV transcript. Initial studies compared the expression with renal histology in NZB/W mice over the times when animals had active disease.
(16 to 52 wk of age) using renal tissue obtained from the longitudinal study described previously. As shown in Figure 2, expression of the EDV transcript was elevated in NZB/W renal cortex compared with that obtained from NZW animals at all time points studied. As disease progressed, the relative expression of the EDV transcript decreased.

In the preceding studies, age-matched NZW mice were used as controls for NZB/W mice with renal disease. To examine...
whether the NZB background in NZB/W mice contributed to EDV transcript expression, additional studies were performed with 26-wk-old NZB mice. As with NZW mice, the EDV transcript was present in NZB kidneys, but at a much lower expression than in NZB/W kidneys. The mean relative expression of EDV/G3PDH in NZB mice was 0.40 ± 0.21 (n = 5), with no animal having a relative expression ratio ≥ 1, while all NZB/W animals of similar age had relative expression ratios between 2.0 and 3.5 (cf. Figure 2), illustrating that it is the unique combination of NZB and NZW genes that leads to heightened expression of the EDV transcript in kidney.

As shown in Figure 2, elevated expression of the EDV transcript occurred by 16 wk of age in NZB/W mice, a time corresponding to the onset of renal inflammatory disease (16). To evaluate whether the EDV transcript preceded histologic renal abnormalities in NZB/W mice, 8-wk-old NZB/W mice were studied and compared with age-matched NZB animals.

As shown in Figure 3, the EDV transcript was elevated in NZB/W animals by 8 wk of age compared with control NZB animals (or NZW mice, not shown). Similarly, EDV expression was more than threefold increased in 4-wk-old NZB/W animals compared with age-matched NZW and NZB mice (P < 0.01). Therefore, increased relative expression of the EDV transcript precedes inflammation in NZB/W mouse kidneys.

Additional studies were performed to confirm the EDV transcript was increased in the kidneys of lupus mice. The relative abundance of EDV transcript in NZB/W mice during the evolution of renal disease was confirmed using kinetic RT-PCR. In this case, primers and the probe spanned bases 2548 to 2669 of the EDV transcript. As shown in Figure 4, data using this quantitative approach confirms the results shown previously, in that the EDV transcript peaked early in the course of disease and was consistently elevated compared with strain control NZW mice at all ages.

To confirm the presence of the EDV transcript and to determine its size, Northern analyses were performed with renal tissue from NZB/W mice. As described previously (30), the EDV transcript was 4.2 kb in size (Figure 5). A smaller transcript of 1.4 kb was also expressed, which followed intensity of the larger transcript in individual animals (Figure 5). The identity of this smaller transcript was not pursued, although this corresponds to the size of ORF A of the EDV (30).
Localization in Renal Tissue

Since renal cortex has a number of cell types, localization of the EDV transcript was accomplished by in situ hybridization studies. For these, IMAGE clone 522713 (AA087673) was digoxigenin-labeled and hybridized cRNA probe was identified by an immunohistochemical technique. As shown in Figure 6A, EDV mRNA was present in the renal cortex of 8 wk old NZB/W animals primarily in renal tubules. A similar localization was seen in NZB/W mice at 12 and 24 wk of age (Figs. 6B and C). At the peak of expression, there appeared to be modest glomerular expression of EDV mRNA, but this was considerably less than the tubular expression (Fig. 6B). NZW mice had low expression of EDV by in situ hybridization (Fig. 6D), consistent with previous RNA expression data. Hybridization of NZB/W kidneys with sense strand as probe was negative (not shown).

Presence of EDV Transcript in Other Organs in Lupus Mice

Additional studies were done to examine the presence of the EDV transcript in other organs in NZB/W mice. Interestingly, in two organs in which inflammation occurs, brain and lung (9–12), there was elevated expression of EDV in NZB/W mice compared with NZW controls (Figure 7). In contrast, there was no statistical difference in liver and spleen expression of EDV between NZB/W and NZW mice. Notably, the relative expression of the EDV transcript in these organs was considerably lower than that for kidney in both strains (note that the y-axis scale in Figure 7 is one tenth of those in Figures 2 and 4). Thus, upregulation of the EDV transcript occurs in organs involved in the inflammatory process in this experimental lupus model.

Discussion

In this study, we used an exploratory approach to identify genes altered in the NZB/W experimental model of lupus nephritis. After following a cohort of NZB/W mice and their control NZW strain, we chose animals at 32 wk of age to study for informative gene changes. The gene that was the most remarkably upregulated (5.5-fold) came from an EST (IMAGE clone 522713) derived from mouse skin. The nucleotide sequence of this EST provided clues to its origin, but we needed to sequence the entire clone to establish its identity. The 1665-bp insert of this clone corresponded to bases 2394 to 4058 of the endogenous MuLV related to the Duplan retrovirus.
This EDV was originally identified in C57BL/6 mouse kidney, in which it was expressed to a much greater extent than other organs (30). Consistent with these findings is that, in addition to NZB/W mice, the EDV transcript was highly expressed in renal cortex of NZB and NZW strains relative to other organs.

Microarrays have a number of uses in biologic experimentation. They can determine complex expression patterns, such as the transcript profile as a cell progresses through the cell cycle (31), or classify samples into biologically meaningful groups, such as different tumor classes and their response to chemotherapy (32,33). The bioinformatic tools to accomplish such tasks are always expanding and include hierarchical and K-mean clustering, self-organizing maps, and principal-component analysis (34). Another utility for microarrays is to define an organism’s transcriptome (35). That is what we have attempted here by screening lupus renal cortical RNA with over 7800 probes and identifying one EST as being the most highly expressed relative to the appropriate strain control.

Although our first clue to the presence of the EDV gene in lupus nephritis came from such a microarray study, we used the traditional approaches of conventional and kinetic RT-PCR, Northern blotting, and in situ hybridization to thoroughly study this gene in lupus mice. These results clearly show that the EDV transcript was highly expressed in the kidneys of NZB/W mice. Our careful studies comparing histopathology with gene expression show that the heightened expression of EDV transcript is at its maximum near the very onset of inflammatory disease in kidney and tends to decline with the histologic progression of kidney disease. As with EDV transcript in kidneys of C57BL/6 mice (30), the full-length transcript in NZB/W mice is 4.2 kb. A smaller transcript of 1.4 kb hybridizing with the probe derived from nucleotides 2447 to 2750 was also present. This size corresponds with the length of ORF A in EDV, and a 1.4-kb transcript was not apparent in Northern analyses with a probe derived from the more 5' gag region (30); it is therefore conceivable that this 1.4-kb transcript is for this ORF A.

Our studies show that both the NZB and NZW parental strains to NZB/W contain the EDV transcript. However, at all ages studied, NZB/W mice consistently had threefold or greater expression of EDV transcript compared with age-matched NZB or NZW mice. These studies were performed when animals were as young as 4 wk old, an age preceding significant renal inflammation; therefore, these results indicate that heightened expression of the EDV transcript cannot be due to inflammation per se. These findings are consistent with some form of epistatic interaction between NZB- and NZW-derived loci, as is known to occur in these and other lupus strains in disease phenomena such as autoantibodies and sera gp70 levels (36–38).

As is true in C57BL/6 mice (30), the EDV transcript was expressed in other tissues of lupus mice, although renal expression was the greatest among the organs examined. Both lung and brain from NZB/W mice had marked upregulation of the EDV transcript compared with tissue from NZW mice; as with kidney, these occurred at a time preceding inflammation. Although the kidney is the most prominently involved organ in NZB/W mice (7,39), lung and brain are also involved in this inflammatory process (7,10,11). In contrast, although the liver and spleen are clearly participants in the pathophysiology of SLE as constituents of the mononuclear phagocyte and immune systems, they had low-level expression of EDV transcript, with no significant differences between NZB/W and NZW mice.
NZB mice. This suggests that the EDV transcript is either a marker for and/or is involved in inflammation.

An etiologic role for retroviruses in human autoimmune diseases and their mouse models has been advanced by a number of investigators (13–15,40). One of the most thoroughly studied is the envelope glycoprotein gp70 derived from xenogenic endogenous retroviruses (7,18,41). All lupus-prone strains, including NZB, NZW, and MRL/+/ mice, have free gp70 protein in sera, the levels of which are no different from NZB/W and MRL/lpr mice (42). In the latter, gp70 in serum precedes the onset of inflammatory renal disease (42). What is unique about lupus mice is that they make pathogenic Abs to gp70, and these resultant immune complexes deposit in glomeruli (17,39). In contrast to gp70, we here show that EDV transcript clearly segregates into NZB/W animals that develop lupus and NZB and NZW strains that are simply lupus-prone.

Our data show that the expression of the EDV transcript is upregulated in the kidneys of lupus mice. We have done all that gene expression profiling can accomplish by detailing the time course, relative amount, and location of EDV expression. Subsequent questions that are raised by this technique are whether the transcript is translated, and if so, whether translated viral protein(s) have a role in SLE. In situ hybridization, EDV transcripts were present primarily in renal tubules and to a lesser extent in glomeruli. These anatomic sites in kidney are involved in immune complex deposition, complement activation, and inflammatory cell accumulation, which lead to endocapillary and extracapillary glomerular proliferation and tubulointerstitial nephritis (2,8,43). As the disease progresses, fibrosis ensues, leading to the pathologic picture of sclerosing glomerulonephritis together with tubulointerstitial fibrosis and atrophy (WHO class VI in humans).

The exact pathogenesis of lupus nephritis remains undefined, but it certainly involves a combination of effects from immune complexes, complement and inflammatory cells (8.44–46). Given the markedly increased expression of EDV transcripts in areas involved in renal pathology, it is conceivable that proteins translated from these viral transcripts are directly immunogenic and are involved in immune complex deposition in these sites or that they interact directly with cells of the immune system to facilitate inflammation. An alternative explanation to link increased expression of EDV transcripts with disease concerns transcription of the EDV gene. Possibilities include that transcription of EDV occurs through the actions of cis or trans elements directly relevant to the pathogenesis of SLE or that the EDV gene promoter fortuitously drives the expression of pathogenic gene(s) involved in lupus end-organ disease. Thus, our observations that the EDV transcript is markedly upregulated in lupus nephritis coupled with future studies designed to determine which of these possibilities are correct may provide key insights into the pathogenesis of this disease.

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