

## Dnase113 deficiency in lupus-prone MRL and NZB/W F1 mice

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### SUMMARY

Loss of deoxyribonuclease I (Dnase1) function is associated with systemic lupus erythematosus (SLE) in humans and mice; however, no coding mutations in *Dnase1* are found in polygenic murine models. Instead, both MRL-*lpr* strains and NZB/W F1 hybrids are homozygous for T89I missense in the macrophage-DNASE, desoxyribonuclease I-like 3 (Dnase113). By *in vitro* expression studies, this substitution decreases this enzyme's nuclease activity against free DNA by only approximately twofold; however, the mutation has a greater effect on the capacity of media conditioned with Dnase113 to confer a barrier to liposomal gene transfection to HeLa cells. The 89I substitution decreases the Dnase113 barrier function *in vitro* by eightfold ( $P < 0.01$ ). In splenocytes and BM-derived macrophages of SLE mice, while cellular Dnase113 levels are induced relative to C57BL/6 (control) mice, levels of FD-nuclease activity are similar. Finally, media conditioned by MRL and NZB/W F1 macrophages, relative to control, contains a weak interferon-gamma (IFN- $\gamma$ ) inducible Dnase113-associated barrier to transfection. This barrier function is hypothesized to reflect the inability of SLE mice to degrade membrane-enveloped DNA-associated antigens, such as apoptotic bodies, which are predicted to stimulate the characteristic autoimmunity of SLE. Our results for these two generally independent models strongly suggest that Dnase113 deficiency increases the susceptibility of these mice to polygenic SLE.

**Keywords** DNase SLE lupus MRL NZB/W F1 genetics

### INTRODUCTION

For over 40 years, deficiency in a deoxyribonuclease, mainly Dnase I or Dnase1, has been proposed to predispose to the autoimmune disease, SLE [1]. In support of this hypothesis, decreased serum Dnase activity was found in human SLE [2,3] and (NZB X NZW) F1 (NZB/W) mice [4]. Recently, *Dnase*<sup>-/-</sup> mice were found to develop SLE manifestations, such as immune complex deposition, glomerulonephritis and elevated antinuclear antibody titres [5]. Finally, a heterozygous null mutation in *Dnase1* was found in two probably related SLE patients with high anti-dsDNA titres [6]. On the other hand, in NZB/W F1 mice and humans, Dnase1 enzyme trials suggested only marginal benefit [7,8]. More importantly, *Dnase1* mutations have not been found commonly in human SLE [2], and coding mutations are absent from the syntenic murine locus in SLE models studied here.

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We hypothesize that deficiency of the homologous but distinct Dnase113 (Dnase1-like 3) enzyme is common in SLE [9]. This macrophage-secreted enzyme shares the serum compartment with Dnase1 and is probably the faint 28 kD DNASE remaining in serum of *Dnase1*<sup>-/-</sup> mice [5]. Unlike Dnase1, Dnase113 can confer a significant barrier to transfection of liposome-associated DNA, a novel enzyme function termed barrier to liposomal (BT) activity [10]. While BT activity is nuclease-dependent, it requires more than just the ability to degrade naked or 'free' DNA (FD-nuclease activity), because Dnase113 truncated for 21 C-terminal amino acids has abundant FD-nuclease activity but no BT activity. This report finds that the missense *Dnase113* variant present in two SLE models is associated with only a mild defect in FD nuclease activity, but with a greater defect *in vitro* and macrophage secreted BT activity.

### METHODS

#### *Murine stocks*

Seven to 8-week-old mice were obtained for our studies (Jackson Laboratories, Bar Harbor, ME, USA). Animals were housed

locally for 10 days prior to tissue harvest. One mouse each from NZB, NZW, MRL-*lpr*, DBA/2, BXSB-*Yaa* and BL/6 was used for RNA isolation from liver (for reverse transcription-polymerase chain reaction (RT-PCR) of *Dnase113* cDNA) and kidney (for RT-PCR of *Dnase1* cDNA). DNA from other strains, including BXD recombinant inbreds, was obtained from Jackson Laboratories. For the studies of splenocyte and macrophage protein and FD-nuclease assays, we used three mice each of C57BL/6 (BL/6, female), MRL-*lpr* (female) and BXSB-*Yaa* (male) strains, as well as NZB/W F1 hybrids. Finally, for BT assays, MRL and BL/6 strains, as well as NZB/W F1 hybrids, were used.

#### Reagents

IFN- $\gamma$  was obtained from Genzyme (Cambridge, MA, USA); chemicals and restriction enzymes from Fisher Scientific (Hampton, NH, USA); recombinant mouse GM-CSF and IL-4, from R&D Systems (Minneapolis, MN, USA); and *Taq* polymerase from Promega (Madison, WI, USA). Deionized water was purified further with a Milli-Q system (Millipore, Bedford, MA, USA).

#### Sequencing and genotyping

*Dnase113* coding sequence was amplified from liver cDNAs. The cloned cDNAs were subjected to double-stranded sequencing of both strands. Amplification primers flanking the entire coding sequence of *Dnase113* and *Dnase1* cDNA have been described previously [10]. The *Dnase1* cDNAs were reverse-transcribed from kidney total RNAs, and all were identical. BL/6 *Dnase113* sequence was identical to NM 007870 (wild-type).

The genotype at base pair (bp) 438 of *Dnase113* was confirmed for all strains by amplifying the surrounding 48 nucleotide genomic fragment surrounding this base pair with 5'-AATGGAAATTC AAGAAGAAGCAC-3' and 5'-CGAGAACTAATCACATAGTTCAAT-3'. The latter primer is mutated (A441T) to create an amplicon susceptible to *SspI* (AATATT) cleaved in 89I (mutant) strains, but not in wild-type strains. After *SspI* digest, products were analysed by 6% acrylamide gels. BXD recombinant inbreds, derived from parental strains discordant at this mutation were genotyped to determine the chromosomal location of *Dnase113* (<http://www.informatics.jax.org>).

#### Immunoblotting experiments

Our polyclonal antisera against a murine *Dnase113* peptide has been described previously [10]. Boiled protein extracts (100  $\mu$ g) were separated electrophoretically by 12% SDS-PAGE and electro-transferred to nitrocellulose (Sigma) in tissue studies, or Immobilon-P (Millipore) in cell culture studies. Protein concentration was determined either by BCA Protein Assay (Pierce) or equalized by immunoblots of  $\beta$ -actin expression. Membranes were blocked for 2 h in PBS-0.1% Tween-20 containing 5% non-fat dry milk and 4% goat serum, then incubated overnight at 4°C with either anti-*Dnase113* antisera at 1 : 4000 or the anti-green fluorescent protein (GFP) at 1 : 7000. ECL detection (Amersham-Pharmacia, Piscataway, NJ, USA) was performed and results recorded by radiography. Images and photographs were scanned as 600 dpi TIFF images, and analysed by measuring pixel area and intensity of traced bands, using Scion Image 4-02 software (<http://www.scioncorp.com/>).

#### Enzyme activity assays

Zymograms and DNASE radial diffusion assays (RDAs) were used to measure FD nuclease activity by previously described

methods [10]. All eukaryotic transfections were performed with FuGENE 6 polycationic liposomal reagent (Roche, Indianapolis, IN, USA).

#### FD nuclease assays

To compare the *in vitro* activity of wild-type and 89I, BL/6 *Dnase113* cDNA (AA 11–305) was amplified with primers 5'-CCACGCCTGAGCTCCCTGCTGCTCT-3' and 5'-GAGCGTG GTACCTAGGAGCGATTG-3' (reverse), and cloned in-frame downstream of a prokaryotic GST cassette (pGEX4T) (Amersham-Pharmacia). Site-directed mutagenesis was used to synthesize the T89I allele using the QuikChange kit (Stratagene, La Jolla, CA, USA). Double-strand sequencing was used to confirm inserts. Transformed BL-21 cells at an OD 600 of 0.7 were induced by 0.05 mM IPTG for 3 h at 25 degrees. GST-fusions were purified from *Escherichia coli* lysates using glutathione-sepharose beads (Amersham-Pharmacia). FD nuclease activity was measured by zymogram, while protein levels were compared by immunoblot.

The cloning of *Dnase113* into the eukaryotic expression pcDNA3.1 vector (Invitrogen) was described previously [10]. Mutant counterparts of this clone were created by site-directed point mutagenesis. Lipofection of wild-type and 89I *pcDNA3.1-Dnase113* vectors (3  $\mu$ g purified using Promega Wizard Plus DNA purification system) was performed into HeLa. Nuclease activity was determined after 36 h by RDAs of conditioned supernatants [2% fetal calf serum (FCS)]. The relative result from zymograms of cell lysates and media gave similar results to those for prokaryotic studies. *Dnase113* protein levels were compared using immunoblots of cell lysates. Immunoblots with anti- $\beta$ -actin demonstrated equal loading of total protein per lane.

#### Assays of murine cells

For tissue assays, three mice were sacrificed for each strain or model (Table 1 and Fig. 3,4). Serum samples were obtained by repeat tail bleeds ( $n = 3$  per mouse). Splenocytes were isolated by filtering the minced organ through cell strainers (Becton Dickinson, Franklin Lakes, NJ, USA), then treating with red blood cell (RBC) lysis buffer (Sigma, St. Louis, MO, USA), and culturing for 24–36 h at 2 million cells/well (6-well Falcon) in RPMI with 10%

**Table 1.** *Dnase113* levels and FD nuclease activity; data from three lupus models (BXSB, NZB/WF1 and MRL-*lpr*) and one control strain (C57BL)

Mice	Macrophages		Splenocytes	
	<i>Dnase113</i> Levels	FD nuclease	<i>Dnase113</i> Levels	FD nuclease
C57BL/6	30 $\pm$ 3‡	314 $\pm$ 33§	83 $\pm$ 22‡	346 $\pm$ 34
BXSB	86 $\pm$ 8	351 $\pm$ 25	422 $\pm$ 53	290 $\pm$ 25
NZB/W	70 $\pm$ 7	256 $\pm$ 32	463 $\pm$ 128	326 $\pm$ 23
MRL	92 $\pm$ 9	251 $\pm$ 20	503 $\pm$ 64	286 $\pm$ 23

All values are expressed in pixels; mean  $\pm$  standard deviation. Statistical significance: ‡ $P < 0.01$  for both macrophage and splenocyte *Dnase113* levels of BL/6 compared to all lupus mice using Wilcoxon rank sum test. § $P < 0.01$  for C57BL macrophage RDA activity versus NZ and MRL-*lpr* (but not BXSB) mice. In addition,  $P < 0.01$  was found for the comparison between BXSB and both NZ and MRL. Both calculations used Wilcoxon rank sum test.

FCS. Half the splenocyte population was used for immunoblot isolation; RDAs were measured after 24–36 h in culture. Bone marrow (BM) cultures were isolated similarly by flushing femurs and tibia, then plated using 2 ml RPMI containing 10% FBS, 2 mM HEPES, 50  $\mu$ M  $\beta$ -mercaptoethanol, 20 ng/ml granulocyte macrophage-colony stimulating factor (GM-CSF) and 1 ng/ml interleukin (IL)-4. The cells were incubated with media changes and removal of non-adherent cells every 3 days. After 7 days of culture, a homogeneous population of adherent cells was obtained. Immunoblots for each sample were performed in duplicate.

#### Barrier to liposomal transfection assays

BT assays have been described previously [10]. To prepare Dnase-conditioned media, 2  $\mu$ g of either pcDNA3-1-*Dnase113* plasmid or its mutagenized (89I) counterpart were lipofected into  $1-2 \times 10^6$  HeLa cells. At 24 h, the media was replaced with DMEM/2% FCS, and conditioned for 36–48 h. A second set of naive HeLa cells are next incubated in Dnase-conditioned media, and then these cells lipofected with 2  $\mu$ g of N1-eGFP plasmid (Clontech, Palo Alto, CA, USA). FD-nuclease activity in conditioned supernatant is measured by RDAs or zymograms using purified salmon sperm DNA (Sigma). GFP transfection efficiency was estimated by immunoblot densitometry of GFP expression or assayed by flow cytometry of 20 000 transfected cells using a Becton-Dickinson FACS Vantage flow cytometer.

BM-derived macrophages were isolated as described previously, plated on plastic 6-well Falcon tissue culture dishes in 2 ml RPMI containing 10% FBS, 2 mM HEPES and 50  $\mu$ M  $\beta$ -mercaptoethanol. After 24 h, the media was replaced with 2 ml RPMI containing 10% FBS  $\pm$  IFN- $\gamma$  and incubated for an additional 48 h. BT activity in macrophage-conditioned media was assayed as described above. NZB/W F1 and MRL mice were used to examine BT assays. Note, the previous macrophage and splenocyte experiments used MRL-*lpr* mice.

#### Statistical analysis

The results (pixel volumes) for each experiment were paired and analysed by Wilcoxon signed rank tests.

## RESULTS

#### Sequence analysis of *Dnase113* and *Dnase1*

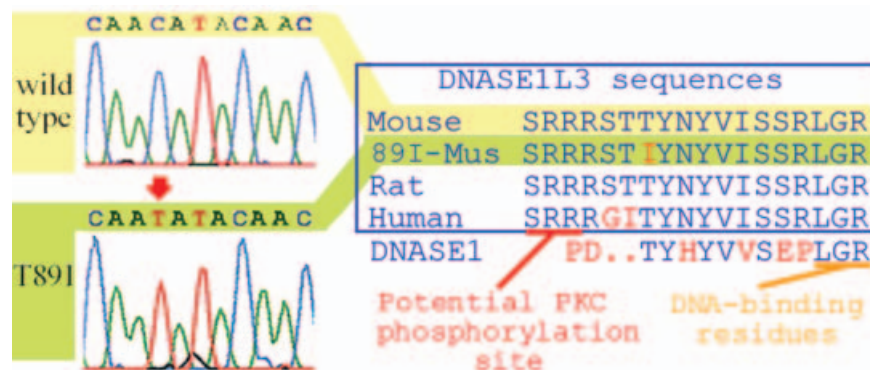
*Dnase1* coding sequences were invariant in the six strains studied. For *Dnase113*, the only difference detected in coding sequences was the transition ACA438ATA[438 C > T] present in *Dnase113* from MRL-*lpr* (Fig. 1), DBA/2, NZB, and NZW strains. The alteration encodes the non-conservative substitution of threonine at amino acid 89 by isoleucine (T89I) (Fig. 1). This change was confirmed by *SspI* restriction analysis of the amplified 48 bp genomic fragment using primers described in Methods. Using this genotyping assay, the mutation was also confirmed in the four strains above, as well as C3H and A strains. The SM, AKR, LG, 129, BALB/c, BXS-B-*Yaa* and C57BL/10 strains were wild-type. Genotypes of BXD (C57BL  $\times$  DBA/2) recombinant inbreds were fully concordant with markers DNASE14Byu1 and DNASE14Mit99, consistent with location on mouse chromosome 14, as expected from syntenic human 3p21 locus (data not shown).

#### Comparison of FD nuclease activity of wild-type (89T) and 89I D

The glutathione-agarose-purified GST-fusion for the 89I (mutant) *Dnase113* showed 54% of the zymographic FD nuclease activity shown by the 89T (wild-type) enzyme (Fig. 2a,  $n = 8$ ,  $P < 0.01$ ). Similar, but not significant results (57%) were obtained after normalized expression in HeLa cells ( $n = 6$  observations,  $28517 \pm 2007$  pixels versus  $16322 \pm 937$  pixels;  $P > 0.05$ ). The nuclease activity on zymograms was normalized relative to the nearly equivalent immunoblotted *Dnase113* levels in lysates. A twofold difference was generally also observed when comparing RDAs of media conditioned by transfected cells and by *Dnase113*-GFP fusions (GFP fused in frame at C-terminus) and immunoblotted with anti-GFP (data not shown). Thus, *in vitro* studies in eukaryotic and prokaryotic systems confirm the 89I substitution decreases FD nuclease activity by about 50%.

#### FD nuclease and cellular DNASE113 levels in murine SLE models and BL/6

Two bands of 28 and 34 kD are immunodetected with anti-*Dnase113* peptide antisera in cell lysates from both splenocytes

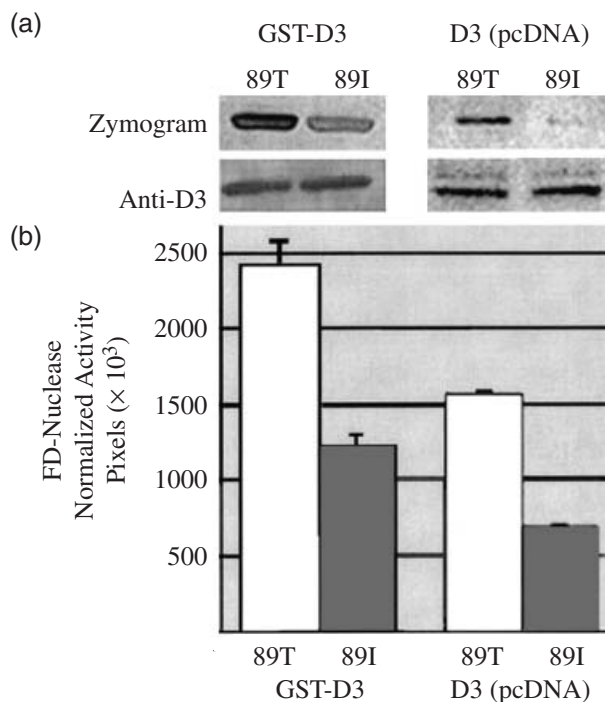


**Fig. 1.** Sequence and alignment of mutant and normal DNASEs. Left register depicts wild-type (a, yellow) and 89I (b, green) sequence traces from BL/6 and MRL-*lpr*, respectively. The ACA to ATA transition at bp 438 encodes the non-conservative substitution isoleucine for threonine as shown in the (c). (c) Alignment shows alteration superimposed occurs relative to other *Dnase113* and annotated bovine DNASE 1. Full alignments of human DNASEs can be found in (9).

and bone-marrow-derived macrophages; the bands represent the enzyme before and after signal peptide cleavage. In conditioned media zymograms, only a single 28 kD band is observed. To determine the expression levels of Dnase113 in mice, we immunoblotted cellular lysates from splenocytes and BM macrophages from BXSB-*Yaa*, BL/6, MRL-*lpr* and NZB/W F1 mice. RDAs were

used to measure FD nuclease activities of media conditioned by these cells.

The most striking observation was induction of Dnase113 in cellular protein lysates from splenocytes (four- to fivefold) and macrophages (two- to threefold) in all three lupus models relative to BL/6 (Fig. 3). Similar levels of induction can be obtained in control BL/6 macrophages, but only after treatment with PMA or IFN- $\gamma$  (data not shown). The latter stimulus also leads to a parallel increase in secreted FD nuclease activity and 28 kD Dnase activity (data not shown). In media conditioned by SLE splenocytes and macrophages, there was a relative deficiency of FD-nuclease activity; hence, while the Dnase113 enzyme levels were increased, the expected parallel increases in FD nuclease activity was not observed (Figs 3,4 and Table 1). In related observations, among the strains/models studied, no significant differences were found in urine FD nuclease activity by RDAs, which is attributed to Dnase1. In addition, we could not reconfirm a significant deficiency of serum FD nuclease activity in SLE strains relative to BL/6 mice [4] (data not shown).



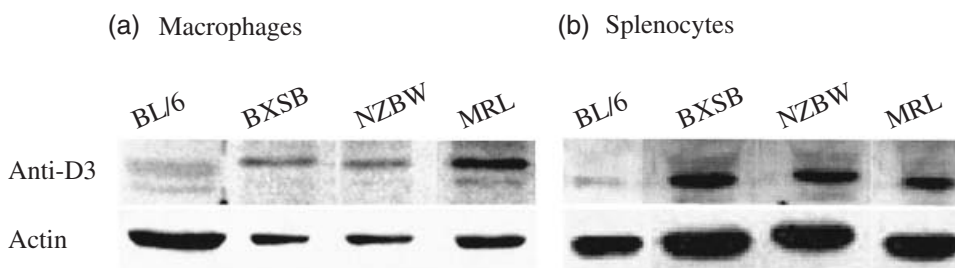
**Fig. 2.** FD-nuclease activities of 89T and 89I Dnase113 s. (a) Corresponding zymograms and anti-Dnase113-peptide Western blots of Dnase113-GST fusions expressed in a prokaryotic system (*E. coli*, left) and Dnase113 expressed in eukaryotic cells (HeLa, right). Anti-Dnase113 immunoblots detect, respectively, the expected column-purified approximately 56 kD GST-fusions (left) or approximately 28 kD enzymes in lysates of cells transfected with pcDNA3-1-Dnase113. Zymograms (top) were performed on parallel and equal loads of the immunoblotted samples. While protein levels are nearly equal, the 89I enzyme has 53% the nuclease activity of the wild-type enzyme ( $n = 8$ , mean values  $2419 \pm 154$  versus  $1211 \pm 84$  pixels; Wilcoxon signed rank sum,  $P < 0.01$ ). Zymogram activity was normalized relative to Dnase113-expression by western. (b) Graph of results.

#### Comparison of BT activity of wild-type and mutant

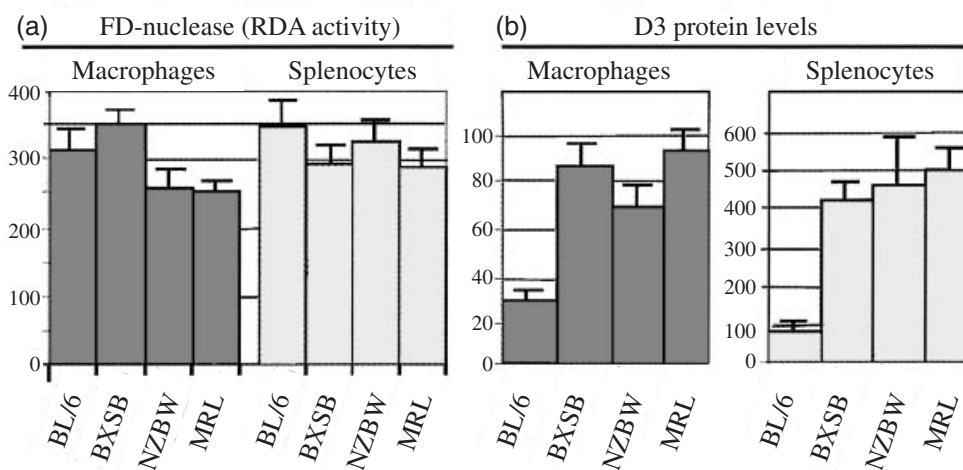
The discovery of Dnase113-specific BT activity led to us to re-evaluate the difference between wild-type and mutant enzyme found in media conditioned by Dnase113-lipofected HeLa (Fig. 5). By BT assays, the 89I enzyme had an approximately eightfold defect in barrier to transfection activity ( $n = 8$ ,  $P$ -value  $< 0.01$ ); in other words, the mutant enzyme was much less efficient in blocking the entry of lipofected DNA. Activity was normalized relative to cellular levels of Dnase113 protein in the FD nuclease activity in the conditioned media was similar in 89I and 89T, probably because of a nearly twofold higher level of enzyme in 89I transfected cell lysates. Undiluted conditioned media in these experiments, which achieves similar levels of FD nuclease activity by RDA to those seen in IFN- $\gamma$  induced macrophage-conditioned media, conferred a near complete barrier to transfection activity with both 89I and 89T, but this was only true with 1 : 10 dilutions of media conditioned with the wild-type enzyme.

#### Comparison of BT activity present in macrophage-conditioned media from NZB/W F, MRL, and BL/6

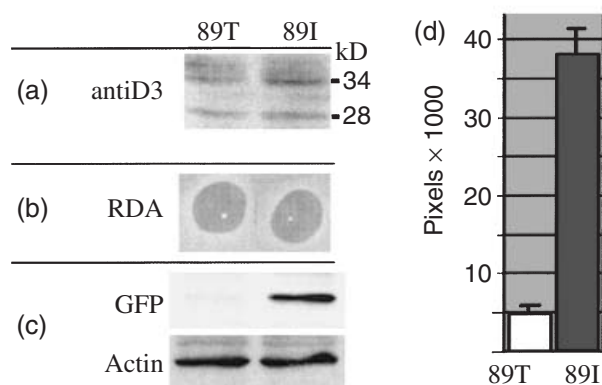
To determine if the relative deficiency of FD nuclease activity seen in media conditioned by SLE macrophages translated into a decreased barrier to transfection activity, we evaluated the ability of media conditioned by BM-derived macrophages from MRL-



**Fig. 3.** Representative anti-Dnase113 Western blots of splenocytes and macrophage cell lysates. Anti-Dnase113 (upper) and anti- $\beta$ -actin (lower) westerns of cell lysates from (a) BM-derived macrophages and (b) splenocytes. The Western blots show induced levels of Dnase113 in all SLE models relative to BL/6. Expression results were normalized relative to actin expression and ( $n = 3$ ) represented in Fig. 4 and Table 1.



**Fig. 4.** Macrophage protein levels of Dnase113 and secreted FD-nuclease activity from BXSB, NZBW, MRL and BL6. This figure represents graphically the mean pixel volumes from Table 1. (a) FD-nuclease activity (RDA) secreted by macrophages was lower in NZBW and MRL-*lpr* mice relative to BL/6 ( $n = 15$ , both  $P < 0.01$ ). (b) Immunoblot levels of Dnase113 protein in cell lysates from both populations were significantly elevated ( $n = 11$  blots,  $P < 0.01$ ). Thus, relative to their induced levels of Dnase113, macrophages from 89I SLE mice were deficient in secreted DNASE activity.



**Fig. 5.** BT assays of *in vitro*-expressed mutant (89I) and wild-type Dnas113. Dnase113 protein levels as assessed in lysates of HeLa cells transfected with nuclease-expressing plasmids. (a) Representative lysates used to condition media show approximately twofold higher levels of the mutant protein by anti-Dnase113 immunoblot. Actin levels were nearly equivalent (data not shown). (b) Representative RDAs of conditioned media show near equivalent FD-nuclease activity. The twofold higher level of 89I expression appears to equalize RDA activity. (c) However, when the respective conditioned media are added to naive HeLa and assayed for the presence of BT activity, media with wild-type 89T enzyme provides a nearly eightfold greater barrier to liposomal transfection of GFP. (d) Graphic demonstration of the results of anti-GF Western blots performed for BT assays. As depicted, media conditioned with wild-type enzyme blocks transfection significantly more than media conditioned with 89I Dnase113.

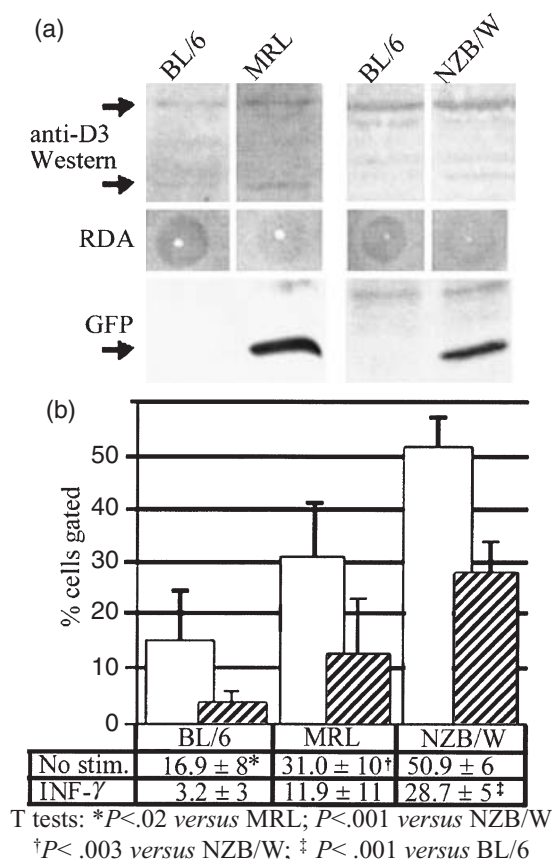
*lpr*, NZB/W F1 mice and wild-type C57BL to confer barrier to transfection activity to HeLa cells (Fig. 6). Media was conditioned for 48 h by adherent cells after IFN- $\gamma$  stimulation. The numbers of adherent cells obtained from both NZB/W F1 and BL/6 mice ( $n = 2$  mice, each marrow isolate split into two wells) were equivalent by visual inspection, and also suggested by actin immunoblotting. Media conditioned by macrophages from MRL

and NZB/W F1 mice has a clear deficiency of barrier to transfection activity. These results support our hypothesis that deficient BT and DNASE1L3 activity are related and predispose to SLE in mice.

## DISCUSSION

Initially, our findings did not identify a clear Dnase113 deficiency in SLE mice, as the defect in nuclease activity against free-DNA was mild and the induced levels of enzyme were expected to compensate for the enzymatic defect. In addition, the 89I alteration was present in non-lupus-prone strains, and prior screens had not linked this locus to SLE in MRL and NZ models. The discovery of a nuclease-dependent, but distinct, enzymatic barrier function of Dnase113 prompted a re-evaluation. The *in vitro* defect in BT activity of the 89I enzyme is not likely to be compensated by the two- to fivefold induction of protein seen in SLE macrophages and splenocytes. In addition, in two independent SLE prone 89I mice, the MRL and NZB/W F1, a dramatic defect of Dnase113-associated macrophage-secreted BT activity was observed relative to the non-lupus-prone, wild-type C57BL/6 mice. The presence of this mutation and defect in two independently derived models strongly suggest Dnase113 deficiency increases their susceptibility to SLE.

We propose that the activity measured by the BT assay reflects a biological function that protects against SLE. The postulated Dnase113 function differs from the janitorial activities assigned to the lysosomal Dnase1, which appears to target naked or free-DNA [11,12]. BT-like functions are predicted to target membrane-associated DNA, for degradation, and prevent this material from inciting autoimmunity against itself and associated components. We propose Dnase113 functions as a macrophage-conferred 'cellular goalie' defending cells from potentially mutagenic [13] or antigen-associated cell-exogenous nucleosomal DNA. We propose that Dnase113 levels are induced in SLE mice to cope for increased load of undegraded material.



**Fig. 6.** BT assays of macrophage-conditioned supernatants of BL/6, MRL-Mp, NZB/W. (a) Despite slightly higher levels of Dnase113 protein (upper 34 kD and lower 28 kD band) in cell lysates from SLE macrophages ( $n = 2$  mice), the FD nuclease activity in macrophage-conditioned media was lower than that of BL/6. Lower register shows an anti-GFP Western blot of lipofected HeLa cells exposed to macrophage-conditioned media. Media conditioned by cells from SLE mice with 89I mutation had less capacity to block GFP gene transfection; hence, SLE macrophages had a deficiency of BT activity. (b) Percentage of gated cells that were GFP positive by flow cytometry of transfected HeLa cells are depicted by graphs with  $\pm$  standard deviation ( $n = 4$  trials). Media conditioned by unstimulated macrophages from both MRL-Mp and NZB/W mice fail to erect a barrier relative to BL/6. NZB/W mice had less BT activity than MRL mice. Even after IFN- $\gamma$  induction, these differences were maintained; however, BT activity remained significantly diminished only in NZB/W mice.

We speculate that the main *in vivo* target of Dnase113 is apoptotic debris, but could include other membrane-associated DNA particles, such as viruses. The phosphatidylserine lipids exposed on apoptotic body surfaces resembles liposomal transfection reagents, and this may flag this material for Dnase113 and macrophage phagocytosis [14]. Dnase113 dysfunction may also compound defects seen in SLE for macrophage phagocytosis [15,16] and clearance of apoptotic bodies [17].

Why the T89I substitution decreases BT more than FD nuclease activity is unknown. When the final 22 residues of Dnase113 are fused to Dnase1, the chimeric enzyme shows substantial BT activity, suggesting that the sequence of the upstream DNASE did not substantially affect BT activity [10]; however, as

the T89 residue is conserved between both nucleases (Fig. 1), these experiments do not address specifically the importance of this residue. The mutation could possibly affect a potential protein kinase C (PKC) phosphorylation site (SRR) present in both Dnases (Fig. 1).

Previous studies of Dnases in SLE mice focused on defects of serum FD nuclease activity [4]. We generally could not confirm significant trends in the differences and, in addition, sera from MRL, NZB/W and BL/6 all showed similar capacities to interfere with liposomal transfection (data not shown). This suggests that serum DNASE-associated activity may not be useful to predict SLE susceptibility, perhaps because it reflects the combined action of multiple enzymes. The putative SLE-protective effects of Dnase113, however, may be derived via plasma, and hence humoral augmentation of DNASE1L3 activity may protect against SLE.

It is unclear why neither Dnase113 nor Dnase1 have been identified as susceptibility loci by genetic screens in mice [18]. The presumed absence of 89I allele from the NZ-derived lupus-prone congenic NZM2410 and its presence in non-lupus-prone strains, such as C3H and DBA/2, suggest this locus is neither necessary nor sufficient by itself to cause SLE. Disease promotion by the 89I allele probably requires other factors, perhaps antigen overproduction or defects in IFN- $\gamma$  signalling. In the human disease, DNASE1L3 defects may play a greater role, since significant association to the 3p21 *DNASE1L3* locus have been reported in African Americans with SLE [19]. The recent observations that peripheral leukocytes from SLE patients commonly express high levels of IFN- $\gamma$  inducible genes, yet have a paradoxical repression of DNASE1L3 support our prediction that deficiency of this gene product is common and associated with SLE progression [20].

Based on the findings, a number of predictions can be made. The induced Dnase113 protein levels and the associated relative deficiency of macrophage-conditioned FD nuclease activity in the 89T BXS mice, suggests a secondary of this nuclease is present. The 89I allele is predicted to exacerbate disease in lupus-prone congenics derived from NZ  $\times$  C57BL/6 crosses [21], and may have synergistic lupus-promoting effects with other antigen clearance defects such as murine DNASE1 [5], C1q [17] or SAP null [22] mice. Finally, we predict human patients with SLE will also have primary or secondary DNASE1L3 abnormalities.

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