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Involvement of DNase γ in the resected double-strand DNA breaks in immunoglobulin genes

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Abstract

Somatic hypermutation (SHM) of immunoglobulin variable (V) region genes occurs in the germinal center (GC) B cells during immune responses, depending on activation-induced cytidine deaminase (AID). SHM is associated with resected double-strand DNA breaks (DSBs) which were shown to occur specifically in rearranged V regions in the GC B cells and CD40-stimulated B cells expressing AID. So far, endonucleases responsible for the DSBs have not been identified. Here we show that DNase γ, a member of DNase I family of endonucleases, is expressed in GC B cells and CD40-stimulated B cells. Overexpression of DNase γ in the mutation-competent Ramos B-cell line resulted in a marked increase in the resected but not blunt DSBs in the V region. Conversely, a selective DNase γ inhibitor, DR396, suppressed the generation of the resected DSBs. These results suggest that DNase γ is involved in the generation of resected DSBs associated with SHM.

Keywords: Somatic hypermutation; Immunoglobulin gene; Variable region; B cells; Double-strand DNA breaks; Endonuclease; DNase γ

Antigen receptors on B lymphocytes and antibodies are encoded by the same immunoglobulin (Ig) heavy chain (H) and light chain (L) genes which have been highly diversified through rearrangements of variable (V) region gene segments during B cell development. In the diversified pool of mature resting B cells, those encountering antigen and helper T cells clonally proliferate to form the germinal center (GC). The GC provides a transient microenvironment in the peripheral lymphatic organs of immunized animals where somatic hypermutation (SHM) and class switch recombination (CSR) of rearranged Ig genes take place. B cells carrying the mutated Ig genes are selected according to the affinity of the Ig for antigen and finally produce high-affinity antigen-specific antibodies [1,2].

SHM occurs in rearranged V regions and their immediate flanking sequences but not in constant (C) region of Ig genes at a rate of 10^{-3} to 10^{-4} per base per cell generation [3]. A similarly high mutation rate has been observed in a few other genes expressed in GC B cells [4]. The mutations in the V region are preferentially targeted to the RGYW/WRCY motif and are more often transitions than transversions [5]. SHM is dependent on the presence of the Ig enhancers, and correlates with transcription [6–10] and histone hyperacetylation at the V region genes [11]. A major advance in our understanding of the molecular mechanism for the SHM process has been the recent discovery of activation-induced cytidine deaminase (AID) [12]. AID is expressed specifically in proliferating B cells in GC, is essential for both SHM

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and CSR [13,14], and appears to be the sole B-cell-specific factor required for these events [15]. AID was also shown to be responsible for gene conversion of Ig genes that occurs in chicken B cells [16]. AID was originally postulated to edit mRNA encoding some crucial factors involved in SHM and CSR [12]. However, accumulating evidence suggests that AID acts as a DNA deaminase, specifically on single-stranded DNA such as the non-template strand of Ig V regions transiently exposed during transcription, converting C to U [17–20]. The strand containing U is either directly replicated, causing transition mutations (C to T, or G to A on the opposite strand), or processed by U-excision activity of uracil-DNA glycosylase (UNG) and/or by some hypothetical error-prone mismatch repair system, resulting in additional transversions and mutations affecting A/T [21–28].

Another line of evidence has suggested that SHM is associated with double-stranded DNA breaks (DSBs) occurring in Ig V region, which was first discovered in Ramos B-lymphoma cell line that undergoes spontaneous mutation at a low frequency [29]. Subsequently, blunt DSBs were identified by ligation-mediated PCR (LM-PCR) in rearranged V genes expressed in Ramos cells as well as in GC B cells, but not in C regions. The breaks were locally associated with mutations, coupled to transcription, and present in cells in the late S/G2 phase of the cell cycle [30–32]. However, it was later shown that such blunt DSBs also occur in unrearranged V genes in GC B cells and are induced independently of AID [33–35]. Finally, a recent study showed that the blunt DSBs in unrearranged V genes are detectable also in non-GC B cells, B-cell precursors or even in non-lymphoid cells. However, DSBs resected to yield free 5′- and 3′-protruding ends specifically occur at rearranged V regions upon induction of SHM, and is AID-dependent. In addition, the resected but not blunt DSBs recruit Rad51/Rad52, proteins involved in homologous recombination [36]. These findings imply that generation of resected DSBs and the subsequent error-prone repair process involving homologous recombination play a role in SHM. However, endonucleases responsible for the resected DSBs are still unknown.

DNase γ, a member of the DNase I family of DNase, is a 33 kDa Ca^{2+}/Mg^{2+}-dependent neutral endonuclease whose activity is detected at high level in lymphoid organs such as spleen, lymph node, and thymus [37–40]. DNase γ has been suggested to catalyze nucleosomal DNA fragmentation that is a hallmark of apoptosis, since forced expression of DNase γ, but not the other members of the same family, induces DNA fragmentation when cells are exposed to apoptotic stimuli [39,41–43]. Unlike DNase I or other family members, DNase γ has two functional nuclear localization signals and is primarily localized in the nucleus [44]. Despite a wealth of information on the biochemical characteristics of DNase γ, its physiological role in vivo is still unclear. We have found that DNase γ expression is induced in mature B cells upon stimulation through CD40 and is abundant in GC B cells as is AID expression. Therefore, we have tested whether DNase γ is involved in the generation of the DSBs at V regions utilizing Ramos cell line. Our results suggest that DNase γ may function as the endonuclease involved in the SHM process.

Materials and methods

Cell culture and transfection. A subclone of a human Burkitt B-lymphoma line Ramos, Ramos 2-2, and its derivatives were cultured in RPMI-1640 medium (Sigma–Aldrich) containing 10% heat-inactivated fetal bovine serum. To generate stable transfectants, cells (1×10^7) were electroporated with the linearized phDNase γ-Myc-His [45] by a Gene Pulser II apparatus (Bio-Rad) at 1 mF, 260 V, and selected with 6 mg/ml G418 (Wako). Cell growth was assessed by counting the number of live cells using the trypan-blue dye. Where indicated, cells (2×10^6) were cultured in the presence of a DNase γ inhibitor, DR396 [46], at the indicated concentration. For RT-PCR, splenic B cells were purified from 9-week-old C57BL/6 mice using a MACS B-cell Isolation Kit (Miltenyi Biotec). 1.5×10^6 B cells (purity was 98%) were stimulated in 1 ml culture medium containing 1 μg/ml anti-CD40 mAb (1C10; Southern Biotechnology) and 10 μg/ml goat anti-mouse IgM $F(ab')_2$ fragment (The Jackson Laboratory) or 100 U/ml recombinant mouse IL-4 (Peprotech) for the indicated period of time.

DNase activity gel system. DNase γ activity was assayed as described previously [39]. In brief, nuclear extract of cells (2×10^6) was separated by electrophoresis in SDS/polyacrylamide gel containing 200 μg/ml of native salmon sperm DNA (Wako). The gels were then treated as described to reactivate DNase γ [39], except that the final buffer contained 3 mM $CaCl_2$/3 mM $MgCl_2$. After staining the gels with ethidium bromide, DNase activity was detected as unstained areas. The amount of nuclear extract was estimated by Western blot analysis with anti-SP1 rabbit polyclonal antibody (sc-59, Santa Cruz Biotechnology) as described previously [47].

Preparation of germinal center B cells from Peyer's patch. Peyer's patches (PPs) were collected from the small intestine of 10-month-old Balb/c mice (Sankyo Labo Service) maintained in specific pathogen free condition. Singly suspended cells from PPs were passed through a nylon mesh, and stained with phycoerythrin (PE)-conjugated anti-B220 (RA3-6B2, Pharmingen, BD Biosciences) and FITC-conjugated peanut agglutinin (PNA, Sigma–Aldrich). $B220^+$ PNA^+ and $B220^+$ PNA^- cells were sorted by FACSVantage SE (BD Biosciences).

RT-PCR. Total RNA was extracted from cells or tissues using TRI reagent (Sigma–Aldrich). cDNA was synthesized using SuperScript III Reverse Transcriptase (Invitrogen) and subjected to standard PCRs with the following heating steps: 95 °C for 3 min, 85 °C for 3 min (during which *Taq* polymerase was added), followed by gene-specific cycling conditions, and then 72 °C for 10 min. The cycling conditions are as follows: for mouse DNase γ and HPRT, 94 °C for 30 s, 62 °C for 30 s, and 72 °C for 30 s, 32 (HPRT) or 40 (mouse DNase γ) cycles; for mouse AID, 94 °C for 45 s, 58 °C for 45 s, and 72 °C for 1 min, 35 cycles; for human DNase γ, 94 °C for 30 s, 58 °C for 30 s, and 72 °C for 30 s, 35 cycles; and for human β-actin, 94 °C for 30 s, 58 °C for 1 min, and 72 °C for 1 min, 30 cycles. The following primers were used: mouse DNase γ, 5′-CACGTACAAAGAGCAGTATGCCTTCG-3′ and 5′-CGAATGTTCTGCCAGGCCTTCTTGGGG-3′; human DNase γ, 5′-GAAGGTCATCAAACGCTGTG-3′ and 5′-GTTTTTCTTCCAAGCCGAGAG-3′; mouse AID, 5′-GGGAATTCAGTCAAGAAAGTCACGCTGG-3′ and 5′-GGGTCGACGTGACATTCCAGGAGGTTGC-3′; mouse HPRT, 5′-TTGCTGGTGAAAAGGA

CCTCTCG-3′ and 5′-CCACAGGACTAGAACACCTGCTAA-3′; human β-actin, 5′-TACAATGAGCTGCGTGTGGC-3′ and 5′-TAGCTCTTCTCCAGGGAGG-3′; and human AID, 5′-GGGAATTCGAGGCAAGAAGACACTCTGG-3′ and 5′-GGGTCGACGTGACATTCCTGGAAGTTGC-3′.

LM-PCR. BW linker was made by annealing oligonucleotides BW-1 and BW-2 [48]. Genomic DNA was prepared from 2×10^6 cells using Genomic Tips (Qiagen), and treated with or without T4 DNA polymerase (Toyobo) in the presence of 200 μM each dNTP at 37 °C for 20 min. After heat inactivation of the enzyme, ethanol-precipitated DNA was ligated with 20 pmol BW linker in a 20 μl ligation buffer (Takara Bio) containing 2 mM ATP and 2.8 Weiss units T4 DNA Ligase (Takara Bio). This ligation mixture was then mixed with 30 μl PCR-L buffer [49] and heated to 95 °C for 10 min. The linker-ligated DNA (the first sample was equivalent to 100 ng of the starting genomic DNA) was 2.5-fold serially diluted into unligated genomic DNA for use as a template in LM-PCR or into sterilized water for Cμ gene amplification. LM-PCRs to detect the linker-ligated Ramos Ig V_HDJ_H and Cμ regions were performed in two rounds as described previously [36]. One-half of each PCR product, and ~1 ng of PCR-amplified Ramos V_H fragment as a hybridization control, were separated on agarose gels, blotted onto Biodyne-B membrane (Pall), and hybridized to P^{32}-labeled oligonucleotide probes for V_H or Cμ [36]. To control the amount of template DNA, Cμ region was amplified with the primers 5′-CAAGACACAGCCATCCGGGTCTTCG-3′ and 5′-TCAGTAGCAGGTGCCAGCTGTGTCG-3′ using the water-diluted DNA samples as templates. In a control experiment, genomic DNA from Ramos 2-2 cells was digested by *Msl*I (New England Biolab) or *Bst*EII (Toyobo) and subsequently processed as above. Sixteen nanograms of the linker-ligated DNA was used as templates for PCR to amplify the enzyme-cut Cμ region as above.

Analysis of apoptosis. The Ramos transfectants were seeded into a 6-well plate (1×10^5/2 ml/well) and cultured for 48 h. Then the cells were fixed with 70% ethanol at 4 °C for 30 min, treated with PBS containing 50 μg/ml RNaseA (Sigma–Aldrich) at 37 °C for 20 min, stained with 50 μg/ml propidium iodide (Sigma–Aldrich), and analyzed for DNA content by FACSort (BD Biosciences).

Results

DNase γ mRNA expression in germinal center B cells

It has been reported that resected DSBs in IgV region genes were detected specifically in B cells in GC where SHM of Ig genes takes place [36]. To study a possible involvement of DNase γ in the DSBs in the hypermutating Ig V region gene, we first examined if DNase γ is expressed in GC B cells. Although it was reported previously that DNase γ mRNA was expressed abundantly in spleen, bone marrow, liver, and to a lesser extent, in thymus, lymph node, and a few other tissues [39,45,50], it has been unclear if B cells express DNase γ. GC B cells and non-GC B cells were purified by flow cytometry from Peyer's patches (PPs) of the small intestine of adult mice (Fig. 1A), and expression of DNase γ mRNA in these cells was examined by RT-PCR (Fig. 1B). DNase γ mRNA was expressed in GC B cells more abundantly than in non-GC B cells. DNase γ mRNA was much more abundant in samples of whole spleen, most likely because macrophages dominantly express DNase γ mRNA in unimmunized spleen as previously

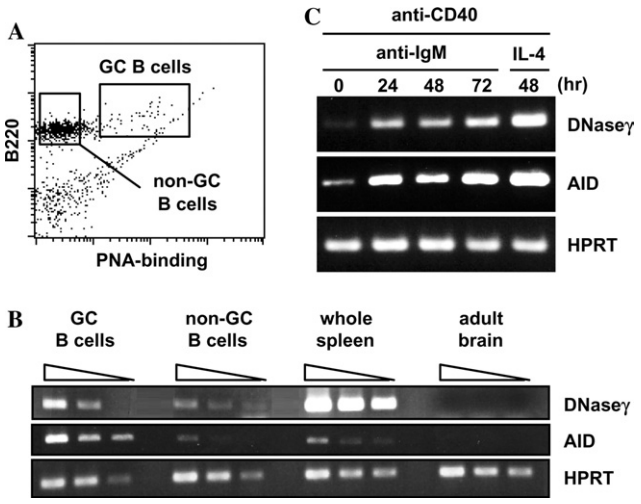

Fig. 1. DNase γ mRNA is expressed in mouse germinal center B cells. (A) The cells from Peyer's patches were stained with anti-B220 and PNA, and then $B220^+$ PNA^+ (GC B cells) and $B220^+$ PNA^- (non-GC B cells) fractions (indicated by windows) were sorted by FACS. (B) RT-PCR analysis. Total RNA from the sorted cells, as well as whole spleen cells and whole adult brain, was converted into cDNA. Equal amount of the cDNA from each sample was serially diluted by 3-fold and used as a template for PCR to amplify DNase γ (top), AID (middle), and HPRT as a dose control (bottom). (C) Purified mouse splenic B cells were cultured with anti-CD40 Ab (1 μg/ml) and anti-IgM Ab [$F(ab')_2$, 10 μg/ml] or IL-4 (100 U/ml) for the indicated length of time (hours). Total RNAs from these cells were subjected to RT-PCR analysis. PCR was performed with 35 cycles for DNase γ and with 30 cycles for HPRT.

shown by in situ hybridization [50]. AID mRNA was also enriched in the GC B cells as previously reported [12]. Neither mRNA was expressed in adult brain, serving as a negative control of this assay. Thus, DNase γ expression appears to be upregulated in hypermutating GC B cells in the peripheral lymphoid organs.

It was reported that the resected DSBs in the rearranged V region DNA were induced in parallel with AID expression in human peripheral blood B cells upon in vitro stimulation for 48 h via CD40. Additional antigen-receptor- or IL-4-stimulation further augmented the generation of the resected DSBs and AID expression [36]. As shown in Fig. 1C, stimulation of mouse splenic B cells by anti-CD40 antibody in conjunction with anti-IgM antibody or IL-4 resulted in a marked increase in the expression of DNase γ as well as AID mRNA. These data indicate that B cell activation that induces AID expression concomitantly induces DNase γ expression, suggesting that DNase γ may be involved in SHM.

Establishment of stable clones overexpressing DNase γ proteins

To investigate the role of DNase γ in the generation of DSBs in V region of Ig genes, we transfected a human DNase γ expression vector into a subclone of Ramos B

cell line (Ramos 2-2) and established stable transfectants (γ/Ramos 12, 25, and 21) expressing a similar level of homogeneous surface IgM. Expression of DNase γ, AID, as well as β-actin as a control, was examined by RT-PCR. Extremely high level of DNase γ transcripts was detected in the γ/Ramos clones compared to the parental Ramos 2-2, whereas the level of AID transcripts was unchanged (Fig. 2A). The enzymatic activity of DNase γ was below the detection limit of this assay in the Ramos 2-2 cells, but readily detectable in the γ/Ramos clones. The overexpression of DNase γ did not affect the cell growth and viability in these transfectants (Fig. 2B), and did not cause spontaneous apoptosis as little subdiploid cells were detected by flow cytometry (Fig. 2C). The lack of DNA fragmentation in these cells

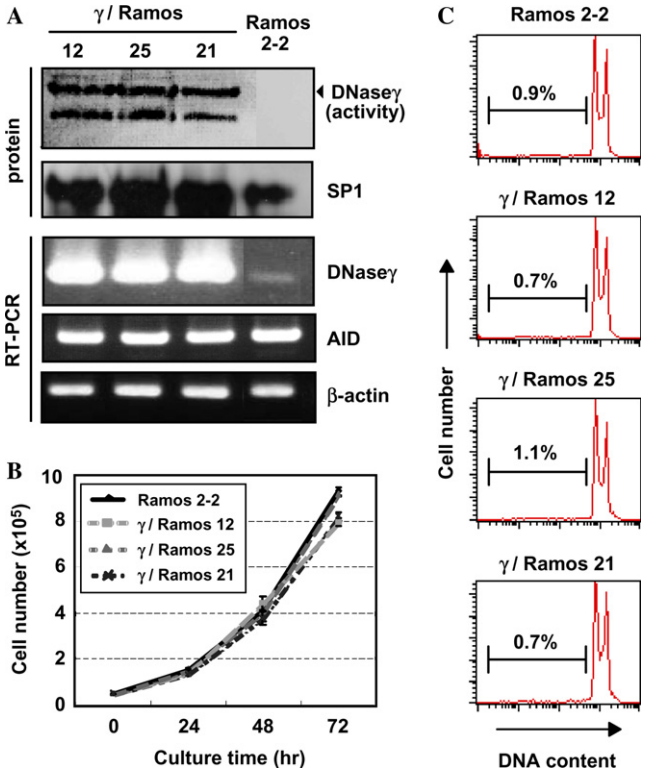

Fig. 2. Establishment of stable transfectants overexpressing DNase γ protein. (A) Nuclear extracts from Ramos 2-2 and γ/Ramos 12, 25, and 21 cells were subjected to the DNase γ activity gel assay (top) and Western blot analysis with anti-SP1 antibody as a nuclear protein loading control (second). Total RNA from the same cells was subjected to RT-PCR analysis to amplify DNase γ (third), AID (fourth) or β-actin cDNA as a control (bottom). (B) Cell growth profiles. 5×10^4 cells were seeded into each well of the culture plates, and the number of viable cells was counted every 24 h. The mean counts of duplicated samples are plotted with standard deviations. (C) Analysis of apoptosis. Exponentially growing cells were stained with propidium iodide (PI) after fixation and analyzed by flow cytometry. The DNA content was expressed as each histogram of PI fluorescence intensity. Apoptotic cells are represented as cells containing less DNA than diploid cells due to a loss of fragmented DNA through the permeabilized cell membrane. The percentage of the apoptotic cells (horizontal bar) is denoted in each histogram.

was also demonstrated by electrophoresis of genomic DNA (data not shown).

Generation of resected DSBs is enhanced in Ramos cells overexpressing DNase γ

To investigate whether DNase γ is involved in the generation of DSBs with either blunt or resected ends in Ig genes, we applied the LM-PCR method utilizing T4 DNA polymerase [36]. Blunt DSBs can be ligated with the linker without T4 DNA polymerase treatment and therefore detected by the PCR using the linker- and the Ramos V_HDJ_H-specific primers, whereas resected DSBs can be ligated and detected only after blunting with T4 DNA polymerase (Fig. 3A). Relatively small amounts of blunt DSBs in the V_H region gene were detected using genomic DNA from Ramos 2-2, and overexpression of DNase γ did not affect the level of the blunt DSBs (Fig. 3B). By contrast, treatment of genomic DNA with T4 polymerase before linker-ligation resulted in a dramatic increase in the level of detectable DSBs in the V_H region gene in the γ/Ramos clones as compared to Ramos 2-2, indicating that the frequency of resected DSBs is increased by the overexpression of DNase γ (Fig. 3B). Neither blunt nor resected DSBs were detectable in the Cμ gene in any of the samples, suggesting that DSBs are V_H region gene-specific and that overexpression of DNase γ did not result in non-specific DSBs (Fig. 3B). The fidelity of this assay was verified using genomic DNA from Ramos 2-2 digested with restriction enzymes (Fig. 3C). A blunt DSB in the Cμ region introduced by *Msl*I was detected by LM-PCR and was not affected by the treatment with T4 DNA polymerase. In contrast, a resected DSB at the same region introduced by *Bst*EII was not detected without, but readily detected with, the T4 DNA polymerase treatment (Fig. 3C, bottom). These data suggest that DNase γ participates in the generation of resected DSBs, which are specific to rearranged Ig V_H genes.

Generation of resected DSBs is suppressed by inhibition of DNase γ activity

To determine whether enzymatic activity is required for DNase γ to generate resected DSBs, we treated Ramos2-2 with DR396, a chemical compound which selectively inhibits DNase γ activity. DR396 was demonstrated to inhibit in vitro activity of recombinant DNase γ completely at 30 μM, but hardly of recombinant DNase I. In addition, DR396 inhibited staurosporine-induced apoptotic DNA fragmentation in vivo, but not any other apoptotic phenotype, in HeLa cells stably transfected with DNase γ. The parental HeLa cells lack DNase γ and do not exhibit DNA fragmentation. Furthermore, DR396 did not inhibit Fas-mediated DNA

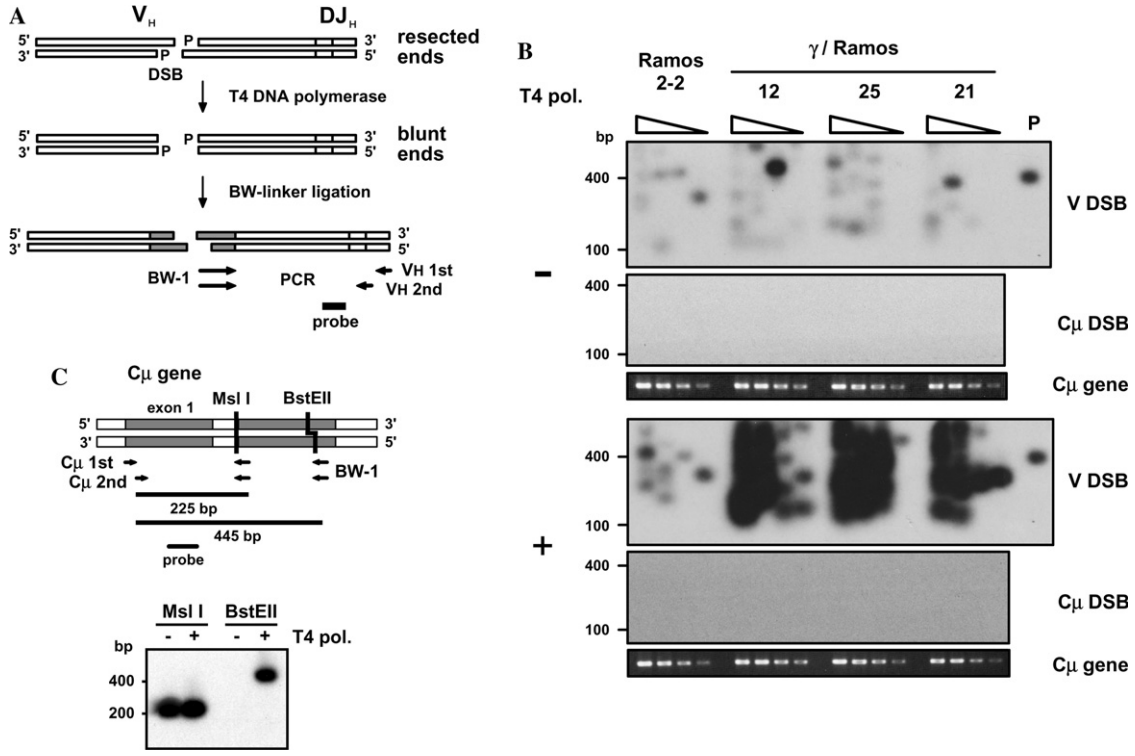

Fig. 3. The generation of resected DSBs is increased in Ramos cells overexpressing DNase γ. (A) Schematic representation of LM-PCR analysis to detect resected DSBs in Ramos Ig V_H region gene. (B) LM-PCR analysis. Genomic DNAs prepared from Ramos 2-2 and the γ/Ramos clone (12, 25, and 21) cells were treated with (+) or without (−) T4 DNA polymerase (T4 pol.), ligated with BW linker, serially diluted (2.5-fold) into unligated homologous genomic DNA (as shown by open triangles), and used as templates for LM-PCR amplifying DSBs at the rearranged V_H region gene (V DSB) or Cμ region gene (Cμ DSB). The PCR products, and a PCR-amplified Ramos V_H fragment as a hybridization control (P), were subjected to Southern blot hybridization with oligonucleotide probes specific for Ramos V_H and Cμ genes. To control the template DNA dose, the linker-ligated DNA was serially diluted (2.5-fold) into sterilized water and used for PCR to amplify Cμ region gene (Cμ gene). Shown is a representative result of three independent experiments with essentially the same results. (C) Verification of the LM-PCR assay utilizing T4 DNA polymerase. DNA from Ramos 2-2 cells was digested with *Msl*I or *Bst*EII to produce blunt or resected ends in Cμ region gene (top), subsequently processed as in (B), and the Cμ DSBs were detected (bottom) with primers shown below the diagram.

fragmentation in Jurkat cells that is known to be catalyzed by another DNase, CAD. These data indicated that DR396 is a highly selective inhibitor of DNase γ [46]. As shown in Fig. 4A, DR396 had no growth inhibitory or cytotoxic effect on Ramos cells at 100 μM up to 48 h. We examined using LM-PCR the DSBs in the V_H region gene of Ramos 2-2 cells that had been cultured with or without 100 μM DR396 for 24 h. Without the inhibitor, more DSBs were detected when genomic DNA was treated with T4 DNA polymerase, indicating a dominant occurrence of resected DSBs in Ramos cells in culture. However, in the presence of DR396, the occurrence of resected DSBs was inhibited. The generation of blunt DSBs was not affected in the presence of this inhibitor (Fig. 4B). The effect of DR396 on the generation of resected DSBs was confirmed with γ/Ramos 25 cells which abundantly and almost exclusively generate the resected DSBs in the V_H region (Fig. 2B). As shown in Fig. 4C, DR396 clearly inhibited the generation of the resected DSBs in a dose-dependent manner, although more DR396 was required for this DNase γ transfectant than for the parental Ramos 2-2 cells. These results support the notion that DNase γ activity is responsible for the generation of resected DSBs in the rearranged V_H region gene in Ramos B cells.

Discussion

It was recently reported that resected DSBs in rearranged V region genes are associated with the induction of SHM [36]. In this study, we demonstrated that DNase γ is involved in the generation of the resected DSBs in the rearranged V_H region in Ramos cells. Overexpression of DNase γ in Ramos cells resulted in a marked increase of resected DSBs in the V_H region, but not in the Cμ region. By contrast, inhibition of DNase γ activity by a specific inhibitory compound resulted in a decrease of the resected DSBs. Thus, DNase γ appears to be a good candidate for the endonuclease that has been implicated in the generation of SHMs. Further experiments utilizing siRNA-mediated knockdown of DNase γ expression, or perhaps more clearly targeted ablation

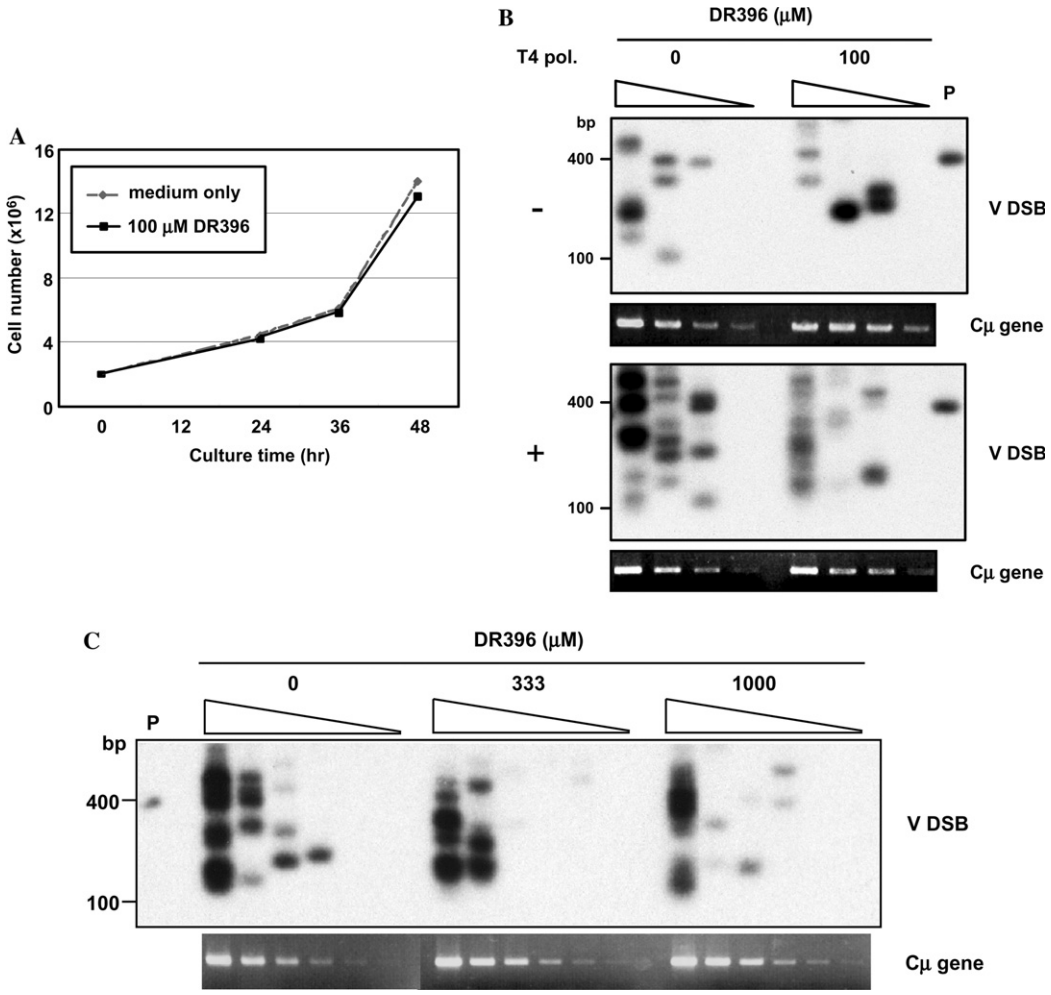

Fig. 4. The generation of resected DSBs is decreased by inhibition of DNase γ activity. (A) Effect of the DNase γ inhibitor, DR396, on cell growth. Ramos 2-2 cells were cultured with or without 100 μM DR396 and the number of live cells was monitored every 12 h after 24 h. Shown is a representative result of two independent experiments. (B) Ramos 2-2 cells were cultured with or without 100 μM DR396 for 24 h. Genomic DNAs from these cells were used for LM-PCR to detect DSBs at the rearranged V_H region gene (V DSB) and for conventional PCR to amplify Cμ region gene (Cμ gene), which was performed as in Fig. 3. Shown is a representative result of two independent experiments with essentially the same results. (C) γ/Ramos 25 cells were cultured for 24 h with the indicated concentration of DR396. Genomic DNAs from these cells were treated with T4 DNA polymerase and the DSBs in the V_H region gene were detected as in (B). Cell growth was not inhibited by DR396: the cell number was increased by 2.2-, 2.3-, or 2.0-fold in the presence of 0, 333, or 1000 μM DR396, respectively, during the 24 h.

of DNase γ gene, would provide a definitive evidence for this assumption.

DNase γ is one of the four members of the DNase I family endonuclease. DNase I is the major nuclease present in serum, urine, and secretions, and its role in clearance of DNA released from dead cells was suggested from an observation of SLE-like autoimmune diseases in DNase I-deficient mice [51]. Physiological roles for the other members have yet to be determined. Unlike other members, DNase γ contains functional nuclear localization signals [44], and through studies using cell lines DNase γ has been implicated in nucleosomal DNA fragmentation during apoptosis at least in some types of cells [39,41–43,52]. Although DNase γ is expressed in activated B cells in GC, overexpression of DNase γ in Ramos B cells did not result in an increase of DNA fragmentation in culture (Fig. 2) but in the increase of resected DSBs in the V_H region (Fig. 3). In addition, expression of DNase γ paralleled with that of AID (Fig. 1) and with the occurrence of resected DSBs in B cells upon stimulation through CD40 [36]. These results together support a possible role for DNase γ in the generation of SHM rather than in the apoptotic DNA fragmentation in GC B cells.

It was reported that generation of resected DSBs in V region genes is AID-dependent [36]. Therefore, DNase γ's function to generate the resected DSBs would be dependent on AID. Honjo et al. [15] have proposed a possibility that AID modifies genetic information, through its cytidine deaminase activity on some RNAs encoding proteins involved in a process of SHM and CSR, and presumed that the proteins may be putative

endonucleases specific to either V or S region DNA. Thus, the RNA encoding DNase γ might be a substrate of AID and edited to encode an active enzyme perhaps acting specifically on V region DNA to generate resected DSBs. Identification of such a modified DNase γ mRNA in AID-expressing B cells would support this hypothesis, although such a modified mRNA might be a minor species and difficult to isolate. Alternatively, DNase γ might be recruited to V region DNA via AID. In this regard, replication protein A (RPA), a single-stranded DNA-binding protein involved in various aspects of DNA metabolism, has recently been identified as a binding partner of AID and proposed to recruit AID to DNA undergoing transcription together with additional mutation factors and DNA repair factors [53]. DNase γ might also interact with AID or the AID–RPA complex to gain access to V region DNA. Studies based on these hypotheses are currently underway.

Acknowledgments

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