ATAD5 Deficiency Decreases B Cell Division and Igh Recombination

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Mammalian ATPase family AAA domain–containing protein 5 (ATAD5) and its yeast homolog enhanced level of genomic instability 1 are responsible for unloading proliferating cell nuclear antigen from newly synthesized DNA. Prior work in HeLa and yeast cells showed that a decrease in ATAD5 protein levels resulted in accumulation of chromatin-bound proliferating cell nuclear antigen, slowed cell division, and increased genomic instability. In this study, B cells from heterozygous (Atad5+/m) mice were used to examine the effects of decreased cell proliferation on Ab diversity. ATAD5 haploinsufficiency did not change the frequency or spectrum of somatic hypermutation in Ab genes, indicating that DNA repair and error-prone DNA polymerase η usage were unaffected. However, immunized Atad5+/m mice had decreased serum IgG1 Abs, demonstrating a functional effect on class switch recombination.

The mechanism of this altered immune response was then examined following ex vivo stimulation of splenic B cells, where Atad5+/m cells accumulated in the S phase of the cell cycle and had reduced proliferation compared with wild-type cells. These haploinsufficient cells underwent a significant decline in activation-induced deaminase expression, resulting in decreased switch region DNA double-strand breaks and interchromosomal translocations in the Igh locus. Class switch recombination to several isotypes was also reduced in Atad5+/m cells, although the types of end-joining pathways were not affected. These results describe a defect in DNA replication that affects Igh recombination via reduced cell division. The Journal of Immunology, 2015, 194: 000–000.

Following stimulation, B cells express activation-induced deaminase (AID) and undergo rapid division to produce Abs with improved affinity by somatic hypermutation (SHM) and with different isotypes by class switch recombination (CSR) (1, 2). Thus, cell division following stimulation underpins the swift response of B cells to stimuli. To understand the effect of DNA replication on SHM and CSR, we studied the role of proliferating cell nuclear antigen (PCNA). PCNA is a sliding clamp protein that forms a homotrimeric ring structure encircling the DNA during replication. Its function is to interact with a plethora of proteins participating in many cellular responses (3), and particularly with DNA polymerases. PCNA holds the replicative polymerases onto the leading and lagging strands to ensure processive synthesis. When damaged bases are encountered, PCNA is monoubiquitinated and helps bypass the lesion by exchanging high-fidelity polymerases for low-fidelity ones, such as polymerase η (pol η) (4, 5).

In addition to ubiquitination, PCNA is regulated by its loading and unloading from DNA. PCNA needs to be recycled because it binds to the many Okazaki fragments on the lagging strand, and unloading guarantees that enough protein is available for the next round of replication. Although the mechanism of PCNA loading by the replication factor C complex has been studied in detail (6), little is known about how the clamp is unloaded. Recent papers indicate that the yeast enhanced level of genomic instability 1 (ELG1) protein (7, 8) and its mammalian counterpart, ATPase family AAA domain–containing protein 5 (ATAD5) (9), remove PCNA after DNA synthesis (10). In HeLa cells with a knockdown of ATAD5, PCNA accumulated on DNA, which slowed progression of replication forks and cell division (9). Additionally, ATAD5 interacts with ubiquitin-specific peptidase 1 at DNA damage bypass sites to deubiquitinate PCNA and promote the exchange of a low-fidelity translesion polymerase back to a high-fidelity replication polymerase (11). Thus, ELG1/ATAD5-dependent processing of PCNA is essential for productive DNA replication.

Because ATAD5 is required for embryonic development, heterozygous mice were generated with a mutant allele (Atad5+/m). These mice are prone to cancers and genomic instability (12). To examine how PCNA manipulation affects the adaptive immune response in B cells, we used Atad5+/m mice to study AID-induced SHM and CSR. It is expected that in wild-type cells, ATAD5 will successfully unload PCNA from newly synthesized DNA, whereas in Atad5+/m cells, PCNA will accumulate on the chromatin (Fig. 1). Amassed PCNA could potentially alter Ab diversity at the Igh locus through both prolonged contact of low-fidelity DNA polymerases during SHM and delayed cell division during CSR.

Materials and Methods
Mice

Atad5+/m and Atad5+/ mice on a C57BL/6 background were previously described (12). Littermate mice were used at 4–9 mo of age. All animal
protocols were reviewed and approved by the Animal Care and Use Committees of the National Institute on Aging and the National Human Genome Research Institute.

**Splenic B cell isolation and ex vivo stimulation**

Resting splenic B cells were collected by negative selection with anti-CD43 and anti-CD11b magnetic beads (Miltenyi Biotec) and cultured in RPMI 1640 media (Invitrogen) containing 10% (v/v) FBS (Sigma-Aldrich), 100 U/ml penicillin-streptomycin (Invitrogen), 2 mM glutamine (Invitrogen), and 50 μM 2-ME (Sigma-Aldrich). Cells were plated at 0.5 × 10^6 cells/ml in 24-well plates and stimulated with 5 μg/ml LPS (Escherichia coli serotype 0111:B4; Sigma-Aldrich) and 5 ng/ml recombinant IL-4 (BioLegend), unless otherwise noted.

**Western blot and quantitative PCR**

B cells were stimulated for 0–3 d, centrifuged, and suspended in Laemmli lysis buffer. Samples were separated by SDS-PAGE gel electrophoresis using a 4% stacking layer with either an 8% polyacrylamide gel for ATAD5 and β-actin, or a 15% polyacrylamide gel for PCNA and AID. Proteins were transferred to a polyvinylidene difluoride membrane (Bio-Rad) and detected with rabbit anti-ATAD5 (12), mouse anti–β-actin (clone AC-15, Sigma-Aldrich), rabbit anti-PCNA (Abcam), or rabbit anti-AID (13). Western blots were quantified using ImageJ software. For ATAD5 transcript levels, RNA was harvested after 3 d stimulation using an RNeasy Mini kit (Qiagen). cDNA was reverse-transcribed using RNase H minus Moloney murine leukemia virus point mutant (Promega) with oligo(dT) primers (Qiagen), followed by quantitation of ATAD5 mRNA (BioLegend), unless otherwise noted.

**Somatic hypermutation**

B cells were obtained either from Peyer’s patches of naive mice or from the spleens of mice immunized with 100 μg 4-hydroxy-3-nitrophenylacetyl hapten (ratio = 30) [NP(30)]–chicken γ-globulin (CGG) (Biosearch Technologies) in CFA for 2–4 wk. Germinal center B cells were detected by staining with FITC-labeled anti-B220 (clone RA3-6B2) and Alexa Fluor 647–labeled anti-GL7 (clone GL-7) (eBioscience). B220⁺GL7⁺ cells were isolated via flow cytometry, and DNA was prepared and amplified by nested PCR using JH4 intron primers as previously described (15). The PCR products were cloned and sequenced; only unique mutations were counted. Mutation spectra percentages were corrected for base composition of the amplified sequence.

**ELISA**

Serum was collected by retro-orbital bleeds from mice on days 0 and 14 after immunization with 100 μg NP(30)-CGG in CFA. Wells were sequentially coated with NP(25)-BSA or NP(8)-BSA, biotinylated anti-NP sera diluted 1:2500 in BSA, and goat anti-mouse IgG1-HRP (SouthernBiotech clonotyping system) diluted 1:1000 in BSA. Ab expression was detected using a TMB peroxidase substrate kit (Vector Laboratories) and was quantitated on a Bio-Rad 680 XR microplate reader.

**Cell cycle and division analyses**

For cell cycle, B cells were stimulated for 30 h, followed by fixation in EtOH. Cells were treated with 200 μg/ml RNase A (Sigma-Aldrich), resuspended in 50 μg/ml propidium iodide (Sigma-Aldrich) in PBS, and incubated at 37°C for 40 min prior to flow cytometry analysis. Cell cycle analysis was performed using a Dean–Jett–Fox model with doublet exclusion in FlowJo 7.6.5 software. For cell division, B cells were labeled with CFSE (Invitrogen) following the manufacturer’s protocol and stimulated for 3 d. Proliferation was measured by flow cytometry, and the

![FIGURE 1.](Image 1)

**FIGURE 1.** Model for DNA replication. Homotrimeric PCNA (yellow ring) binds to Okazaki fragments (short red lines) and holds the high-fidelity DNA polymerase δ (green circle) on the template DNA. In wild-type cells, ATAD5 (blue oval) unloads PCNA from the replication fork after DNA synthesis is complete. In Atad5^+/m^ cells, PCNA accumulates on the chromatin and may affect the immune response.

![FIGURE 2.](Image 3)

**FIGURE 2.** ATAD5 downregulation in heterozygous B cells. (A) ATAD5, β-actin, and PCNA protein levels in Atad5^+/+ and Atad5^+/m^ cells. Representative Western blot analysis of splenic B cells stimulated with LPS and IL-4 for 0–3 d is shown. (B) Quantification of Western blot signal for ATAD5 normalized to β-actin for cells from wild-type (diamond) or heterozygous (circle) mice stimulated for 0 or 1 d. Bars indicate average signal. Data are from three to four mice per genotype using one mouse per experiment. The p value was determined with a one-tailed equal variance Student t test. (C) Western blot signal for PCNA normalized to β-actin, similar to (B). Data are from five to seven mice of each genotype with one mouse per experiment.
number of cells per generation was calculated using FlowJo 7.6.5 software. Viability was measured by 7-aminoactinomycin D staining.

**Double-strand break detection**

DNA was isolated from cells that had been stimulated for 2 d, ligated to a blunt double-strand linker, and amplified by ligation-mediated PCR (LM-PCR) as previously described (16). PCR products were separated by electrophoresis, blotted onto a membrane, and hybridized to a probe located in the switch $\mu$ ($S_\mu$) region. Hybridization intensity was quantified using ImageQuant TL software.

**Class switch recombination**

B cells were stimulated with LPS as described above and with 0.5 $\mu$g/ml anti-mouse CD40 (clone FGK45, Enzo Life Sciences). The following cytokines were then added for specific switching: for IgG1, IL-4 as described above; for IgG2b, 2 ng/ml TGF-$\beta$ (R&D Systems); for IgG3, no additional cytokines; and for IgA, IL-4, TGF-$\beta$, and 1.5 ng/ml mouse IL-5 (R&D Systems). Flow cytometry analysis of switched populations was conducted after 4 d using cells stained with FITC- or PerCP-labeled anti-B220 (clone RA3-6B2, eBioscience) and either allophycocyanin-conjugated anti-IgG1 (clone M1-14D12, eBioscience), recombinant PE-conjugated IgG2b, IgG3, or IgA Abs (SouthernBiotech). For germine transcripts, B cells were stimulated 4 d as described above. mRNA was harvested and converted to cDNA as stated for AID qPCR. PCR was performed as given previously (17, 18) using primers synthesized by Integrated DNA Technologies.

**Microhomology and Igh/c-myc translocations**

For microhomology, cells were stimulated with LPS for 4 d, and DNA containing $S_{\mu}$–$S_{\gamma}3$ joins was amplified, cloned, and sequenced (19). The nucleotide overlap length was based on perfect homology with no insertions. For translocations, cells were stimulated with LPS and IL-4 and harvested after 3 d. Igh–c-myc translocations were detected on chromosomes 12 or 15 using nested PCR analysis as previously described (20, 21) with $10^5$ cells per PCR amplification. PCR products were separated by gel electrophoresis, blotted, and probed sequentially with Igh- and c-myc-specific oligonucleotides.

**Results**

**Atad5$^{+/m}$ B cells have decreased ATAD5 expression**

To test the model in Fig. 1 that heterozygous mice had down-regulated ATAD5, splenic B cells were stimulated ex vivo with LPS and IL-4, and protein expression was measured over time. ATAD5 was not observed in total cell extracts prior to stimulation, but was detected as the cells began to enter the S phase at day 1 (Fig. 2A). ATAD5 levels continued to increase at day 2 and plateaued by day 3. ATAD5 downregulation in heterozygous cells was readily apparent 1 d after stimulation, with a significant 56% reduction in protein levels compared with wild-type (Fig. 2B). PCNA levels were likewise measured in cell extracts to test whether ATAD5 deficiency affects PCNA expression. As with ATAD5, PCNA upregulation occurred upon B cell entry into the S phase (Fig. 2A). There was no significant difference in PCNA expression in Atad5$^{+/m}$ cells relative to wild-type (Fig. 2C), which is similar to a report that deletion of ELG1 in yeast had no effect on PCNA levels (7). Thus, Atad5$^{+/m}$ cells express less cognate protein after stimulation but still maintain normal PCNA levels, indicating that the heterozygous mice are an appropriate model to study the effect of ATAD5 deficiency on B cell responses.

**FIGURE 3.** Effect of ATAD5 downregulation on SHM and CSR in mice. (A) SHM frequency and spectra in Peyer’s patch germinal center B cells from 4- and 9-mo-old mice. Data are from four to five independent experiments each using two to three mice per genotype. (B) SHM frequency and spectra in splenic germinal center B cells 14 and 28 d after immunization with NP-CGG. Data are from four independent experiments with five to seven mice per genotype. (C–E) Serum NP-specific IgG1 expression measured by ELISA after immunization. Bars indicate average signal. Data are from six independent experiments with five to seven mice per genotype. (C–E) Serum NP-specific IgG1 expression measured by ELISA after immunization. Bars indicate average signal. Data are from six independent experiments with five to seven mice per genotype, using one mouse per experiment. (C) High- and low-affinity Abs bound to NP(25)-BSA. The p value was determined with a two-tailed equal variance Student t test. (D) High-affinity Ab bound to NP(8)-BSA. (E) Ratio of NP (8) binding to NP(25) binding.
**Atad5<sup>+/m</sup> mice have unaltered SHM and decreased serum IgG1 in vivo**

ATAD5 regulates the access of the low-fidelity pol η to DNA through monoubiquitinated PCNA (11, 22), and a deficiency in pol η results in reduced mutations of A and T bases during SHM (15, 23–25). If pol η activity was altered in Atad5<sup>+/m</sup> cells, mutations at A:T could be changed. To test this, SHM was examined in a 492-bp region spanning the intron downstream of the rearranged J<sub>W4</sub> gene segment in the I<sub>gh</sub> locus. Germinal center B cells were isolated from either chronically stimulated Peyer’s patches in 4- or 9-mo-old mice (Fig. 3A), or spleens from mice 14 or 28 d after immunization with NP-CGG (Fig. 3B). In Peyer’s patch cells, mutation frequencies were similar in mice from both genotypes and increased with age in accord with previous studies (16, 26). In immunized spleen cells, the frequencies were also similar between genotypes and did not significantly change between days 14 and 28. Importantly, there was no difference in the spectra of mutations, particularly mutations of A and T bases, in Atad5<sup>+/m</sup> cells compared with wild-type cells from both Peyer’s patches and immunized spleens. These results indicate that ATAD5 haploinsufficiency did not alter DNA repair (no change in mutation frequency) or DNA polymerase η usage (no change in A:T mutations).

However, a significant impairment in IgG1 Ab production occurred in Atad5<sup>+/m</sup> mice. Mice were immunized with NP-CGG, and serum IgG1 levels were measured by ELISA on days 0 and 14. The average Ab affinity was determined using different conjugates of NP-conjugated BSA on microtiter plates. Both high- and low-affinity Abs bind to high-density NP(25) Ag, but only high-affinity Ab binds to low-density NP(8) Ag. At 14 d post-immunization, Atad5<sup>+/m</sup> mice exhibited a significant 22% decrease in total NP-specific Ab binding to NP(25) conjugates relative to wild-type mice (Fig. 3C). There was no difference in the amount of high-affinity Ab binding to NP(8) conjugates (Fig. 3D) or in the ratio of Ab binding to NP(8) and NP(25) (Fig. 3E). These in vivo studies indicate that ATAD5 deficiency negatively affects IgG1 Ab production but does not impact SHM or affinity maturation.

**Atad5<sup>+/m</sup> cells accumulate in the S phase and have decreased proliferation**

To probe the mechanism behind reduced IgG1 serum Ab in Atad5<sup>+/m</sup> mice, we examined CSR in cells simultaneously stimulated ex vivo with LPS and IL-4. Prior work has shown that small interfering RNA knockdown of ATAD5 in HeLa cells (9) and depletion of ELG1 protein in yeast cells (7) resulted in a delayed S phase. It has been proposed that decreased ATAD5 levels inhibit the unloading of PCNA at replication forks, and these cells are unable to progress through cell division until PCNA is unloaded after replication. Thus, cells pile up in the S phase of the cell cycle and DNA replication is slowed. To test whether a similar effect occurred in Atad5<sup>+/m</sup> heterozygous mice, the number of cells in G<sub>1</sub>, S, or G<sub>2</sub> phases was measured. Stimulated B cells, which initially enter the S phase at 24 h, were collected at 30 h. Cells were fixed with EtOH and stained with propidium iodide to measure cell cycle phase. A significant number of Atad5<sup>+/m</sup> cells accumulated in S phase relative to wild-type (Fig. 4A). No significant changes were apparent in the number of cells in G<sub>1</sub>, when AID and uracil DNA glycosylase create breaks for CSR (27, 28), or G<sub>2</sub>.

The increased number of cells in the S phase may correlate with decreased cell division. Cell division was tested via CFSE labeling prior to ex vivo stimulation. At 3 d, cells were analyzed by flow cytometry (Fig. 4B) and the number of cells per generation was calculated (Fig. 4C). There was a significant difference between

**FIGURE 4.** Cell cycle, proliferation, and AID analyses. (A) Percentage of cells in cell cycle phases after 30 h of stimulation. Data were averaged from three experiments with one mouse per genotype per experiment. The p values were determined with a two-tailed paired Student t test. (B) Representative CFSE labeling histogram from cells prior to stimulation (dashed line), or poststimulation wild-type (shaded black line) or Atad5<sup>+/m</sup> (red line) cells. (C) Quantification of CFSE-labeled cells. Error bars signify the SD of values from five independent experiments with one mouse per genotype. *p < 0.05 by a two-tailed equal variance Student t test. (D) AID expression in Atad5<sup>+/+</sup> or Atad5<sup>+/m</sup> B cells after 3 d stimulation. AID mRNA transcript levels were measured by qPCR and normalized to β-actin. The p value was determined with a one-tailed equal variance Student t test. AID protein expression was measured by Western blot, as shown in a representative blot. Quantification of AID protein was normalized to β-actin, and bars indicate the average signal. The p value was determined from a two-tailed equal variance Student t test. Both mRNA and protein data are from three experiments each using one mouse per genotype.
the two genotypes, with a greater percentage of Atad5+/m cells in generations three and four, whereas a higher percentage of wild-type cells was present in generation five, signifying that the haploinsufficient cells had slower cell division. This was not due to increased apoptosis, as cell viability was not affected by Atad5 heterozygosity during the stimulation period (data not shown). The cell cycle and division data indicate that ATAD5 aids in the progression of B cells through the S phase during cell division.

Because levels of AID protein have been shown to increase with successive cell divisions (29), AID expression was measured by both qPCR of mRNA transcripts and Western blot of protein in Atad5+/m B cells after 3 d of stimulation. The amount of AID mRNA and protein was significantly lower in the heterozygous cells (Fig. 4D), likely due to their decreased proliferation. It is thus probable that these cells will be impaired for CSR.

**Atad5+/m cells have reduced double-strand breaks, CSR, and translocations**

RNA polymerase II accumulates in the Sγ region of DNA (30, 31) and interacts with cofactors to recruit AID protein (32, 33). AID then deaminates cytosine to uracil (34), and uracils are processed into double-strand breaks, which serve as substrates for CSR and translocations. We therefore tested whether the decreased proliferation of Atad5+/m cells leads to reduced recombination in Sγ. Recombination in ex vivo–stimulated B cells was examined at multiple levels: double-strand break formation, CSR, and Igh translocation to the c-myc locus.

To measure double-strand breaks, an oligonucleotide linker was ligated to genomic DNA from cells after 2 d of stimulation. The samples were then amplified by LM-PCR (Fig. 5A) (16, 35, 36), separated by gel electrophoresis, and the products containing Sγ DNA were identified by hybridization (Fig. 5B). When the hybridization intensity was quantified, Atad5+/m cells possessed a significant decrease in breaks compared with Atad5+/+ cells (Fig. 5C).

The reduction in double-strand breaks will likely decrease CSR. A significant average decline of 37% in IgG1 switching relative to wild-type occurred in Atad5+/m cells stimulated ex vivo with LPS, anti-CD40, and IL-4 (Fig. 6A), similar to the decrease in serum IgG1 14 d after immunization. To test whether switching to other isotypes was also decreased, we activated cells under different conditions. There were significant declines of 34 and 29% relative to wild-type, respectively, for IgG2b and IgA, and a modest decrease in IgG3. It is possible that the decreased CSR is caused by insufficient germline transcription of acceptor switch regions to promote AID binding. Transcript levels following stimulation were measured for γ1, γ2b, γ3, and γ regions, but there was no change relative to wild-type (data not shown). This suggests that the decline in Atad5+/m CSR is not due to reduced access of AID to acceptor switch regions.

CSR can also be affected by the type of nonhomologous end-joining using either the classical or alternate pathways, which is determined by the microhomology length of the joined products. Classical end-joining is characterized by blunt or short overlapping sequences, whereas alternate end-joining uses longer overlaps (37, 38). Lengths of joins were measured in B cells stimulated with LPS for 4 d to induce switching to IgG3. Sβ, Sγ, and Sα were chosen because it is shorter than other S regions, which makes it easier to identify homology after recombination. DNA containing Sβ→Sγ joins was amplified, sequenced, and analyzed for the number of identical bases, or microhomology, shared between Sβ and Sγ at the break site. There was no difference in the mean length in Atad5+/m cells (1.5 bp) compared with Atad5+/+ cells (1.3 bp) (Fig. 6B), suggesting that the decline in CSR was not due to changes in end-joining.

**Double-strand breaks can instigate translocations between Sγ on chromosome 12 and exon 1 of the c-myc gene on chromosome 15.** After 4 d in culture, translocations were measured using primers to amplify the recombinated loci (Fig. 7A), and products were separated by gel electrophoresis and identified by Southern hybridization with both Igh and c-myc probes (Fig. 7B). An analysis of ~350 independent amplification reactions per genotype showed that Atad5+/m cells had significantly reduced levels of translocations, with 2.1 translocations per 107 cells, compared with wild-type cells, with 4.6 translocations per 107 cells (Fig. 7C). These results indicate that fewer double-strand breaks in short-term cultures of Atad5+/m primary cells correlated with fewer translocations. Taken together, the data show that the role of ATAD5 in DNA replication substantially restricted AID activity in recombination at the Igh locus.
Concerning SHM, although AID generates uracils during the G1 cell cycle phase (27), fixation of uracils into permanent mutations occurs during DNA replication across the mutation site in the S phase. The SHM frequency and spectra in the J_{H4} intronic region were measured in germinal center cells from chronically stimulated Peyer’s patches and from immunized spleens. There was no statistical difference in mutation frequencies between Atad5^{+/+} and Atad5^{+/m} cells in either case, suggesting that ATAD5 haploinsufficiency and accumulation of cells in the S phase did not alter fixation of uracils and their subsequent repair. In addition to releasing unmodified PCNA at replication forks, ATAD5 has been shown to regulate the levels of monoubiquitinated PCNA after DNA damage (11). Because pol η is dependent on PCNA monoubiquitination during SHM, we looked for a change in mutations at A and T bases (25, 41). Surprisingly, there were no alterations to the mutational spectra in either Peyer’s patch or immunized spleen cells, suggesting proper pol η function during SHM. Thus, decreased levels of the PCNA handler, ATAD5, did not affect SHM.

Concerning CSR, PCNA accumulation did impair switching in vivo, as evidenced by significantly reduced serum IgG1 Abs in Atad5^{+/m} mice 14 d postimmunization. There was no change in affinity maturation in the heterozygous serum pool compared with wild-type, indicating that selection for affinity was unaffected, which is consistent with the similar SHM frequencies. The cellular processes responsible for reduced switching were examined in more detail in ex vivo studies, where cells could be synchronously stimulated. Compared to wild-type cells, Atad5^{+/m} cells exhibited a build-up in the S phase that led to significantly decreased proliferation. CSR is known to be linked to the number of cell divisions (42, 43) through increased AID expression (29). In agreement with these reports, the delayed cell division observed in Atad5^{+/m} cells corresponded with a decrease in AID expression. This reduced the amount of double-strand breaks in the S_{μ} region, which act as the initiating lesions for recombination. Interchromosomal recombinations between S_{μ} and c-myc were likewise decreased. The frequency of CSR to several isotypes was also diminished, which might be due to different usage of the two pathways regulating nonhomologous end-joining. Classical, or blunt, end-joining yields predominantly productive recombination between two switch regions, resulting in CSR. Alternative, or microhomology-mediated, end-joining is associated with intraswitch recombination within an S region, producing no CSR (44, 45). The decrease in CSR and translocations does not appear to be due to defects in nonhomologous recombination pathways, be-
cause there was no difference in microhomology lengths between Atax15<sup>+/−</sup> and wild-type cells. Rather, decreased proliferation lowered AID production, which then diminished strand breaks and yielded fewer recombination events. Our results are similar to the diminished CSR in mice haploinsufficient for AID (46), but emphasize that delayed division, which also decreases AID, compromises Igh recombination as well. In total, our data suggest that ATAD5 deficiency indirectly reduces AID expression and CSR through delayed DNA replication.

Numerous proteins affect CSR through the mechanics of break recognition and joining (Ku70/80, DNA-PKcs, XRCC4, Lig4), chromatin-associated complexes at double-strand breaks (ATM, H2AX, MDC1, cohesin), and break processing (53BP1, Mre11, CtIP value, Rad9, RecQ helicases, Exo1, mismatch repair proteins, DNA polymerase ζ) (47–53). Mice with genetic defects in these proteins often have complicated phenotypes that simultaneously change multiple components of CSR. Therefore, it becomes difficult to ascertain the specific role of DNA replication in isotype switching. The experiments presented in the present study demonstrate that a mutation that delays completion of DNA replication and cell division alters the immune response by reducing AID-dependent double-strand breaks and CSR.

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Disclosures

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