CORRESPONDENCE

Uracil-DNA glycosylase is not implicated in the choice of the DNA repair pathway during B-cell class switch recombination

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Mature B-cells express membrane IgM and IgD (of same specificity) through alternative splicing of a pre-mRNA encompassing constant $(C)_\mu$ and C_δ genes. After encountering antigen, B-cells undergo class switch recombination (CSR) that substitutes the C_μ gene with C_γ, C_ε, or C_α, thereby generating IgG, IgE, and IgA antibodies with the same antigenic specificity but new effector functions. DNA-editing enzyme activation-induced deaminase (AID) is essential for CSR by targeting switch (S) regions preceding C_μ (namely, the S_μ donor region) and the C_γ, C_ε, and C_α genes (namely, the $S_{\gamma,\varepsilon,\alpha}$ acceptor regions).[1, 2] CSR is controlled in *cis* by IgH locus super-enhancers[3] and in *trans* by a wide spectrum of enzymes and proteins.[1, 2] Among them, the role of the uracil DNA glycosylase (UNG) remains controversial. UNG is a key enzyme of base excision repair, which carries out faithful repair. Some authors estimate that during CSR, the UNG enzymatic activity removes the AID-induced dC to dU converted base of single-strand DNA, generating abasic sites and leading to DNA strand breaks.[1] For other authors, the role of UNG is to stabilize the S–S synapse and to recruit DNA repair factors that facilitate the end-joining process.[4, 5] Thus, the classical non-homogenous end joining pathway would be increased over the alternative end joining (A-EJ) pathway in UNG-deficient mice,[4] suggesting an intriguing role of UNG in promoting the A-EJ pathway. These results were based on the analysis of several S_μ–$S_{\gamma1}$ sequences obtained in UNG-deficient conditions due to technical limitations (conventional Sanger sequencing) to investigate S–S junction molecular signatures. We recently reported a new computational tool (CSReport) for automatic analysis of CSR junctions sequenced by high-throughput sequencing.[6] We used this tool to analyze the rare S_μ–σ_δ junctions formed during IgD CSR[7, 8] and S_μ–$S_{\gamma3}$, S_μ–$S_{\gamma1}$, and S_μ–S_α junctions in wild-type (wt) mice.[9] We thus used CSReport and high-throughput sequencing to analyze the molecular signature of S_μ–$S_{\gamma3}$, S_μ–$S_{\gamma1}$, and S_μ–S_α junctions in UNG-deficient mice in detail.

Our research has been approved by our local ethics committee review board (Comité Régional d'Ethique sur l'Expérimentation Animale du Limousin, Limoges, France) and carried out according to the European guidelines for animal experimentation. Wt mice and UNG-deficient mice (a gift from Dr. Tomas Lindahl, UK) were used. Single-cell suspensions of spleen cells were cultured for 4 days at 1×10^6 cells/ml in RPMI 1640 with 10% fetal calf serum and 5 µg/ml LPS, with (CSR toward IgG_1) or without (CSR toward IgG_3) addition of 20 ng/ml IL-4 or 2 ng/ml TGFβ (PeproTech, Rocky Hill, NJ) (CSR toward IgA).[3, 9, 10] Splenocytes were washed in PBS and stained with various antibodies: anti-B220-PC5, anti-CD138-APC, anti-IgM PECy7, anti-IgG_3-FITC, anti-IgG_1-FITC, and anti-IgA-FITC. Cells were analyzed on a Fortessa LSR2 (Beckman Coulter). In parallel experiments, stimulated splenocyte DNA was extracted for investigation of S_μ–$S_{\gamma3}$, S_μ–$S_{\gamma1}$, and S_μ–S_α junctions. As previously described in detail,[6] junctions were amplified with PCR. Libraries of 200 bp were prepared from the 1–2 kb PCR products of S_μ–$S_{\gamma1}$, S_μ–$S_{\gamma3}$, and S_μ–S_α amplified for Ion Proton sequencing ("GénoLim platform" of the Limoges University, France). Sequenced reads were then mapped to the S_μ, $S_{\gamma1}$, $S_{\gamma3}$, and $S_\alpha$regions using BLAST algorithms. The computational tool developed for experiments performs junction assembly; identifies breakpoints in S_μ, $S_{\gamma1}$, $S_{\gamma3}$, and S_α; identifies junction structure (blunt, micro-homology, large-homology, or junction with insertions) and outputs a statistical summarization of the identified junctions.

The UNG deficiency markedly reduced CSR toward IgG_3 (mean 0.1 vs 6.9%), IgG_1 (mean 1.9 vs 14.7%), and IgA (mean 1.4 vs 2.5%) compared to that of wt mice (Fig. 1a). After DNA extraction, the molecular signatures of the S_μ–$S_{\gamma1}$, S_μ–$S_{\gamma3}$, and S_μ–S_α junctions were investigated. The structural profiles of the S_μ–$S_{\gamma1}$, S_μ–$S_{\gamma3}$, and S_μ–S_α junctions (blunt, micro-homology, large-homology, or junction with insertions) for UNG-deficient and wt mice are shown in Fig. 1b. The distributions of the IgG_1, IgG_3, and IgA junctions in terms of distance from the forward PCR primer in S_μ and from one of the reverse primers in $S_{\gamma3}$, $S_{\gamma1}$, and S_α are reported in Fig. 1c. Localizations of breakpoints within AID hotspots (AGCT, WRCY, RGYW) and other motifs are shown in Fig. 1d (displayed along specifically targeted segments within S regions).

The data indicated that if UNG deficiency markedly affected CSR efficiency, it did not significantly affect the pattern of blunt vs micro-/large-homology remaining junctions. Similarly, the positions of IgG_3, IgG_1, and IgA junctions (in term of distance from S_μ and $S_{\gamma1,\gamma3,\alpha}$) and their colocalization with AID-attack motifs were not affected. Summarization of these 1568 independent junctions provided a description of the CSR sequences in UNG-deficient mice at an unprecedented level. To date, only a few had been reported after cloning and subsequent sequencing by Sanger's method.[4] In this study, the panel of wt junctions was markedly different (in terms of blunt vs micro-homology junctions) from ours and those of Panchakshari and colleagues.[2] Our results undoubtedly demonstrate that CSR in UNG-deficient conditions did not affect the balance between N-HEJ and A-EJ. These results also showed that µ–γ_1, µ–γ_3, and µ-α CSR in UNG-deficient mice is regulated and that double-strand breaks for IgG1, IgG3, and IgA CSR are not random breaks. In conclusion, our results strengthen the hypothesis that during AID-induced CSR, UNG in association

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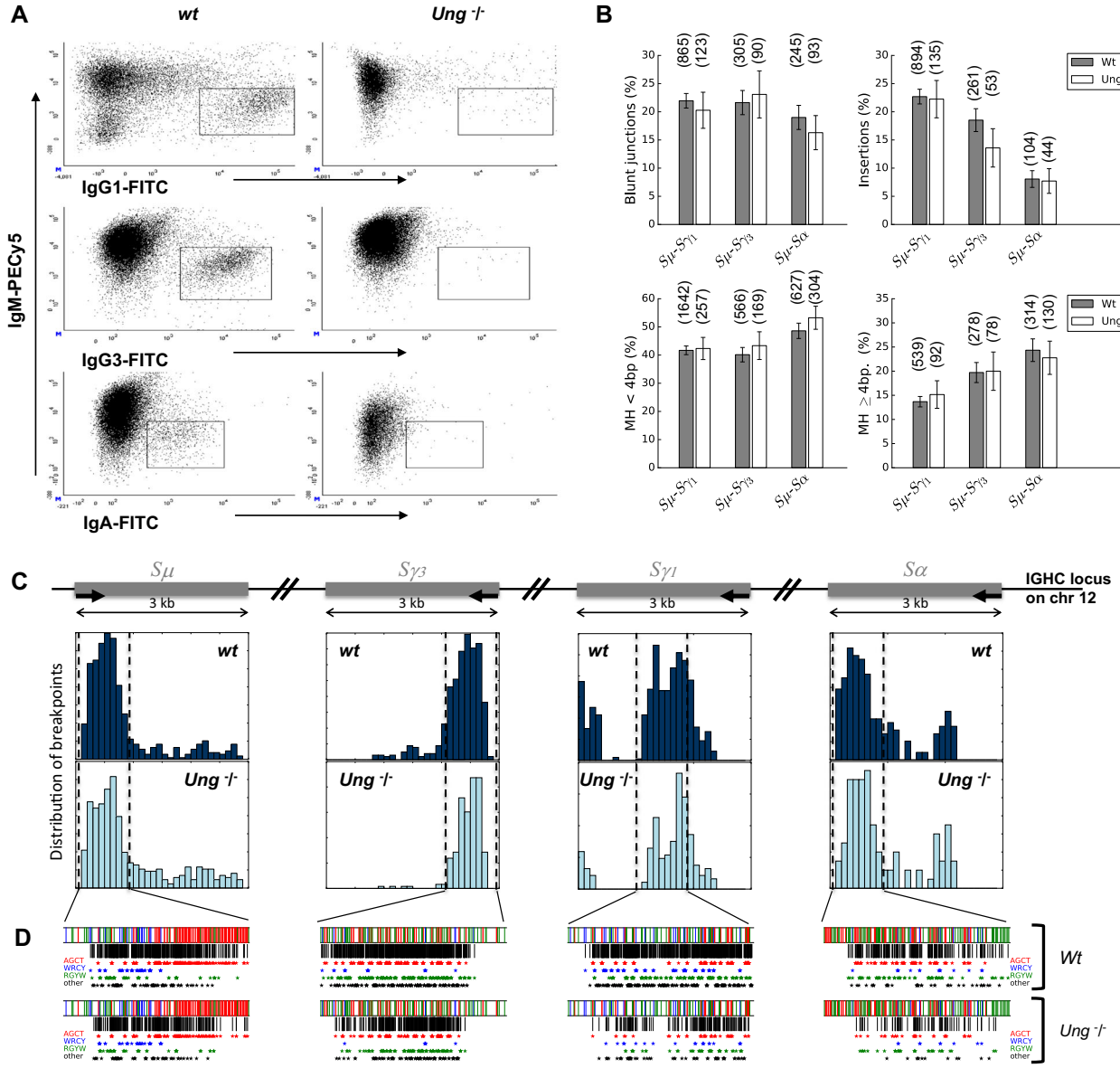

Fig. 1 Class switch recombination in UNG-deficient and wt mice. **a** IgG_1, IgG_3, and IgA CSR in UNG-deficient and wt mice. Spleen cells were cultured with LPS for 4 days with (CSR toward IgG_1) or without (CSR toward IgG_3) addition of IL-4 or TGFβ (CSR toward IgA). Cells gated on $B220^+$ and/or $CD138^+$ cells were investigated with anti-IgMPECy5, anti-IgG_3-FITC, anti-IgG_1-FITC, and anti-IgA-FITC antibodies. One representative experiment out of three to five for each genotype is shown. **b** Structure profiles of S_μ–$S_{\gamma 1}$, S_μ–$S_{\gamma 3}$, and S_μ–S_α junctions in UNG-deficient and wt mice. Junctions are classified in terms of junction types (junction with insertions, blunt junction, junction with micro- or large-homology). Numbers in parenthesis indicate the number of junctions of each type. The junction profile was not significantly different in the wt and UNG-deficient mice ($p = 0.65$, $p = 0.15$, and $p = 0.28$ for S_μ–$S_{\gamma 1}$, S_μ–$S_{\gamma 3}$, and S_μ–S_α, respectively; chi-squared test). The results are pooled from three to five mice per experiment. **c** Breakpoint localizations in S_μ–$S_{\gamma 1}$, S_μ–$S_{\gamma 3}$, and S_μ–S_α junctions in wt and UNG-deficient mice (same junctions as in Fig. 1b). **d** Motif targeting in S_μ–$S_{\gamma 1}$, S_μ–$S_{\gamma 3}$, and S_μ–S_α junctions. Stars identify breakpoint positions in AGCT, WRCY, and RGYW AID hotspots and others (same junctions as in Fig. 1b)

with recombination factors may facilitate the stabilization of the S–S synapse to facilitate efficient recombination. In contrast, our results do not argue in favor of an UNG role in the recruitment of specific DNA repair factors.

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AUTHOR CONTRIBUTIONS

Y.D. designed the research. N.G., H.I., and A.S. performed the research. A.S., F.B., and Y. D. analyzed the data. A.S., F.B., and Y.D. wrote the paper.

ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

REFERENCES

1. Chaudhuri, J. & Alt, F. W. Class-switch recombination: interplay of transcription, DNA deamination and DNA repair. *Nat. Rev. Immunol.* **4**, 541–552 (2004).
2. Panchakshari, R. A. et al. DNA double-strand break response factors influence end-joining features of IgH class switch and general translocation junctions. *Proc. Natl Acad. Sci. USA* **115**, 762–767 (2018).
3. Saintamand, A. et al. Elucidation of IgH 3′ region regulatory role during class switch recombination via germline deletion. *Nat. Commun.* **6**, 7084 (2015).
4. Yousif, A. S., Stanlie, A., Mondal, S., Honjo, T. & Begum, N. A. Differential regulation of S-region hypermutation and class-switch recombination by noncanonical functions of uracil DNA glycosylase. *Proc. Natl Acad. Sci. USA* **111**, E1016–E1024 (2014).
5. Yousif, A. S., Stanlie, A., Begum, N. A. & Honjo, T. Opinion: uracil DNA glycosylase (UNG) plays distinct and non-canonical roles in somatic hypermutation and class switch recombination. *Int. Immunol.* **26**, 575–578 (2014).
6. Boyer, F. et al. CSReport: a new computational tool designed for automatic analysis of class switch recombination junctions sequenced by high-throughput sequencing. *J. Immunol.* **198**, 4148–4155 (2017).
7. Ghazzaui, N., Issaoui, H., Saintamand, A., Boyer, F. & Denizot, Y. Analysis of IgD CSR junctions by high-throughput sequencing. *Immunol. Lett.* **188**, 86–88 (2017).
8. Issaoui, H., Ghazzaui, N., Saintamand, A., Denizot, Y. & Boyer, F. IgD class switch recombination is not controlled through the immunoglobulin heavy chain 3′ regulatory region super-enhancer. *Cell. Mol. Immunol.* **14**, 871–874 (2017).
9. Issaoui H., Ghazzaui N., Saintamand A., Denizot Y., Boyer F. High throughput sequencing reveals similar molecular signatures for class switch recombination junctions for the γ and α isotypes. *Cell. Mol. Immunol.* https://doi.org/10.1038/s41423-018-0025-z (2018).
10. Issaoui, H. et al. The IgH 3′ regulatory region super-enhancer does not control IgA class switch recombination in the B1 lineage. *Cell. Mol. Immunol.* **15**, 289–291 (2018).