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Letters to the Editor-in-Chief

The role of Apex2 in class-switch recombination of immunoglobulin genes

To the Editor-in-Chief,

In the recent publication titled 'Apex2 is required for efficient somatic hypermutation but not for class switch recombination' by Sabouri *et al.* (1), the authors reached conclusions opposite to that which we published in the Journal of Experimental Medicine in 2007 (2). Sabouri *et al.* showed that IgA switching in the CH12F3-2 cell line was not affected by Apex1 knockdown by siRNA and that class switching to IgG1 in Apex2-deficient and control splenocytes was equally efficient. The authors concluded that Apex1 and Apex2 are not required for immunoglobulin class switching and suggested that the culture conditions might be at the basis of this discrepancy. To study the role of Apex2 in class switching, Sabouri *et al.* cultured total splenocytes (consisting of ~50% T cells) from *apex2*$^{Y/-}$ and control mice with LPS + IL-4 for 4 days, whereas we cultured T-cell-depleted splenocytes (consisting of at least 90% B cells) with LPS + IL-4 for 3 days and added BLyS to our cultures. We chose to use purified B cells for these experiments to ensure that the observed phenotype is B cell intrinsic. We have now replicated the experiment presented by Sabouri *et al.* and found that indeed IgG1 was not affected by Apex2 deficiency when culturing total splenocytes without the addition of BLyS (Supplementary Figure 1, available at *International Immunology* Online and data not shown). However, class switch recombination (CSR) to IgG3 and IgG2a in total splenocyte cultures activated with LPS + anti-δ-dextran or LPS + anti-δ-dextran+IFNγ, respectively, were reduced in Apex2-deficient cells (Supplementary Figure 1, available at *International Immunology* Online). The amount of reduction is identical to that obtained using purified B cells, i.e. 30% for IgG3 and 50% for IgG2a. In contrast to the total splenocyte cultures, switching to IgG1 in purified B-cell cultures is consistently reduced by 30% (2). Moreover, class switching was reduced to a lesser extent in cultures to which BLyS was added (data not shown), opposite to what was suggested by Sabouri *et al.* In our opinion, the fact that switching to the IgG1 isotype is not affected by Apex2 deficiency in total splenocyte cultures does not warrant the general conclusion that Apex2 is not required for efficient class switching and illustrates that such firm conclusions should be based on the assessment of class switching to more than just one Ig isotype. In that respect, it is unfortunate that the authors chose to limit their analysis to IgG1 switching, since IgG1 switching is often less affected by DNA repair deficiencies than are other isotypes (3–5). Also, IgA switching is marginally affected by apurinic/apyrimidinic (AP) endonuclease deficiency (2), indicating that the CH12F3-2 cell line (which switches to IgA) is an inappropriate model to study the role of AP endonucleases in CSR. Our data suggest that there is considerable redundancy between the enzymatic activities of Apex1 and Apex2. We found that double-stranded breaks in the μ switch region were not reduced in *apex2*$^{Y/-}$ or in *apex1*$^{+/-}$ B cells but were substantially reduced in *apex1*$^{+/-}$ *apex2*$^{Y/-}$ (DBL) B cells. However, this double deficiency did not result in a further reduction in CSR, consistent with the idea that the remaining Apex1 and the few DNA double-strand breaks in these cells are sufficient for CSR. It would have been interesting if Sabouri *et al.* had knocked down Apex2 in addition to Apex1 in the CH12F3-2 cells to address this issue.

Supplementary material

Supplementary Figure 1 is available at *International Immunology* Online.

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References

1 Sabouri, Z., Okazaki, I. M., Shinkura, R. *et al.* 2009. Apex2 is required for efficient somatic hypermutation but not for class switch recombination of immunoglobulin genes. *Int. Immunol.* 21:947.
2 Guikema, J. E., Linehan, E. K., Tsuchimoto, D. *et al.* 2007. APE1- and APE2-dependent DNA breaks in immunoglobulin class switch recombination. *J. Exp. Med.* 204:3017.
3 Manis, J. P., Dudley, D., Kaylor, L. and Alt, F. W. 2002. IgH class switch recombination to IgG1 in DNA-PKcs-deficient B cells. *Immunity* 16:607.
4 Schrader, C. E., Edelmann, W., Kucherlapati, R. and Stavnezer, J. 1999. Reduced isotype switching in splenic B cells from mice deficient in mismatch repair enzymes. *J. Exp. Med.* 190:323.
5 Schrader, C. E., Vardo, J., Linehan, E. *et al.* 2004. Deletion of the nucleotide excision repair gene Ercc1 reduces immunoglobulin class switching and alters mutations near switch recombination junctions. *J. Exp. Med.* 200:321.

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