Aoufouchi et al., http://www.jem.org/cgi/content/full/jem.20070950/DC1

SUPPLEMENTAL MATERIALS AND METHODS

Anti-AID monoclonal antibody production.

Female BALB/c mice (8-wk-old; Janvier) were immunized intraperitoneally with a priming dose of 20 μg purified AID protein (produced in *Escherichia coli*, as described in [1]) in complete Freund's adjuvant (Sigma-Aldrich), followed by 2 booster immunizations at 3-wk intervals with 10 μg AID in incomplete Freund's adjuvant. 3 d before fusion, the mouse with the highest antigen-specific antibody titer received a final boost of 10 μg of AID protein in PBS. Hybridomas were generated using standard protocols. Primary hybridomas supernatants were screened by ELISA for anti-AID antibody production, and positive wells were cloned by limiting dilution in 60-well Terazaki plates in growing medium supplemented with 2.5% hybridokine (Interchim). Wells positive after two rounds of cloning were expanded for further analysis. For large-scale mAb production, hybridoma cells were cultured in RPMI, 5% FCS supplemented with the OptiMAb Monoclonal Antibody Production Enhancer (Invitrogen) according to the manufacturer's instructions. A mixture of two selected clones, 4.26.1 (IgG2a) and 4.18.1 (IgG2b), was used in this study (Fig. S2).

Knock-in of EGFP at the *AICDA* locus in the BL2 cell line.

A vector for gene targeting at the *AICDA* locus was constructed by stepwise cloning in the pBluescript SK vector (Stratagene), with an MluI–SfiI adaptor inserted in its SacI site. This vector comprises (Fig. 1 A) the following: (a) a 2.4-kb fragment amplified from the BL2 *AICDA* locus flanking the natural AatII I site in the intron between exons 2 and 3 (i.e., extending 1.555 bp upstream from exon 2); (b) a hygromycin resistance gene flanked by loxP sites inserted in AatII; a 1.9-kb fragment spanning from AatII to the end of the last AID exon, amplified with addition of a SalI site in 3′; (c) the EGFP coding sequence, inserted in the SalI site, thus introducing a Leu-Glu dipeptide between the last amino acid of AID and the first one of EGFP (amplified without its first Met residue); and (d) a 3.1-kb fragment flanking the last coding exon of AID (i.e., extending 1.112-bp downstream from the AATAAA poly(A) addition signal). The vector, linearized with NotI, was used to transfect either the BL2 Burkitt's lymphoma cell line, or clones in which one AID allele has been inactivated (1). 9 clones were diagnosed positive for homologous recombination and for the presence of the EGFP gene among 671 transfectants. Three clones of each genotype (i.e., with either a germline or a knockout configuration of the second *AICDA* allele) were transiently transfected with a Cre-expressing vector (under the CMV promoter) and EGFP-positive cells purified by two rounds of cell sorting with a FACS Vantage apparatus (Becton Dickinson).

Analysis of hypermutation.

Mutation analysis was performed on genomic DNA extracted from sorted EGFP-positive cells by the DNeasy tissue kit (QIAGEN). Amplification and sequence analysis of the rearranged V4-39 gene and of a 443-bp fragment of the Cμ locus were performed, as previously described (1). *PAX5* (exon 1B), *TP53*, and *BCL6* gene fragments were analyzed according to Pasqualucci et al. (2).The AID cDNA sequence was amplified from genomic DNA with *Pfu* DNA polymerase (Stratagene) using the following primers located in the CMV promoter and the EGFP sequence, respectively: GACCTCCATAGAAGACACCG and AGTCGTGCTGCTTCATGTGG (94°C for 30 s, 60°C for 90 s, 72°C for 2 min, 35 cycles). The PCR products were gel purified with a QIAquick gel extraction kit (QIAGEN) and cloned with the Zero blunt cloning kit (Invitrogen). Plasmid DNAs extracted from individual bacterial colonies were sequenced in an automated sequencer (ABI Prism 3100 Genetic Analyzer; Applied Biosystems).

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