PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B

Phil. Trans. R. Soc. B (2009) **364**, 577–582
doi:10.1098/rstb.2008.0210
Published online 13 November 2008

Immunoglobulin class switch recombination: study through human natural mutants

Anne Durandy[1,2,3,*]

[1]*INSERM, U768, Paris 75015, France*
[2]*Université René Descartes-Paris 5, Paris 75006, France*
[3]*Centre d'Etudes des déficits Immunitaires (C.E.D.I.), Paris 75015, France*

Immunoglobulin class switch recombination deficiencies in humans are exquisite models to analyse the mechanisms of class switch recombination (CSR). Besides defects in CD40L/CD40 interaction, others result from an intrinsic B-cell deficiency. The recent elucidation of the molecular basis of some of them has made it possible to delineate the molecular events involved in antibody maturation. Activation-induced (cytidine) deaminase (AID) and uracil-N-glycosylase deficiencies have demonstrated the role of AID as the inducer of DNA lesions in switch and variable regions. However, most of these CSR deficiencies remain molecularly undefined. Their characterization would lead to a better understanding of the complex machinery involved in CSR.

Keywords: class switch recombination; activation-induced (cytidine) deaminase; mismatch repair; non-homologous end-joining repair; double-strand DNA breaks

1. INTRODUCTION

Immunoglobulin class switch recombination (Ig-CSR) deficiencies are rare primary immunodeficiencies whose frequency is estimated as approximately 1 in 100 000 births. These deficiencies are characterized by normal or increased serum IgM levels and a contrasting, marked decrease or absence of IgG, IgA and IgE (Notarangelo *et al.* 1992). As a result of this molecular defect, defective CSR may be associated with faulty generation of somatic hypermutations (SHM) in the Ig variable (V) region. The molecular identification and analysis of several Ig-CSR deficiencies has made it possible to better describe the mechanisms underlying CSR and SHM, both of which are key elements in the maturation of antibody responses (Durandy *et al.* 2007).

Maturation of the antibody repertoire produces several antibody isotypes with high antigen affinity, a necessary feature for optimized antibody responses. Antibody maturation occurs mostly in the germinal centres of the secondary lymphoid organs, following antigen and T-cell-driven activation. CSR results in the production of antibodies of different isotypes (IgG, IgA and IgE) with the same V(D)J specificity and therefore the same antigen affinity (Iwasato *et al.* 1990). SHM primarily introduces stochastic mutations (10^{-3} bp/cell cycle) into the variable (V) region of the Ig, a genetic modification that is followed by the positive selection of B cells harbouring a B-cell receptor (BCR) with high antigen affinity (Storb *et al.* 1998). CSR and SHM occur together in germinal centres with BCR/CD40 activation; however, neither is a prerequisite for the other because IgMs may be mutated in the absence of any such feature in IgG or IgA isotypes (Kaartinen *et al.* 1983).

Mutations in the gene encoding the CD40 ligand (CD40L, CD154; Korthauer *et al.* 1993), a molecule highly expressed in activated follicular helper T cells (Breitfeld *et al.* 2000), results in an Ig-CSR deficiency. The defect is generally associated with reduced SHM generation. This observation has been corroborated by the description of a similar phenotype caused by mutations in *CD40* (Ferrari *et al.* 2001) and demonstrates the essential role of the CD40 signalling pathway in B cells for both CSR and SHM.

Other Ig-CSR deficiencies are a consequence of an intrinsic defect in the CSR machinery (Durandy *et al.* 1997). The autosomal recessive Ig-CSR deficiency, caused by mutations in the *AICDA* gene (encoding the activation-induced (cytidine) deaminase, AID), is characterized by the impairment of both CSR and SHM (Revy *et al.* 2000). This finding, together with the description of the phenotypic characteristics of $AID^{-/-}$ mice (Muramatsu *et al.* 2000), has demonstrated the master role of AID in antibody maturation. In fact, AID selectively changes cytosine residues into uracils in the switch (S) and V regions (Bransteitter *et al.* 2003). Uracil-N-glycosylase, which is also mutated in another reported CSR deficiency (Imai *et al.* 2003*b*), removes uracil residues (introduced into DNA by AID; Rada *et al.* 2002) and thus produces an abasic site that is cleaved by a specific endonuclease (Guikema *et al.* 2007). This event ultimately leads to scattered, single-stranded DNA breaks.

In fact, AID- and UNG-induced DNA lesions are differently repaired in S and V regions, respectively. In S regions, CSR-induced double-strand break (DSB) repair requires phosphorylation of the H2AX histone (γH2AX) (Celeste *et al.* 2002) and the presence of the

*Author and address for correspondence: Hôpital Necker-Enfants Malades, 149 rue de Sèvres, 75015 Paris, France (anne.durandy@inserm.fr).

One contribution of 17 to a Discussion Meeting Issue 'DNA deamination in immunity, virology and cancer'.

MRE11/RAD50/NBS1 complex (Theunissen *et al.* 2003; Reina-San-Martin *et al.* 2005), 53BP1 (Ward *et al.* 2003), MDC1 (Lou *et al.* 2006) and some of the molecules in the non-homologous end-joining (NHEJ) repair pathway, as shown by the phenotype of mice or B cells deficient in each of these molecules (Rolink *et al.* 1996; Casellas *et al.* 1998). It is very likely that other DNA repair molecules are required, since a B-cell-intrinsic CSR defect (associated with increased sensitivity to ionizing radiation) has been shown not to be linked to any defects in these molecules (Peron *et al.* 2007). Mutations in the *MRE11* (leading to ataxia-like disease), Nijmegen breakage syndrome (*NBS1*) and *ATM* (ataxia-telangiectasia) genes can lead to Ig-CSR deficiencies and thus reveal the role of these molecules in S region DNA repair in humans (Pan *et al.* 2002; Lahdesmaki *et al.* 2004). The mismatch repair (MMR) system is known to play a role in CSR in mice, as shown by diminished, switched, isotype serum levels and abnormal switch junctions (Ehrenstein & Neuberger 1999; Schrader *et al.* 1999): the MSH2/MSH6 complex appears to recognize AID-induced DNA mismatch in the absence of UNG, leading to back-up CSR and a skewed SHM pattern, as shown by the CSR-defective phenotype of a double UNG–MSH2 knockout mutant (Rada *et al.* 2004). Recently, it has also been reported that MSH5 variants in humans can be associated with common variable immunodeficiency and IgA deficiency (Sekine *et al.* 2007). The role of the PMS2/MLH1 complex is less clear; it has been recently proposed that it can convert DNA single-strand breaks (SSBs) into DSBs, as suggested by the low occurrence of blunt DSB observed in S regions of murine PMS2-deficient B cells (Schrader *et al.* (2007).

The error-prone repair of SHM-induced DNA breaks requires the MMR and error-prone DNA polymerases but not NHEJ (Rolink *et al.* 1996; Casellas *et al.* 1998), γH2AX (Reina-San-Martin *et al.* 2003), 53BP1 (Manis *et al.* 2004), MRE11, NBS1 or ATM (Pan-Hammarstrom *et al.* 2003). The MSH2/MSH6 complex is essential in SHM for (i) recognizing the U/G residues induced by cytidine deamination by AID and (ii) recruiting EXO1 and polymerase η (Xue *et al.* 2006). The role of the PMS2/MLH1 complex in SHM remains subject to debate (Winter *et al.* 1998; Phung *et al.* 1999).

All steps of antibody maturation are not thus perfectly understood, but their understanding has been greatly improved by the characterization of inherited Ig-CSR deficiencies. We herein describe two new Ig-CSR deficiencies, one is very likely due to a defect in AID targeting on S regions and the other caused by an abnormal repair of S regions.

2. RESULTS

(a) *Ig-CSR deficiency possibly due to a defect in AID CSR-specific cofactor?*

In our experience, a subgroup of 16 patients are affected by a novel Ig-CSR deficiency, the phenotype of which has been extensively studied.

(i) *Patients' phenotype*

This syndrome is probably transmitted as an autosomal recessive disease, according to the M/F ratio (=1) and the frequency of consanguinity (20%). Patients present with a similar phenotype as AID-deficient patients, i.e. recurrent bacterial infections, auto-immune manifestations and lymphadenopathies. However, lymphadenopathies are less impressive than those observed in AID deficiency and no typical AID-deficient giant germinal centres are observed.

(ii) *Ig-CSR deficiency*

The *in vivo* CSR defect, as judged by serum Ig levels, is severe with intense decrease or absence of IgG and complete lack of IgA. No switched IgM–IgD B cells are observed in peripheral blood, although CD27+ B cells are normally present.

The *in vitro* CSR defect is intense, with no recombination towards switched isotypes in CSR-activated B cells. In order to localize the CSR defect, we checked for the expression of germline, circular and functional IgE transcripts in CD40L+IL4-activated B cells. Germline transcripts were normally present, but circular and functional transcripts were missing, in correlation with lack of IgE in supernatants. Moreover, blunt DNA DSBs were not detected by the LM-PCR sensitive technique in Sμ regions. Since these characteristics were reminiscent of those of AID deficiency, we sequenced the *AICDA* gene: no abnormality was found, *AICDA* transcripts were normally expressed and AID protein expression was normally found by Western blot in activated B cells.

Thus, the defect is located downstream from the transcription step and upstream from the S region DNA cleavage, and is not caused by AID deficiency.

(iii) *Normal uracil excision assay*

The uracil excision as shown in mice (Rada *et al.* 2002) and humans (Imai *et al.* 2003*b*) and the APEX-induced abasic site cleavage as shown in mice (Guikema *et al.* 2007) are the following steps required for repair of the AID-induced DNA lesions. Although UNG, APEX1 and 2 sequences were normal as well as their RNA transcripts' expression, we checked for their activities on a nucleotidic substrate containing a U:G mismatch. Protein extracts from patients' EBV-B cell lines did normally cleave the probe, ruling out a defect in this pathway.

(iv) *Normal SHM*

AID deficiency is known to induce a defect in both CSR and SHM (Revy *et al.* 2000), and UNG deficiency to lead to a skewed pattern of SHM. We therefore checked for SHM occurring in VH of IgM in patients' CD27+B cells: normal frequency and pattern of SHM was observed.

(v) *Normal radiation sensitivity*

Since some Ig-CSR deficiencies are associated with a DNA repair defect (Peron *et al.* 2007) and see below, we checked for radiosensitivity of patients' fibroblasts and EBV-B cell lines: radiosensitivity was found in normal ranges.

This phenotype is very similar to that caused by AID C^{ter} mutations. Indeed, we have previously shown that mutations located in the C^{ter} of AID lead to a profound Ig-CSR deficiency without affecting the SHM process (Ta *et al.* 2003), strongly suggesting that AID, besides its cytidine deaminase activity, exerts another activity in CSR, possibly by recruiting a CSR-specific cofactor. Similar observation was obtained when the activity of an artificial AID mutant with deletion of its C^{ter} part was studied after transduction in $AID^{-/-}$ murine B cells (Barreto *et al.* 2003). This Ig-CSR deficiency could thus be caused by a defect in this AID CSR-specific cofactor. The nature of this cofactor remains unknown.

One hypothesis could be that the cofactor is required for targeting of AID on S regions since the CSR-induced DSBs are shown not to occur in Sμ regions from patients' B cells, localizing the CSR defect, as in AID deficiency, downstream from the transcription step but upstream from the DNA cleavage. AID targeting remains indeed unelucidated: there are neither consensus nor homologous sequences around the S junctions, in contrast to the RSS regions recognized specifically by Rag enzymes during the V(D)J recombination. The sequence structure of S regions (Honjo 2002; Yu *et al.* 2003) or the DNA conformation during transcription could be required for AID targeting (Yu *et al.* 2003; Shen *et al.* 2005), but no molecular characterization of the interaction is available, making possible the role of cofactors. An interaction between the 32 Kd subunit of the DNA-binding replication protein A (RPA) complex and AID has been reported as essential for AID binding to DNA in both S and V regions (Chaudhuri *et al.* 2004). However, this interaction is probably unaffected in this Ig-CSR deficiency since SHM are normally found.

Another possibility could be a defect in AID phosphorylation. Indeed, AID phosphorylation by protein kinase A (PKA) on S38 and T27 amino acids has been shown to be required for AID interaction with RPA, binding to double-stranded DNA, and activity in CSR. Artificial mutants carrying alanine substitutions on S38 and T27 amino acids are unable to induce CSR when transduced in $AID^{-/-}$ murine splenic B cells (Basu *et al.* 2005). However, the same requirement of AID phosphorylation for interaction with RPA and SHM has also been recently described (McBride *et al.* 2006).

Other additional post-transcriptional modification of AID, required for CSR and dispensable for SHM, could thus be responsible for this Ig-CSR deficiency if defective.

Conversely, study of mutations occurring in Sμ regions under AID activation has revealed that they normally occur in mice $AID^{-/-}$ B cells when transduced by AID C^{ter} mutants, strongly suggesting that the AID cofactor binding the C^{ter} part of AID is not involved in AID targeting on S regions but in downstream steps (Barreto *et al.* 2003). Several cofactors, DNA-PKcs and murine double minute 2 (MDM2), have been reported as binding the C terminal of AID (Macduff *et al.* 2005; Wu *et al.* 2005). They could represent CSR-specific AID cofactors since (i) the NHEJ system has been shown to be required for CSR but dispensable for SHM (Rolink *et al.* 1996) and (ii) MDM2 binds the NBS1 molecule (Alt *et al.* 2005), for which a role in CSR-induced DNA repair has been described (Reina-San-Martin *et al.* 2005). MDM2 has been further shown to be required for gene conversion in the chicken DT-40 cell line, but no data on CSR are available. The involvement of both molecules has been excluded as causative of the Ig-CSR deficiency observed in the patients. Its molecular characterization would make it possible to define the CSR-specific cofactor that AID recruits by its C terminus, up to now elucidated.

(b) *Ig-CSR deficiency probably due to a defect in DNA repair of S regions*

A novel Ig-CSR deficiency, so far found in 36 patients, has been defined, even if its molecular basis remains unknown (Imai *et al.* 2003*a*).

(i) *Patients' phenotype*

This syndrome is probably transmitted as an autosomal recessive disease, according to the M/F ratio (=1.2) and the frequency of consanguinity (17%). The prognosis is worsened by the severity of infections (four patients died from infections), the occurrence of auto-immune manifestations found in eight patients and of B-cell lymphomas (seven patients). Lymphadenopathies can be present and biopsies reveal disorganized germinal centres.

(ii) *Ig-CSR deficiency*

The *in vivo* CSR defect, as judged by serum Ig levels, is less severe than AID deficiency with often residual levels of IgG and IgA. No switched IgM–IgD B cells are observed in peripheral blood, and CD27+ B cells counts are strongly decreased.

The *in vitro* CSR defect is intense, with no recombination towards switched isotypes in CSR-activated B cells. In order to localize the CSR defect, we checked for the expression of germline, circular and functional IgE transcripts in CD40L+IL4-activated B cells. Germline transcripts were normally present, but circular and functional transcripts were missing, in correlation with lack of IgE in supernatants. In contrast to what is observed in AID deficiency, blunt DNA DSBs are normally detected in Sμ regions of CSR-activated B cells. Thus, the CSR defect is located downstream from DSB.

The residual IgA production allowed us to characterize Sμ–Sα junctions *ex vivo*, from patients' B cells. The majority of junctions displayed a perfectly matched homology (microhomology) of 1 bp or above and no junction showed precisely joined blunt ends, whereas control junctions were either blunt or use 1 bp homology.

(iii) *Skewed pattern of SHM*

SHM frequency was found slightly decreased or normal variably within the CD27+ B cells. Of note, the count of this population was lower than normal (less than 10%). Nucleotide substitution reveals a skewed pattern with an excess of transitions on G:C residues.

(iv) *Normal uracil excision assay*
Since the skewed pattern observed in SHM was very similar to that observed in UNG deficiency (Imai *et al.* 2003*b*), we checked for UNG activity using a nucleotidic probe containing a U:G mismatch. Protein extracts from patients' EBV-B cell lines and fibroblasts did normally cleave the probe, ruling out a defect in this pathway.

(v) *Increased radiosensitivity*
The occurrence of B lymphomas, the CSR defect located downstream from the DSB and the abnormality of switch junctions were strongly evocative of a DNA repair defect in S regions. Since it is known that AID is sufficient in fibroblasts to induce CSR on an appropriate substrate (Okazaki *et al.* 2002), we postulate that the defect in the DNA repair factor should be detectable in patients' fibroblasts. The radiosensitivity of patients' fibroblasts was thus tested, giving evidence for a reproducibly increased radiosensitivity. Although the increased radiosensitivity was less marked than that of fibroblasts from patients suffering from ataxia-telangiectasia (A-T) or Artemis deficiency used as controls, it was significantly different from that of healthy fibroblasts. These results were confirmed by using EBV-B cell lines. Thus, this Ig-CSR deficiency is associated with a DNA repair defect.

(vi) *Characterization of the DNA repair defect*
Excessive radiosensitivity could be due to a DNA repair defect caused by an impaired recruitment of repair proteins to DSB. We therefore studied one of the earliest responses to DNA damage, namely the induction of histone H2AX (γH2AX) phosphorylation (Rogakou *et al.* 1998; Paull *et al.* 2000). γH2AX is essential for keeping DNA ends together and for stabilizing the association of DNA repair factors such as the MRE11/RAD50/NBS1 (MRN) complex, 53BP1 (tumour protein p53-binding protein 1) and mediator of DNA damage checkpoint 1 (MDC1) at the site of the damage. DNA repair foci, including γH2AX, MRE11, 53BP1 and MDC1 were equally recruited after irradiation in control and patients' cells, both in fibroblasts and in EBV-B cell lines.

Another early event occurring rapidly after DNA damage is cell-cycle progression arrest. We thus studied the inhibition of cycle progression in patients' fibroblasts after irradiation. Both arrests of entry into S phase (G1/S checkpoint) and into mitosis (G2/M checkpoint) were normally observed in patients' cells in contrast to A-T fibroblasts that exhibited, as expected, a drastic defect in both checkpoints.

The major DSB DNA repair pathway used in mammals is the NHEJ pathway. Some NHEJ factors have been shown to be necessary during CSR (Casellas *et al.* 1998; Manis *et al.* 1998). To study the ability of patients' cells to join double-stranded DNA ends by this pathway, we analysed the *in vitro* end joining of linearized plasmid DNA by using patients' fibroblast and/or EBV-B cell-line extracts (Buck *et al.* 2006). The DNA-end ligation assay resulted in the formation of DNA concatemers with extracts from both patients and controls. We next examined the ability of patients' cells to join incompatible DNA DSB formed *in vivo* and thus requiring DNA break processing before ligation. Patients' and controls' fibroblasts were transfected with restriction enzyme-digested, linearized plasmids containing incompatible 3′–3′ overhang ends. Recircularized plasmids were recovered 72 hours after transfection and their junctions were studied by DNA sequencing. Most junctions in plasmids recovered from both patient and control fibroblasts showed similarly accurate repair.

Altogether, these results show that the increased sensitivity of cells to γ-irradiation observed in patients results from neither a defect in the initial DNA damage sensing nor a defect in the cell-cycle checkpoints induced by DNA damage, nor a defect in the NHEJ pathway. Thus, an as yet uncharacterized defect in a DNA repair pathway can be postulated to account for a unique phenotype characterized by defective CSR and SHM, associated with an abnormality of the switch junction repair and increased cell radiosensitivity. This factor could be required for efficient NHEJ in S regions and DNA repair of V regions. It could also be NHEJ independent. Of note, AID-dependent illegitimate recombination events occurring between the IgH locus and c-myc in B-cell lymphomagenesis have been shown to be mediated by an as yet unknown, NHEJ-independent process (Ramiro *et al.* 2006). It is thus attractive to consider that this as yet uncharacterized DNA repair pathway might be physiologically involved in the CSR and SHM processes.

3. CONCLUDING REMARKS

The ongoing delineation of inherited HIGM syndromes is shedding new light on the process of physiological antibody maturation in humans. However, although some steps of antibody maturation have been clarified during the last years by the characterization of human Ig-CSR deficiencies, such as AID or UNG deficiencies, some others remain undefined: how does AID target switch and variable Ig loci, how are the double-strand DNA breaks generated and repaired? The characterization of Ig-CSR deficiencies could allow answering these important questions in the near future.

This work was supported by grants from the Institut National de la Santé et de la Recherche Médicale, the Association de la Recherche contre le Cancer, the Association Nationale pour la Recherche and EURO-PADnet (FP7 European Programme).

REFERENCES

Alt, J. R., Bouska, A., Fernandez, M. R., Cerny, R. L., Xiao, H. & Eischen, C. M. 2005 Mdm2 binds to Nbs1 at sites of DNA damage and regulates double strand break repair. *J. Biol. Chem.* **280**, 18 771–18 781. (doi:10.1074/jbc.M413387200)

Barreto, V., Reina-San-Martin, B., Ramiro, A. R., McBride, K. M. & Nussenzweig, M. C. 2003 C-terminal deletion of AID uncouples class switch recombination from somatic hypermutation and gene conversion. *Mol. Cell* **12**, 501–508. (doi:10.1016/S1097-2765(03)00309-5)

Basu, U., Chaudhuri, J., Alpert, C., Dutt, S., Ranganath, S., Li, G., Schrum, J. P., Manis, J. P. & Alt, F. W. 2005 The

AID antibody diversification enzyme is regulated by protein kinase A phosphorylation. *Nature* **438**, 508–511. (doi:10.1038/nature04255)

Bransteitter, R., Pham, P., Scharff, M. D. & Goodman, M. F. 2003 Activation-induced cytidine deaminase deaminates deoxycytidine on single-stranded DNA but requires the action of RNase. *Proc. Natl Acad. Sci. USA* **100**, 4102–4107. (doi:10.1073/pnas.0730835100)

Breitfeld, D., Ohl, L., Kremmer, E., Ellwart, J., Sallusto, F., Lipp, M. & Forster, R. 2000 Follicular B helper T cells express CXC chemokine receptor 5, localize to B cell follicles, and support immunoglobulin production. *J. Exp. Med.* **192**, 1545–1552. (doi:10.1084/jem.192.11.1545)

Buck, D. *et al*. 2006 Cernunnos, a novel nonhomologous end-joining factor, is mutated in human immunodeficiency with microcephaly. *Cell* **124**, 287–299. (doi:10.1016/j.cell.2005.12.030)

Casellas, R. *et al*. 1998 Ku80 is required for immunoglobulin isotype switching. *EMBO J.* **17**, 2404–2411. (doi:10.1093/emboj/17.8.2404)

Celeste, A. *et al*. 2002 Genomic instability in mice lacking histone H2AX. *Science* **296**, 922–927. (doi:10.1126/science.1069398)

Chaudhuri, J., Khuong, C. & Alt, F. W. 2004 Replication protein A interacts with AID to promote deamination of somatic hypermutation targets. *Nature* **430**, 992–998. (doi:10.1038/nature02821)

Durandy, A. *et al*. 1997 Abnormal CD40-mediated activation pathway in B lymphocytes from patients with hyper-IgM syndrome and normal CD40 ligand expression. *J. Immunol.* **158**, 2576–2584.

Durandy, A., Taubenheim, N., Peron, S. & Fischer, A. 2007 Pathophysiology of B-cell intrinsic immunoglobulin class switch recombination deficiencies. *Adv. Immunol.* **94**, 275–306. (doi:10.1016/S0065-2776(06)94009-7)

Ehrenstein, M. R. & Neuberger, M. S. 1999 Deficiency in Msh2 affects the efficiency and local sequence specificity of immunoglobulin class-switch recombination: parallels with somatic hypermutation. *EMBO J.* **18**, 3484–3490. (doi:10.1093/emboj/18.12.3484)

Ferrari, S. *et al*. 2001 Mutations of CD40 gene cause an autosomal recessive form of immunodeficiency with hyper IgM. *Proc. Natl Acad. Sci USA* **98**, 12 614–12 619. (doi:10.1073/pnas.221456898)

Guikema, J. E., Linehan, E. K., Tsuchimoto, D., Nakabeppu, Y., Strauss, P. R., Stavnezer, J. & Schrader, C. E. 2007 APE1- and APE2-dependent DNA breaks in immunoglobulin class switch recombination. *J. Exp. Med.* **204**, 3017–3026. (doi:10.1084/jem.20071289)

Honjo, T. 2002 Does AID need another aid? *Nat. Immunol.* **3**, 800–801. (doi:10.1038/ni0902-800)

Imai, K. *et al*. 2003*a* Hyper-IgM syndrome type 4 with a B lymphocyte-intrinsic selective deficiency in Ig class-switch recombination. *J. Clin. Invest.* **112**, 136–142.

Imai, K. *et al*. 2003*b* Human uracil-DNA glycosylase deficiency associated with profoundly impaired immunoglobulin class-switch recombination. *Nat. Immunol.* **4**, 1023–1028. (doi:10.1038/ni974)

Iwasato, T., Shimizu, A., Honjo, T. & Yamagishi, H. 1990 Circular DNA is excised by immunoglobulin class switch recombination. *Cell* **62**, 143–149. (doi:10.1016/0092-8674(90)90248-D)

Kaartinen, M., Griffiths, G. M., Markham, A. F. & Milstein, C. 1983 mRNA sequences define an unusually restricted IgG response to 2-phenyloxazolone and its early diversification. *Nature* **304**, 320–324. (doi:10.1038/304320a0)

Korthauer, U. *et al*. 1993 Defective expression of T-cell CD40 ligand causes X-linked immunodeficiency with hyper-IgM. *Nature* **361**, 539–541. (doi:10.1038/361539a0)

Lahdesmaki, A., Taylor, A. M., Chrzanowska, K. H. & Pan-Hammarstrom, Q. 2004 Delineation of the role of the Mre11 complex in class switch recombination. *J. Biol. Chem.* **279**, 16 479–16 487. (doi:10.1074/jbc.M312796200)

Lou, Z. *et al*. 2006 MDC1 maintains genomic stability by participating in the amplification of ATM-dependent DNA damage signals. *Mol. Cell* **21**, 187–200. (doi:10.1016/j.molcel.2005.11.025)

Macduff, D. A., Neuberger, M. S. & Harris, R. S. 2005 MDM2 can interact with the C-terminus of AID but it is inessential for antibody diversification in DT40 B cells. *Mol. Immunol.* **43**, 1099–1108. (doi:10.1016/j.molimm.2005.07.024)

Manis, J. P., van der Stoep, N., Tian, M., Ferrini, R., Davidson, L., Bottaro, A. & Alt, F. W. 1998 Class switching in B cells lacking 3′ immunoglobulin heavy chain enhancers. *J. Exp. Med.* **188**, 1421–1431. (doi:10.1084/jem.188.8.1421)

Manis, J. P., Morales, J. C., Xia, Z., Kutok, J. L., Alt, F. W. & Carpenter, P. B. 2004 53BP1 links DNA damage-response pathways to immunoglobulin heavy chain class-switch recombination. *Nat. Immunol.* **5**, 481–487. (doi:10.1038/ni1067)

McBride, K. M., Gazumyan, A., Woo, E. M., Barreto, V. M., Robbiani, D. F., Chait, B. T. & Nussenzweig, M. C. 2006 Regulation of hypermutation by activation-induced cytidine deaminase phosphorylation. *Proc. Natl Acad. Sci. USA* **103**, 8798–8803. (doi:10.1073/pnas.0603272103)

Muramatsu, M., Kinoshita, K., Fagarasan, S., Yamada, S., Shinkai, Y. & Honjo, T. 2000 Class switch recombination and hypermutation require activation-induced cytidine deaminase (AID), a potential RNA editing enzyme. *Cell* **102**, 553–563. (doi:10.1016/S0092-8674(00)00078-7)

Notarangelo, L. D., Duse, M. & Ugazio, A. G. 1992 Immunodeficiency with hyper-IgM (HIM). *Immunodefic. Rev.* **3**, 101–121.

Okazaki, I. M., Kinoshita, K., Muramatsu, M., Yoshikawa, K. & Honjo, T. 2002 The AID enzyme induces class switch recombination in fibroblasts. *Nature* **416**, 340–345. (doi:10.1038/nature727)

Pan-Hammarstrom, Q., Dai, S., Zhao, Y., van Dijk-Hard, I. F., Gatti, R. A., Borresen-Dale, A. L. & Hammarstrom, L. 2003 ATM is not required in somatic hypermutation of VH, but is involved in the introduction of mutations in the switch mu region. *J. Immunol.* **170**, 3707–3716.

Pan, Q., Petit-Frere, C., Lahdesmaki, A., Gregorek, H., Chrzanowska, K. H. & Hammarstrom, L. 2002 Alternative end joining during switch recombination in patients with ataxia-telangiectasia. *Eur. J. Immunol.* **32**, 1300–1308. (doi:10.1002/1521-4141(200205)32:5<1300::AID-IMMU1300>3.0.CO;2-L)

Paull, T. T., Rogakou, E. P., Yamazaki, V., Kirchgessner, C. U., Gellert, M. & Bonner, W. M. 2000 A critical role for histone H2AX in recruitment of repair factors to nuclear foci after DNA damage. *Curr. Biol.* **10**, 886–895. (doi:10.1016/S0960-9822(00)00610-2)

Peron, S. *et al*. 2007 A primary immunodeficiency characterized by defective immunoglobulin class switch recombination and impaired DNA repair. *J. Exp. Med.* **204**, 1207–1216. (doi:10.1084/jem.20070087)

Phung, Q. H., Winter, D. B., Alrefai, R. & Gearhart, P. J. 1999 Hypermutation in Ig V genes from mice deficient in the MLH1 mismatch repair protein. *J. Immunol.* **162**, 3121–3124.

Rada, C., Williams, G. T., Nilsen, H., Barnes, D. E., Lindahl, T. & Neuberger, M. S. 2002 Immunoglobulin isotype switching is inhibited and somatic hypermutation perturbed in UNG-deficient mice. *Curr. Biol.* **12**, 1748–1755. (doi:10.1016/S0960-9822(02)01215-0)

Rada, C., Di Noia, J. M. & Neuberger, M. S. 2004 Mismatch recognition and uracil excision provide complementary paths to both Ig switching and the A/T-focused phase of somatic mutation. *Mol. Cell* **16**, 163–171. (doi:10.1016/j.molcel.2004.10.011)

Ramiro, A. R. *et al*. 2006 Role of genomic instability and p53 in AID-induced c-myc-Igh translocations. *Nature* **440**, 105–109. (doi:10.1038/nature04495)

Reina-San-Martin, B., Difilippantonio, S., Hanitsch, L., Masilamani, R. F., Nussenzweig, A. & Nussenzweig, M. C. 2003 H2AX is required for recombination between immunoglobulin switch regions but not for intra-switch region recombination or somatic hypermutation. *J. Exp. Med.* **197**, 1767–1778. (doi:10.1084/jem.20030569)

Reina-San-Martin, B., Nussenzweig, M. C., Nussenzweig, A. & Difilippantonio, S. 2005 Genomic instability, endoreduplication, and diminished Ig class-switch recombination in B cells lacking Nbs1. *Proc. Natl Acad. Sci USA* **102**, 1590–1595. (doi:10.1073/pnas.0406289102)

Revy, P. *et al*. 2000 Activation-induced cytidine deaminase (AID) deficiency causes the autosomal recessive form of the hyper-IgM syndrome (HIGM2). *Cell* **102**, 565–575. (doi:10.1016/S0092-8674(00)00079-9)

Rogakou, E. P., Pilch, D. R., Orr, A. H., Ivanova, V. S. & Bonner, W. M. 1998 DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. *J Biol. Chem.* **273**, 5858–5868. (doi:10.1074/jbc.273.10.5858)

Rolink, A., Melchers, F. & Andersson, J. 1996 The SCID but not the RAG-2 gene product is required for S mu-S epsilon heavy chain class switching. *Immunity* **5**, 319–330. (doi:10.1016/S1074-7613(00)80258-7)

Schrader, C. E., Edelmann, W., Kucherlapati, R. & Stavnezer, J. 1999 Reduced isotype switching in splenic B cells from mice deficient in mismatch repair enzymes. *J. Exp. Med.* **190**, 323–330. (doi:10.1084/jem.190.3.323)

Schrader, C. E., Guikema, J. E., Linehan, E. K., Selsing, E. & Stavnezer, J. 2007 Activation-induced cytidine deaminase-dependent DNA breaks in class switch recombination occur during G1 phase of the cell cycle and depend upon mismatch repair. *J. Immunol.* **179**, 6064–6071.

Sekine, H. *et al*. 2007 Role for Msh5 in the regulation of Ig class switch recombination. *Proc. Natl Acad. Sci. USA* **104**, 7193–7198. (doi:10.1073/pnas.0700815104)

Shen, H. M., Ratnam, S. & Storb, U. 2005 Targeting of the activation-induced cytosine deaminase is strongly influenced by the sequence and structure of the targeted DNA. *Mol. Cell Biol.* **25**, 10 815–10 821. (doi:10.1128/MCB.25.24.10815-10821.2005)

Storb, U., Peters, A., Klotz, E., Kim, N., Shen, H. M., Hackett, J., Rogerson, B. & Martin, T. E. 1998 *Cis*-acting sequences that affect somatic hypermutation of Ig genes. *Immunol. Rev.* **162**, 153–160. (doi:10.1111/j.1600-065X.1998.tb01424.x)

Ta, V. T. *et al*. 2003 AID mutant analyses indicate requirement for class-switch-specific cofactors. *Nat. Immunol.* **4**, 843–848. (doi:10.1038/ni964)

Theunissen, J. W., Kaplan, M. I., Hunt, P. A., Williams, B. R., Ferguson, D. O., Alt, F. W. & Petrini, J. H. 2003 Checkpoint failure and chromosomal instability without lymphomagenesis in Mre11(ATLD1/ATLD1) mice. *Mol. Cell* **12**, 1511–1523. (doi:10.1016/S1097-2765(03)00455-6)

Ward, I. M., Minn, K., van Deursen, J. & Chen, J. 2003 p53 Binding protein 53BP1 is required for DNA damage responses and tumor suppression in mice. *Mol. Cell Biol.* **23**, 2556–2563. (doi:10.1128/MCB.23.7.2556-2563.2003)

Winter, D. B. *et al*. 1998 Altered spectra of hypermutation in antibodies from mice deficient for the DNA mismatch repair protein PMS2. *Proc. Natl Acad. Sci USA* **95**, 6953–6958. (doi:10.1073/pnas.95.12.6953)

Wu, X., Geraldes, P., Platt, J. L. & Cascalho, M. 2005 The double-edged sword of activation-induced cytidine deaminase. *J. Immunol.* **174**, 934–941.

Xue, K., Rada, C. & Neuberger, M. S. 2006 The *in vivo* pattern of AID targeting to immunoglobulin switch regions deduced from mutation spectra in msh2$^{-/-}$ ung$^{-/-}$ mice. *J. Exp. Med.* **203**, 2085–2094. (doi:10.1084/jem.20061067)

Yu, K., Chedin, F., Hsieh, C. L., Wilson, T. E. & Lieber, M. R. 2003 R-loops at immunoglobulin class switch regions in the chromosomes of stimulated B cells. *Nat. Immunol.* **4**, 442–451. (doi:10.1038/ni919)