

Pathophysiology of B-Cell Intrinsic Immunoglobulin Class Switch Recombination Deficiencies

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Abstract

B-cell intrinsic immunoglobulin class switch recombination (Ig-CSR) deficiencies, previously termed hyper-IgM syndromes, are genetically determined conditions characterized by normal or elevated serum IgM levels and an absence or very low levels of IgG, IgA, and IgE. As a function of the molecular mechanism, the defective CSR is variably associated to a defect in the generation of somatic hypermutations (SHMs) in the Ig variable region. The study of Ig-CSR deficiencies contributed to a better delineation of the mechanisms underlying CSR and SHM, the major events of antigen-triggered antibody maturation. Four Ig-CSR deficiency phenotypes have been so far reported: the description of the activation-induced cytidine deaminase (AID) deficiency (Ig-CSR deficiency 1), caused by recessive mutations of AICDA gene, characterized by a defect in CSR and SHM, clearly established the role of AID in the induction of the Ig gene rearrangements underlying CSR and SHM. A CSR-specific function of AID has, however, been detected by the observation of a selective CSR defect caused by mutations affecting the C-terminus of AID. Ig-CSR deficiency 2 is the consequence of uracil-N-glycosylase (UNG) deficiency. Because UNG, a molecule of the base excision repair machinery, removes uracils from DNA and AID deaminates cytosines into uracils, that observation indicates that the AID-UNG pathway directly targets DNA of switch regions

from the Ig heavy-chain locus to induce the CSR process. Ig-CSR deficiencies 3 and 4 are characterized by a selective CSR defect resulting from blocks at distinct steps of CSR. A further understanding of the CSR machinery is expected from their molecular definition.

1. Introduction

B-cell intrinsic immunoglobulin class switch recombination (Ig-CSR) deficiencies are rare primary immunodeficiencies, usually denominated hyper-IgM syndromes, the frequency of which is evaluated to 1 in 100,000 births. All are characterized by a defective Ig-CSR, as shown by the serum Ig level determination: IgM levels are either normal or increased, contrasting with a strong decrease or an absence of IgG, IgA, and IgE (Notarangelo *et al.*, 1992). All mature B cells carry membrane IgM and IgD or IgM only, since no switched Ig (IgG or IgA) is produced. As a function of the molecular defect, the defective CSR is associated or not with a defective generation of somatic hypermutations (SHMs) into the Ig variable (V) region. The definition of different Ig-CSR deficiencies made it possible a better delineation of the mechanisms underlying CSR and SHM, both required for maturation of antibody responses.

Maturation of the antibody repertoire results in the production of antibodies of various isotypes with high affinity for antigen, a process that is required for providing an efficient humoral response. Antibody maturation occurs in the germinal centers of the secondary lymphoid organs following antigen and T-cell-driven activation: (1) the CSR results in the production of antibodies of different isotypes (IgG, IgA, and IgE) with the same V specificity, and therefore the same antigen affinity (Iwasato *et al.*, 1990; Kinoshita and Honjo, 2000; Matsuoka *et al.*, 1990). CSR is necessary for adaptative antibody response since the different Ig isotypes exhibit distinct biological activities: IgG has a longer half-life than IgM (21 and 5 days, respectively), binds to Fc γ receptors increasing phagocytosis of phagocytic cells and activates the complement system. IgA, which is mostly produced in mucosae, is a first means of defense toward pathogens. IgE plays a major role against antihelminthic infection. (2) The SHM introduces with high-frequency ($1/10^3$ bp/cell cycle) stochastic mutations mostly in the V region of the Ig, a genetic modification that is followed by the positive selection of B cells carrying a B-cell receptor (BCR) with high affinity for antigen (Jacobs *et al.*, 2001; Storb *et al.*, 1998). This process requires interaction with follicular dendritic cells (Frazer *et al.*, 1997; Rajewsky, 1996). SHM-induced self-reactive B cells are either deleted or inactivated by receptor revision (Itoh *et al.*, 2000; Meffre *et al.*, 1998; Wilson *et al.*, 2000). CSR and SHM occur simultaneously in germinal centers under BCR/CD40 activation but neither is a prerequisite for the other because IgM may be mutated while

IgG or IgA can remain unmutated (Jacob and Kelsoe, 1992; Kaartinen *et al.*, 1983; Liu *et al.*, 1996).

The description of an X-linked Ig-CSR deficiency caused by mutations in the gene encoding the CD40 ligand molecule (CD40L, CD154) (Allen *et al.*, 1993; Aruffo *et al.*, 1993; Disanto *et al.*, 1993; Korthauer *et al.*, 1993), a molecule highly expressed on follicular helper T cells (Breitfeld *et al.*, 2000), has demonstrated the essential role of the CD40 signaling pathway in B cells for both events of antibody maturation. CD40L interacts with CD40 constitutively expressed on B cells, but also on monocytes and dendritic cells (Castle *et al.*, 1993; Fuleihan *et al.*, 1993; Nonoyama *et al.*, 1993). Due to a CD40 transactivation defect, the B cells of patients with this syndrome cannot proliferate or form germinal centers in secondary lymphoid organs or produce IgG and IgA *in vivo*. However, B cells are intrinsically normal as they can be induced to proliferate and to undergo CSR to generate IgG, IgA, and IgE on *in vitro* activation by CD40 agonists and appropriate cytokines (Durandy *et al.*, 1993). The defective CSR is often associated with decreased SHM (Agematsu *et al.*, 1998), an observation that indicates that CD40L/CD40 interaction is necessary for induction of both events in germinal centers. CD40— deficiency has been observed in a few patients as an autosomal recessive inherited disease and diagnosed on the lack of CD40 expression on B cells and monocytes. The clinical and immunologic findings are identical to those of CD40L deficiency, with the exception that the B cells cannot undergo CSR on *in vitro* sCD40L+ cytokine activation (Ferrari *et al.*, 2001). CD40 signaling pathway in B cells necessary for CSR and SHM involves NF- κ B, as shown by the observation that hypomorphic mutations of the gene encoding the NF- κ B essential modulator or NEMO (IKK γ), a regulatory unit of the IKK α , IKK β and IKK γ complex, can also lead in some patients to a CSR deficiency. Presumably because of the heterogeneity of the mutations, the *in vitro* CD40-mediated CSR and the SHM are found variably defective (Jain *et al.*, 2004; Durandy, unpublished data). Such variability could be also related to the fact that CD40 activation induces two NF- κ B pathways: the NF- κ B1 (p50 and its precursor p106) that requires IKK α , IKK β and IKK γ complex, and the NF- κ B2 (p52 and its precursor p100) that does not (Coope *et al.*, 2002).

Surprisingly, CD40L deficiency is in some occasions associated with detectable IgA amounts, despite a complete absence of CD40L expression. This observation points to CD40L-independent pathway(s) triggering CSR. CSR can occur through the B-cell-activating factor receptor (BAFF-R) and the transmembrane activator and calcium-modulating cyclophilin-interacting protein (TACI). Following exposure to BAFF or the proliferation-inducing ligand (APRIL), and in the presence of appropriate cytokines and BCR engagement, B cells undergo an entirely CD40-independent CSR leading to the production

of IgG and IgA. This CSR can occur in the splenic marginal zone or intestinal lamina propria (Litinskiy *et al.*, 2002). The role of BAFF and APRIL in CSR has been emphasized by the observation of IgG and/or IgA defects in patients deficient in TACI (Castigli *et al.*, 2005; Salzer *et al.*, 2005). Other T-cell molecules also play a role, such as the inducible costimulator molecule (ICOS), also expressed on follicular T cells in germinal centers. Indeed, ICOS deficiency, first described as a rare cause of common variable immunodeficiency (Grimbacher *et al.*, 2003), can lead to a defective CSR likely because of defective germinal center reaction (Warnatz *et al.*, 2006). Another molecule, the complement protein C4b-binding protein (C4BP), can also play a role in B-cell survival and CSR. It has been shown to bind CD40, to colocalize with B cells in secondary follicles and to induce CSR toward IgE in presence of IL-4 in an NF- κ B-dependent signaling pathway (Blom *et al.*, 2003; Brodeur *et al.*, 2003; Morio *et al.*, 1995).

SHMs have been shown also to occur in IgM+CD27+ B cells in the absence of CD40L expression (Weller *et al.*, 2001). These mutated IgM B cells very likely produce antibodies against encapsulated bacteria in the splenic marginal zone (Kruetzmann *et al.*, 2003). This B-cell subset displays similarity to murine B-1a B cells, which secrete natural antibodies and are required for the T-cell-independent antibody response to polysaccharide antigens (Martin and Kearney, 2000; Wardemann *et al.*, 2002). Pyk2-deficient mice, which lack marginal zone B cells, exhibit an impaired antibody response to polysaccharides (Fagarasan and Honjo, 2000; Guinamard *et al.*, 2000). The mechanism underlying the generation of SHM in the absence of CD40L/CD40 signaling remains unknown (Weller *et al.*, 2004). Another evidence for the possible occurrence of SHM outside the germinal centers is the observation that self-reactive-mutated B cells are usually generated in the T zone-red pulp border of the spleen rather than in the germinal centers (William *et al.*, 2002).

Besides these Ig-CSR deficiencies secondary to defective T- and B-cell interaction or B-cell CD40 signaling pathway, half of Ig-CSR deficiencies are caused by an intrinsic B-cell defect which directly impairs the complex machinery of the CSR process (Callard *et al.*, 1994; Durandy *et al.*, 1997). These Ig-CSR deficiencies are characterized by a selective CSR defect resulting from blocks at distinct steps of CSR. Patients are prone to infections with bacterial pathogens but not to opportunistic infections, in contrast to patients affected by Ig-CSR deficiency caused by a defective CD40 signaling pathway. The prognosis is therefore much better for these patients, although continuous Ig substitution treatment is required. Unlike patients with defective CD40 signaling pathway or ICOS deficiency, patients with these Ig-CSR deficiencies frequently present with enlargement of lymphoid organs such as the spleen, tonsils, and lymph nodes.

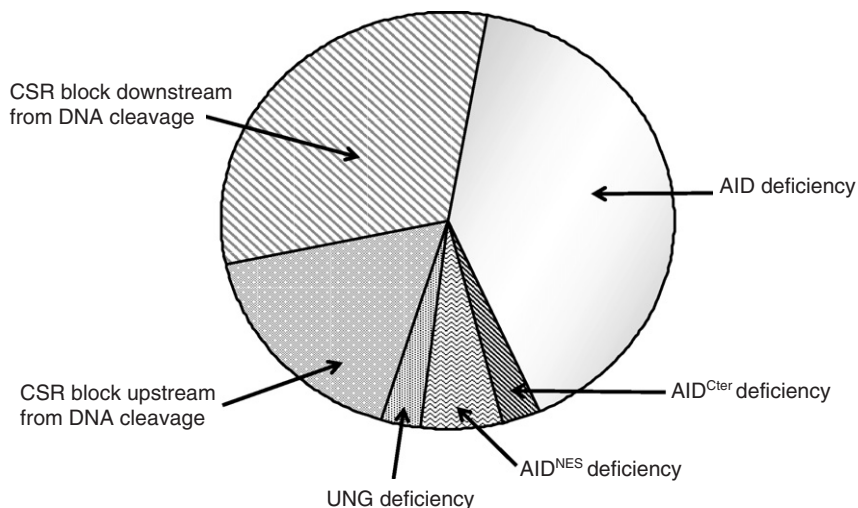


Figure 1 Relative frequency of the four different subsets of B-cell intrinsic Ig-CSR deficiencies. Frequency was evaluated according to the number of affected families.

As in the other Ig-CSR deficiencies, patients have normal or high serum IgM levels, contrasting with markedly reduced serum IgG and IgA levels. Consistent with serum Ig levels, IgG antibodies specific for pathogens or immunization antigens are absent, whereas isohemagglutinins and antipolysaccharide antigen antibodies of the IgM isotype are detected. It is, at present, unclear whether the ability of patients' B cells to produce antipolysaccharide antibodies provides a significant protection against infections by *Streptococcus pneumoniae* and *Haemophilus influenzae*.

The dissection of B-cell abnormalities and molecular definition of these B intrinsic Ig-CSR deficiencies has made possible to delineate some of the molecular events involved in the antibody maturation processes. Four different subsets are defined (Fig. 1).

2. Ig-CSR Deficiency Type 1 Caused by Activation-Induced Cytidine Deaminase Deficiency

This Ig-CSR deficiency was found to be the consequence of a defect in the activation-induced cytidine deaminase (AID), a molecule specifically expressed in activated B cells.

2.1. Classical AID Deficiency

2.1.1. Phenotype

Sixty-two patients from 43 unrelated families and different ethnic backgrounds were referred for investigation because of susceptibility to bacterial infections, mostly of the upper respiratory and digestive tracts. Impressive enlargement of the tonsils and lymph nodes was observed in 47 patients, leading in some to multiple biopsies. Pathological examination revealed the presence of giant germinal centers (5–10 times larger than normal) filled with highly proliferating B cells (Fig. 2). Proliferating B cells coexpress at plasma membrane IgM, IgD, and CD38, all phenotypic characteristics of a small B-cell subset known as germinal center founder cells (Lebecque *et al.*, 1997). These cells correspond to a transitional stage between follicular mantle and germinal center B cells, at the stage at which SHM of the IgV region gene and antigen-driven selection occur. The expression of CD95 on B cells and the presence of numerous macrophages filled with apoptotic bodies appeared to rule out a defect in apoptosis to account for germinal center enlargement, which is presumably the consequence of an intense B-cell proliferation (Revy *et al.*, 2000). IgM-mediated autoimmunity was not infrequent (12 patients), mostly affecting blood cells (autoimmune hemolytic anemia or thrombocytopenia). Other autoimmune manifestations were rarely observed, including rheumatoid arthritis (one case), autoimmune hepatitis (two cases), uveitis (one case), diabetes (one case), and systemic lupus erythematosus disease (one case). One patient suffered

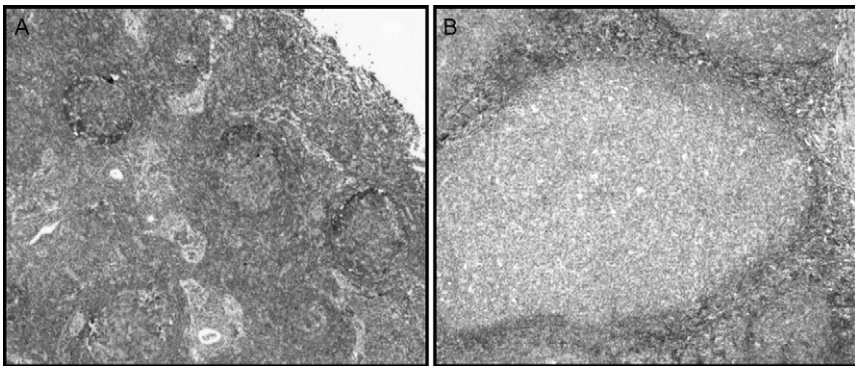


Figure 2 Giant germinal centers in AID deficiency. (A) Reactive cervical lymph node from an age-matched control. (B) Cervical lymph node from an AID-deficient patient (p.68X+p.H56-E58 del). Immunocytochemistry with an AID-specific monoclonal antibody.

from inflammatory bowel disease (Quartier *et al.*, 2004). Malignancies were not reported with the exception of an Ewing sarcoma.

These patients suffered from a drastically defective CSR, as IgG, IgA, and IgE levels were barely detectable in serum, in contrast to elevated IgM levels in all cases, except in six who were diagnosed and treated early in life. Although serum IgM levels decreased in most patients after Ig substitution was initiated, they frequently remained above normal values. Isohemagglutinins and antipolysaccharide IgM antibodies were detected, contrasting to the complete absence of IgG antibody production in response to immunization antigens or microorganisms. *In vitro*, B cells normally proliferated, produced large amounts of IgM, and upregulated the CD23 activation marker when activated by sCD40L+ appropriate cytokines. In contrast, they could not undergo CSR to generate IgG, IgA, or IgE on the same stimulation (Durandy *et al.*, 1997). Analysis of the successive events of the *in vitro* CSR enabled to place the block inside the CSR pathway. The first step of CSR resulting in the generation of Ig germ line transcripts was detectable, whereas the further steps were not: double-stranded DNA breaks (DSB) in $S\mu$ regions, as judged by a very sensitive ligation-mediated PCR technique (Catalan *et al.*, 2003), could not be detected, excision and functional Ig transcripts were equally undetectable, and no switched Ig isotype could be found in culture supernatants (Revy *et al.*, 2000). Therefore, the CSR defect was shown to be located downstream from the transcription step and upstream from the DSB creation (Table 1; Fig. 3).

All peripheral blood B cells expressed membrane IgM and most (90%) coexpressed IgD. A normal percentage (20–50%) carried CD27, a marker that has been related to the memory-mutated phenotype of B cells (Klein *et al.*, 1998). Such cells could have undergone SHM in normal conditions. SHM was investigated in the VH3–23 Ig region of purified CD19+/CD27+ B cells from 29 patients. SHM was found constantly absent or drastically decreased (0– < 1.2%) as compared to age-matched controls (2.5–6.3% mutations/bp) (Fig. 4B). Analysis of the nucleotide substitution of the few observed mutations did not reveal any obviously abnormal pattern.

2.1.2. AICDA Mutations

The observation of consanguineous families led to check for a genetic locus cosegregating with the disease. A homozygosity mapping was performed in eight families, which determined a 4.5-cM region on chromosome 12p13 with a lod score of 10.45, a locus in which *AICDA* gene encoding the B-cell-specific molecule AID (Muramatsu *et al.*, 1999) was localized (Muto *et al.*, 2000). We therefore sequenced *AICDA* and found biallelic mutations scattered all along the gene, affecting as well the nuclear localization signal (NLS), the active

Table 1 Phenotype of B-Cell Intrinsic Ig-CSR Deficiencies^a

Deficiency	Inheritance	Relative frequency (%)	CSR step block	CD27+ B cells	SHM	Autoimmunity	Lymphoma
AID	AR	43	Upstream for DSB	N	0 or ↘↘	+	—
AID ^{Cter}	AR	3	ND	N	N	—	—
AID ^{NES}	AD	6	Downstream for DSB	N	N	—	—
UNG	AR	3	Upstream for DSB	N	N (bias)	+	Possible
AID cofactor?	AR?	18	Upstream for DSB	N	N	+	—
DNA repair?	AR?	33	Downstream for DSB	↓	N	+	+

^aND, not done; N, normal; AR, autosomal recessive; AD, autosomal dominant.

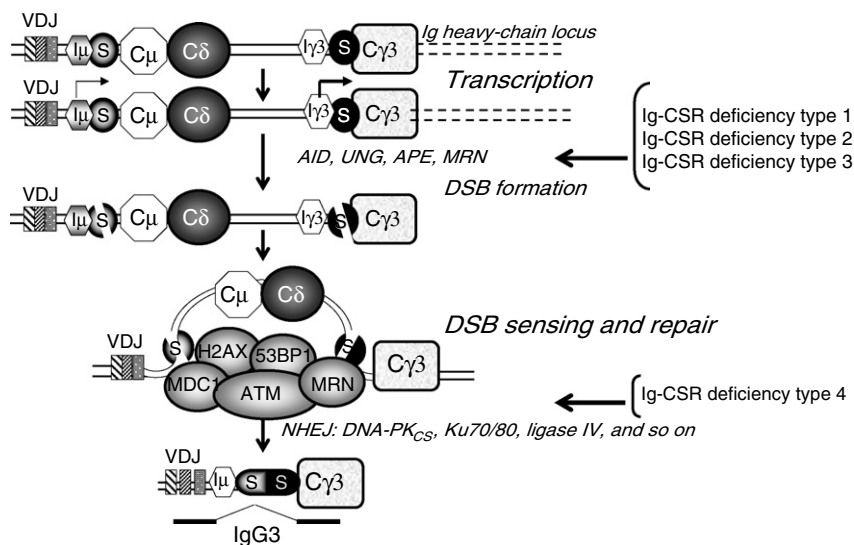


Figure 3 Schematic representation of Ig-CSR and its defects. A schematic representation of the successive steps of CSR toward IgG3 as an example is shown. The transcription step is induced by cytokines. AID induces the DNA lesion by deamination of cytidine into uracil residues. The uracil misintegrated into DNA are removed by UNG2, resulting in an abasic site that is eventually cleaved by an apyrimidinic endonuclease (APE) or the MRN complex (M, MRE11 for meiotic recombination homologue 11; R, hRAD50; N, NBS1 for Nijmegen Breakage syndrome 1). The precise mechanisms leading to DSB in switch (S) regions are not perfectly understood. Multimolecular complexes including phosphorylated γ H2AX, the p53-binding protein (53BP1), the ataxia telangiectasia mutated (ATM), the complex MRN, and MDC1 (DNA damage checkpoint 1) recognize and repair the CSR-induced DSB, in association with the NHEJ enzymes. Defects in the Ig-CSR processes are shown.

cytidine deaminase domain, and the APOBEC1-like domain (Fig. 4A). Eighteen were either missense mutations or in-frame small insertions or deletions, while five led to a premature stop codon, and one was a large deletion of all the coding sequence. Mutations were found mostly in the homozygous state (32 families) or compound heterozygous mutations (11 families) (Durandy *et al.*, 2006; Quartier *et al.*, 2004). Some of them were recurrent and found in unrelated families but no clear mutation hot spot was observed. Of note, we did not detect mutations located either at the phosphorylation sites (S38 and T27) or the dimerization domain (G47–G54) that have been respectively described (Basu *et al.*, 2005; Wang *et al.*, 2006).

In vivo data obtained with these natural human mutations were corroborated by three different *in vitro* assays: analysis of cytidine deaminase activity

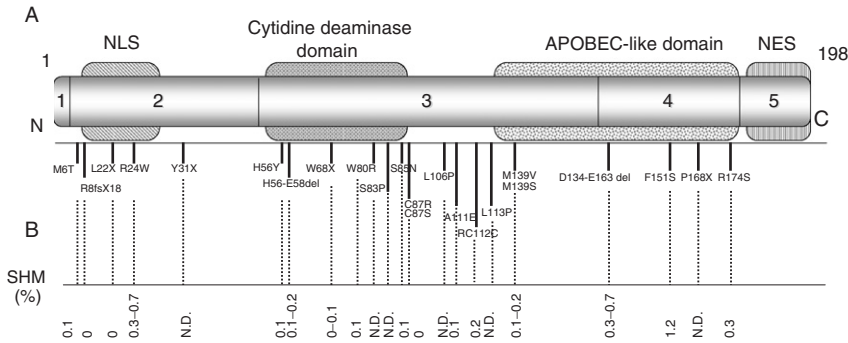


Figure 4 Autosomal recessive Ig-CSR deficiency due to mutations in *AICDA* gene. (A) Twenty-three different mutations (18 missense mutations or in-frame small deletions, 5 nonsense mutations or frameshift deletions) were observed in 62 patients from 43 unrelated families. Mutations were found homozygous in 32 families; others presented with compounded mutations. (B) SHM frequency was studied in 29 patients on VH3-23 region of IgM on CD19+CD27+ purified B-cell population. Results are expressed as the percentage of mutated nucleotides on all studied nucleotides. Age-matched controls, 2.5–6.3%; ND, not done. When several patients carrying same mutations were tested, the results show the range of SHM observed.

on DNA after transfection in *Escherichia coli*, as described by Petersen-Mahrt *et al.* (2002), induction of CSR after expression in AID^{-/-} murine splenic B cells, and study of SHM on an appropriate substrate after transduction in fibroblasts (Okazaki *et al.*, 2002). Mutants located in the NLS (p.R8fsX18, p.R24W), in the cytidine deaminase domain (p.H56Y, p.H56-E58 del, p.W68X, p.W80R), in the domain located between the cytidine deaminase domain and the APOBEC1-like domain (p.L106P, p.D143-E163 del), and in the APOBEC1-like domain (p.M139V) exerted no detectable cytidine deaminase activity in *E. coli*, with the exception of p.M139V in which a residual activity was found (Ta *et al.*, 2003). All mutants exerted neither CSR in B cells nor SHM activity in fibroblasts. The expression of the *AICDA* mutants, assessed after transfection in 293T cells, was found positive except for in p.R8fsX18, p.W86X, and p.D143-E163 del (Ta *et al.*, 2003). However, endogenous AID expression was either decreased or even undetectable by Western blot analysis in EBV B-cell lines from all these patients and in germinal centers by immunohistochemistry in available biopsies, suggesting that, in most cases, mutations lead to an unstable protein (Durandy, unpublished data).

The description of a defect of both CSR and SHM in AID deficiency, in concordance with the phenotype concomitantly described in AID-deficient mice (Muramatsu *et al.*, 2000), provided the key findings to determine the master role of AID in CSR and SHM.

2.2. AID Deficiency Associated with Preserved SHM

In three other patients with AID deficiency, a normal frequency and pattern of SHM were observed, contrasting with a drastically impaired *in vivo* and *in vitro* CSR (Table 1; Fig. 5A). The giant germinal centers usually observed in secondary lymphoid organs in the other AID-deficient patients were not detected as noted in a biopsy specimen obtained in one case. This preliminary observation suggests a direct role for SHM in the control of B-cell proliferation inside

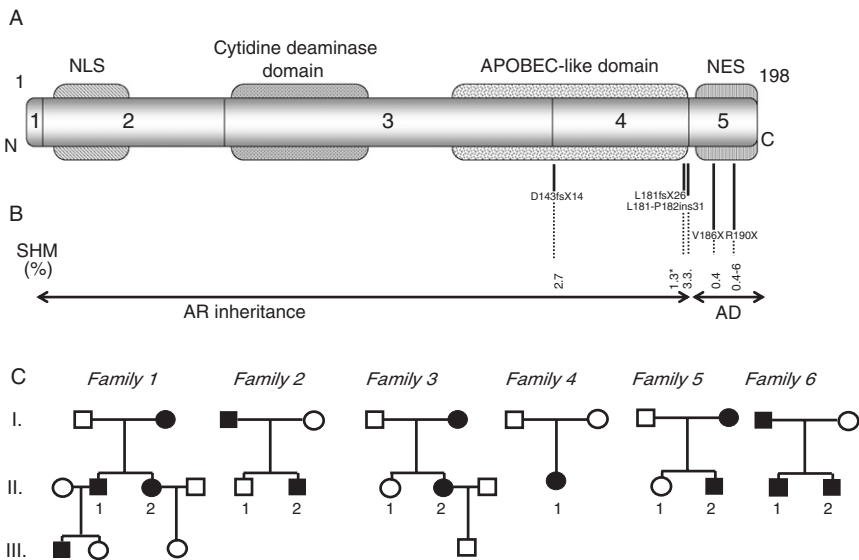


Figure 5 Ig-CSR deficiencies due to mutations in *AICDA* C-terminus. (A) *AICDA* C-terminal mutations. Three patients carried mutation on splice sites in intron 4, the consequences of which were different. In one patient, a homozygous mutation led to the in-frame insertion of 31 nucleotides between exon 4 and exon 5 (p.L181-182 ins31). In the two other patients, the splice site mutation was heterozygous and associated with a missense mutation located either in the region between the cytidine deaminase domain and the APOBEC-like domains (p.R112C) or in the cytidine deaminase domain (p.S83P). The predicted protein was, in one case, frameshift replacement of the C-terminus by 26 amino acids (p.L181fsX26) and in the other case, deletion of exon 4 and frameshift replacement by 4 amino acids (D143-L181fsX4). Both mutations abrogated the NES. Fourteen patients from 6 families carried heterozygous nonsense mutations located in the NES: R190X in 11 patients (5 unrelated families) and V186X in 3 patients (1 family). (B) SHM was performed on CD19+CD27+ B-cell population, except in * in which SHM was studied on CD19+ B-cell population (controls: 0.5–5%). AR, autosomal recessive; AD, autosomal dominant. (C) Autosomal dominant transmission of *AICDA* pedigrees of autosomal dominant AID deficiencies: p.R190X/+ (families 1–5), p.V186X/+ (family 6).

the germinal centers. Strikingly, these three patients carried mutations located in the C-terminal part of *AICDA* (*AID*^{Cter}) (Fig. 5A). In one, a homozygous mutation in the splice acceptor site of intron 4 leads to the in-frame insertion of 31 amino acids (p.L181-P182ins31), leading to expression of an abnormally large protein. Two other patients exhibited compound mutations: (1) a missense mutation in domain located between the cytidine deaminase domain and the APOBEC1-like domain (p.R112C) and a mutation in the splice donor site of intron 4, leading to a 26 amino acid frameshift replacement of the C-terminus leading to the NES sequence loss (p.L181fsX26), (2) a mutation in the cytidine deaminase domain (p.S83P) and a splice donor site mutation of intron 4 resulting in deletion of exon 4 and frameshift insertion of four amino acids also excluding the NES (p.D143fsX4). In *in vitro* assays, the p.L181-182ins.31 mutant exerted a slightly reduced cytidine deaminase activity in *E. coli* that is likely the consequence of decreased expression of the protein. Both p.L181-182ins.31 and p.L181fsX26 mutants were shown to induce SHM in fibroblasts but not CSR in *AID*^{-/-} B cells, although, as expected, the p.R112C, an *AICDA* mutation located in the APOBEC1-like domain, was unable to sustain both CSR and SHM (Ta *et al.*, 2003). Interestingly, these three mutations located in the C-terminal part of *AID* (p.L181-182ins.31, p.L181fsX26, and p.D143fsX4) do not exert a dominant negative effect since heterozygous carriers exhibited normal serum Ig levels (Durandy, unpublished data).

The observation of normal SHM but defective CSR induced by these natural mutants was confirmed by the analysis of an artificial mutant deleted of the last 10 amino acids. This mutant was found to catalyze SHM, gene conversion but not CSR (Barreto *et al.*, 2003). No data concerning the occurrence of CSR-induced mutations and DSB in S regions are up to now available in patients causing mutations in *AID*^{Cter}. However, results found with the artificial mutant strongly suggest that *AID* has a CSR-specific activity possibly by binding through its C-terminus to one or several CSR-specific cofactor(s). Such an interaction could account for the *AID* selectivity in targeting DNA in S regions of Ig genes of germinal center B cells. Alternatively, because the artificial mutant, although unable to induce CSR when transduced into *AID*^{-/-} mice splenic B cells, led to a normal frequency and pattern of mutations in the S μ region, the CSR cofactor could also be involved in the CSR-specific DNA repair (Barreto *et al.*, 2003). Two proteins, DNA-dependent protein kinase (DNA-PK_{CS}) and murine double minute 2 (MDM2), have been reported as binding the C-terminus of *AID* (MacDuff *et al.*, 2006; Wu *et al.*, 2005). They could represent CSR-specific *AID* cofactors since (1) the nonhomologous end joining (NHEJ) repair pathway to which DNA-PK_{CS} belongs has been shown to be required for CSR but dispensable for SHM (Rolink *et al.*, 1996) and (2) MDM2 binds the Nijmegen breakage syndrome 1 (NBS1) molecule

(Alt *et al.*, 2005), a molecule necessary for the CSR-induced DNA repair (Reina-San-Martin *et al.*, 2005). MDM2 has been shown to be required for gene conversion in the chicken DT-40 cell line, but no data on CSR are available. Such an interaction between AID and MDM2 could suggest that the AID-induced DNA breaks are sensed to induce appropriate DNA repair, following NBS1 recruitment.

Conversely, several artificial murine *AICDA* mutants with mutations located in the NLS in AID N-terminus have been shown to exert, as expected, a normal cytidine deaminase activity after transfection in *E. coli*, but strikingly a normal CSR activity when transduced in murine splenic AID^{-/-} B cells, contrasting with a defective SHM induction in fibroblasts (Shinkura *et al.*, 2003). This feature, which is not only related to impaired NLS, raises the hypothesis that an interaction of AID N-terminus with SHM cofactor(s) could exist. However, we could not confirm this observation in six Ig-CSR-deficient patients harboring homozygous missense mutations in the N-terminus of AID immediately upstream from the NLS (p.M6T) or within the NLS (p.R24W) (Fig. 4A). All present with both a typical CSR defect as shown by their Ig levels and *in vitro* B-cell activation and a complete lack of SHM. This observation suggests several possibilities: (1) the recruitment of cofactors could be not identical in humans and mice or (2) these natural mutants lead either to an unstable protein or to a protein unable to properly shuttle into the nucleus. However, AID was detectable by Western blot in a p.R24W-mutated EBV-B-cell line. Finally, one cannot exclude that an immunodeficiency consisting in defective antibody affinity maturation but normal CSR could be caused by AID mutations located at the N-terminus remains to be described. It implies that AID-mutated protein is expressed and traffics normally between cytoplasm and nucleus on activation.

2.3. Autosomal Dominant Transmission of AID Deficiency

In 14 patients from 6 unrelated families, we observed an Ig-CSR deficiency, the inheritance of which was suggestive of an autosomal dominant transmission (Fig. 5C). It was characterized by variable susceptibility to infections and serum Ig level abnormalities: serum IgM was increased, IgG was absent or only decreased, and IgA was undetectable. Interestingly, these patients carry heterozygous nonsense mutations located in the nuclear export signal (NES): p.R190X in five families (Imai *et al.*, 2005) and p.V186X in one (Fig. 5A). Both normal and mutated allele transcripts were equally expressed in activated B cells as judged by semiquantitative RT-PCR amplification. Although the *in vitro* CSR was drastically defective, the DSB were normally found in S_μ regions, localizing the CSR defect downstream from the DNA cleavage, a

finding compatible with the hypothesis that CSR-specific AID-cofactor(s) is (are) involved in CSR-DNA repair of S regions (Table 1).

SHM frequency was normal, except in two patients (2 II-2 and 6 II-2) in whom SHM was undetectable (Fig. 5C). The reason of this defect remains unknown. Because the available AID-specific monoclonal antibody recognizes its 10 last amino acids, it was not possible to check for the presence of the truncated protein. However, full-length AID protein was observed in the available p.R190X EBV B-cell lines.

The variable immunodeficiency found in p.R190X or p.V186X heterozygous patients is not caused by haploinsufficiency, considering the normal Ig levels observed in subjects carrying heterozygous mutations located elsewhere in *AICDA* gene (Imai *et al.*, 2005). Two nonmutually exclusive hypotheses can, thus, be proposed to account for this dominant negative effect: (1) The truncation affects the NES domain of the protein, leading to nuclear accumulation of the mutant allele, which could interfere with normal AID trafficking and function. While wild-type AID is only detected in the cytoplasm of activated B cells and is able to shuttle into the nucleus for a short period on activation, the p.R190X-mutated allele was found mostly in the nucleus after overexpression in AID-deficient B cells (Ito *et al.*, 2004). However, the observation that two different splice site mutations leading also to NES truncation are responsible for an autosomal recessive CSR defect, with conserved SHM (see above), suggests that the loss of NES may not be sufficient to create a dominant negative mutant. (2) The mutated allele, incorporated in an AID homomultimeric complex, disrupts AID activity. It has been reported that AID forms homomultimeric complexes *in vitro* (Ta *et al.*, 2003), even in the absence of its C-terminal part, as shown by detectable *in vitro* multimerization of artificial mutants truncated for the last 10 amino acids (Barreto *et al.*, 2003). An AID motif involved in homodimerization has been delineated between p.G47 and p.G54 and thus is present in these C-terminal mutants (Wang *et al.*, 2006). One possibility to consider is that intact AID multimers could be necessary to recruit CSR-specific AID cofactor(s).

Molecular target of AID has been a matter of debate. As the sequence of AID shares similarity with that of the RNA-editing enzyme APOBEC1, it has been suggested that AID edits an mRNA encoding a substrate common to CSR and SHM, probably an endonuclease (Chen *et al.*, 2001; Honjo *et al.*, 2002; Kinoshita and Honjo, 2001). The requirement for *de novo* protein synthesis downstream from AID expression in CSR could suggest the synthesis of a recombinase, and is consistent with the RNA-editing model (Doi *et al.*, 2003). The findings of a CSR-specific deficiency associated with given *AICDA* mutations would, in this context, suggest that AID can edit two different endonucleases, making it more complex and less likely. Alternatively, several lines of

evidence indicate that AID is a DNA-editing enzyme. Transfection of AID into *E. coli* results in deamination of DNA deoxycytidine residues into deoxyuridine (Petersen-Mahrt *et al.*, 2002). Furthermore, it has been demonstrated that AID can induce deoxycytidine deamination on single-stranded DNA in cell-free experimental conditions (Bransteitter *et al.*, 2003; Chaudhuri *et al.*, 2003; Dickerson *et al.*, 2003; Ramiro *et al.*, 2003). However, these data were obtained in nonphysiological conditions, involving overexpression in *E. coli* or in *in vitro* assays, in which the well-known RNA-editing protein APOBEC1 has a similar effect (Harris *et al.*, 2002; Petersen-Mahrt and Neuberger, 2003). The description of the *in vivo* consequences on antibody maturation of the defect in uracil-*N*-glycosylase (UNG), an enzyme that recognizes uracil residues on DNA, as described below, is, however, only compatible with a DNA-editing activity of AID.

3. Ig-CSR Deficiency Type 2 Caused by UNG Deficiency

We reported three patients from three families, two being consanguineous, affected by a severe Ig-CSR deficiency with high levels of IgM and very low levels or absence of IgG and IgA, leading to recurrent bacterial infections (Imai *et al.*, 2003b). Two of the three patients presented with lymphadenopathies, one with autoimmunity (hemolytic anemia). The CSR defect was also observed *in vitro* as shown by the complete lack of production of IgG, IgA, or IgE by patients' B cells on activation by sCD40L+ appropriate cytokines, although their B cells proliferate in the same culture conditions. As observed in most cases of AID deficiency, the CSR defect occurs downstream from the germ line transcript induction and upstream from the DSB occurrence (Imai *et al.*, 2003b) (Table 1). In contrast with what is observed in most patients affected by AID deficiency, the frequency of SHM was found normal in the CD19+CD27+ B-cell population. However, the pattern of SHM was strikingly abnormal in the three patients since a slight increase in mutations targeting G/C residues (75%) as compared to controls (63%) was observed. It was associated with a drastic skewed nucleotide substitution pattern on G/C since nearly all found mutations were transitions (G > A, C > T 95%) whereas, in controls, transitions represent only 60% of mutations. Conversely, transition frequency was normal on A/T nucleotides (50%) (Imai *et al.*, 2003b).

Because the Ig-CSR deficiency phenotype observed in these patients resembled the phenotype described as a consequence of homozygous *UNG* gene inactivation in mice (Rada *et al.*, 2002), although in the latter a much milder CSR defect was found, we explored the possibility of UNG deficiency in the patients. In humans, as in mice, UNG has two different promoters and two alternative splice products, resulting in two isoforms: UNG1, the mitochondrial

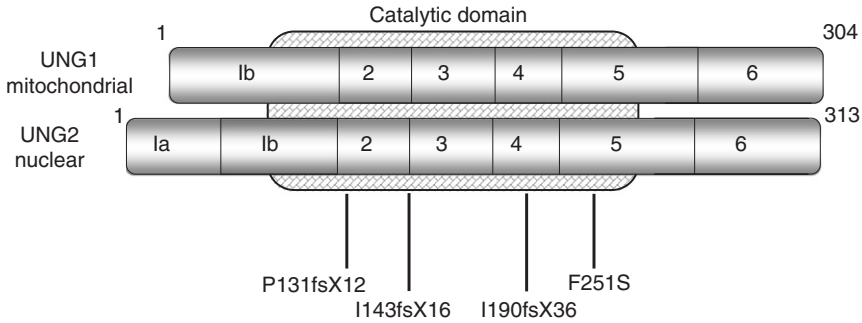


Figure 6 Autosomal recessive Ig-CSR deficiency due to mutations in *UNG* gene. Three patients presented with mutations located in the catalytic domain of both UNG1 and UNG2. Number of amino acids are those of UNG2. One patient carried two heterozygous small frameshift deletions (p.P131fsX12 + p.I190fsX36), the two other were homozygous for a small frameshift deletion (p.I143fsX16) or a missense mutation (p.F251S), respectively. In three patients, UNG1 and UNG2 were undetectable in EBV B-cell lines by immunoprecipitation.

isoform, is ubiquitously expressed while UNG2, the nuclear isoform, is only expressed in proliferating cells (Nilsen *et al.*, 2000; Otterlei *et al.*, 1998), including B cells activated for CSR (Imai *et al.*, 2003b). The above-mentioned *de novo* protein synthesis-dependent step downstream from AID activity in CSR described in murine splenic B cells (Doi *et al.*, 2003) could, thus, possibly correspond to UNG2 induction. We found mutations in all three patients: two heterozygous small deletions leading to premature stop codons (p.P131fsX12 + p.I190fsX36 in nuclear UNG2) in one patient, a homozygous missense mutation (p.F251S) in another patient, and a homozygous small deletion leading to a stop codon in the third one (p.I143fsX16) (Fig. 6). All four mutations were located in the active site of UNG1 and UNG2.

UNG mutations, as studied in EBV B-cell lines, led to an absence of any detectable protein by immunoprecipitation using a polyclonal antibody to UNG1 and UNG2, and no UNG activity neither in cytoplasmic nor in nuclear extracts (Imai *et al.*, 2003b; Kavli *et al.*, 2005). In accordance with this observation, accumulation of genomic uracil residues was present in the three EBV B-cell lines, while undetectable in controls. These results indicate that no other UNG, such as SMUG1 (Di Noia *et al.*, 2006), can compensate for the absence of UNG2, at least in EBV B cells. In contrast, although lacking the UNG1 isoform, patients do not present with metabolic abnormalities, suggesting a compensatory activity exerted by other uracil-DNA glycosylases in mitochondria.

The description of a drastic CSR defect and a skewed pattern of SHM in the absence of UNG is only compatible with a DNA-editing activity of AID. Hence, in the proposed model, AID deaminates dC on DNA to generate dU residues (Di Noia and Neuberger, 2002; Rada *et al.*, 2002). Following the deglycosylation and removal of the misintegrated dU residues by UNG, an abasic site is created, which can be attacked by an apyrimidinic endonuclease, resulting in DNA breaks (Fig. 3). It has been shown *in vitro* that the MRE11/hRad50/NBS1 complex could exert this endonucleasic activity (Larson *et al.*, 2005). In the absence of UNG2 and of abasic site, the DNA cleavage cannot occur, leading to a defect in CSR and a skewed pattern of SHM because SHMs occur by replication of U:G residues leading to transitions (G > A, C > T) only or by their repair by the mismatch repair (MMR) enzymes (MSH2, MSH6) leading to transitions and transversions on bystander nucleotidic residues (Di Noia and Neuberger, 2002; Rada *et al.*, 2004; Xue *et al.*, 2006). The discrepancy between the drastic CSR defect observed in UNG-deficient humans and the more subtle defect observed in UNG^{-/-} mice could be related to a variable involvement of MMR in CSR in both species (Ehrenstein and Neuberger, 1999; Schrader *et al.*, 1999).

An identical phenotype was observed in the three patients, although two patients carried UNG mutations encoding truncated proteins, and the third one a missense mutation (p.F251S in UNG2). To assess the consequences of this last mutation on protein expression and activity, it was shown that, although the mutant protein was weakly expressed after transfection into *E. coli*, the purified protein exerted a normal enzymatic activity. However, when coexpressed in HeLa cells, the p.F251S mutant interacted with wtUNG1, an interaction that led to its abnormal translocation from nucleus to mitochondria and its rapid degradation (Kavli *et al.*, 2005). Such a mechanism can very likely underlie the accumulation of genomic uracil residues as well as the drastic Ig-CSR deficiency observed in the patient. This result is of importance since it has been reported that the mouse counterpart of this mutant (denoted F242S) can remove uracil and restore CSR when transduced in UNG^{-/-} B cells (Begum *et al.*, 2004). One explanation for this discrepancy could be that the UNG2 F251S mutant effect depends on the balance between its expression level and that of UNG1. Overexpression of UNG2 mutant, in the absence of UNG1 in UNG^{-/-} B cells, does not lead to abnormal redirection to mitochondria and degradation, thus enabling CSR since the protein is active.

It has also been proposed that enzymatically dead UNG is required for CSR, suggesting that UNG is either involved in the recruitment of DNA repair molecules or play a role by folding the CSR-induced DSB (Honjo *et al.*, 2004). However, this hypothesis does not fit with (1) the defective occurrence of DSB in S μ regions in UNG-deficient B cells on activation to CSR (Imai *et al.*, 2003b)

and (2) the accumulation of genomic uracils found in UNG-deficient EBV B-cell lines (Kavli *et al.*, 2005).

UNG deficiency, as detected so far in three patients, is not associated with other clinical features than Ig-CSR deficiency. However, UNG is part of the DNA base excision repair involved in the repair of spontaneously occurring base lesions and therefore constitutes an antimutagenic defense strategy. UNG-deficient mice do develop B-cell lymphomas when aging (Nilsen *et al.*, 2003). It is, thus, possible that UNG deficiency predisposes to such tumors in adulthood. Another consequence of UNG deficiency has been reported in UNG-deficient mice since postischemic brain injury is much more severe than in control mice, a likely consequence of the mitochondrial DNA repair defect (Endres *et al.*, 2004).

Therefore, the complete absence of UNG leads in humans to a unique Ig-CSR deficiency characterized by defective CSR and a skewed pattern of SHM, an observation providing a clue to demonstrate the DNA-editing activity of AID.

4. Molecularly Undefined Ig-CSR Deficiency with Normal SHM

Half cases of the Ig-CSR deficiency caused by an intrinsic B-cell defect are neither related to AID nor UNG deficiency and remain molecularly unelucidated (Fig. 1). We had the opportunity to study the clinical and immunologic phenotype of 52 patients affected by an Ig-CSR deficiency, previously published as HIGM4, although its molecular defect is still undefined (Imai *et al.*, 2003a). Although most cases were sporadic, the observation of patients in a few multiplex or consanguineous families is compatible with an autosomal recessive inheritance. The clinical phenotype is very similar to that of AID deficiency, including susceptibility to bacterial infections of the respiratory tract and the gut and autoimmunity. Autoimmune hemolytic anemia and thrombocytopenia were not infrequent (eight cases) while other autoimmune manifestations (uveitis and systemic lupus erythematosus) were noted in a few cases (1 and 2, respectively). Lymphoid hyperplasia was less frequent and milder than in AID deficiency, characterized by moderate follicular hyperplasia, whereas the giant germinal centers typical of AID deficiency were not observed.

The CSR defect appears to be milder than in AID deficiency since residual levels of IgG could be detected in the serum from 18 patients of 52. *In vitro*, B cells from these patients (expressing IgM and IgD or IgM only), although able to proliferate, to produce IgM, and to up-regulate CD23, did not undergo CSR on activation by sCD40L and appropriate cytokines. *AICDA*, *UNG2*, and Ig germ line transcripts were normally induced on CSR induction, whereas Ig excision circles and functional transcripts were undetectable. Even B cells from

patients with residual serum IgG levels lacked functional γ transcripts, indicating that either the *in vitro* test is less sensitive than the *in vivo* Ig level determination or a residual CSR had occurred *in vivo* as a result of repeated stimulations, or via another signaling pathway. The overexpression of BAFF or APRIL on activated dendritic cells stimulates marginal zone B cells to undergo CSR in a CD40-independent pathway—a compensatory mechanism absent in AID-deficient cells which cannot undergo CSR whatever the stimulation.

Isohemagglutinins and IgM antipolysaccharide antibodies were present in normal amounts but no IgG antibodies against immunization agents or infectious antigens were detected (Imai *et al.*, 2003a). SHM was found normal in frequency and pattern, an observation not in favor of AID or UNG deficiencies, a diagnosis definitively excluded by both gene sequencing and protein expression studies.

Further analysis of the precise location of the CSR block led to the delineation of two different groups.

4.1. Ig-CSR Deficiency Type 3 Characterized by a CSR Block Located Upstream from the S μ Region DNA Cleavage

This subset, up to now observed in 17 patients, is always associated with a normal fraction of CD19+CD27+ “memory” B-cell population. This phenotype is similar to the one of patients carrying mutations in the C-terminus of AID.

The Ig-CSR deficiency, herein described, could be the consequence of a defective targeting of AID on S regions since the CSR-induced DSB are shown not to occur in S μ regions from patients’ B cells, localizing the CSR defect, as in AID deficiency, downstream from the transcription step and upstream from the DNA cleavage (Table 1; Fig. 3). 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 both DNA in S and V regions (Chaudhuri *et al.*, 2004). However, this interaction is likely unaffected in this Ig-CSR deficiency since SHMs 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 been also 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.

An alternative mechanism could also consist in AID inaccessibility to S regions because of a defect in an activation-dependent change in chromatin unrelated to transcription.

4.2. Ig-CSR Deficiency Type 4 Characterized by a CSR Block Downstream from the S μ Region DNA Cleavage

The clinical phenotype of this subset differs by the occurrence of B-cell lymphomas (5 of 35 patients). In one available biopsy of an affected cervical lymph node, numerous monoclonal immunoblastic B cells, strongly expressing AID, were observed in interfollicular areas surrounding follicles of various sizes (Durandy, unpublished data). Lymphoma was not associated with EBV.

In contrast to most cases of AID deficiency, the DSB were normally detected in S μ regions in CSR-activated B cells, although the *in vitro* CSR was defective. However, the next step, DNA repair, was impaired since excision circles and functional transcripts of switched isotypes were not found (Table 1; Fig. 3). The occurrence of DSB in S μ regions ruled out a defect in AID targeting to S regions. SHMs were found normal in frequency and pattern within the CD27+CD19+ B-cell-purified population. Of note, however, this B-cell subset was strikingly decreased as compared to controls (<10% of B cells). Several hypotheses can be discussed to account for this unique phenotype.

4.2.1. A Defect in the Repair of the AID-Induced DNA Lesion in S Regions

The occurrence of B-cell lymphomas, the decreased number of CD27+ B cells, and the CSR defect located downstream from the DNA breaks suggest that a DNA repair defect during the CSR process could be responsible for this Ig-CSR deficiency. The discrepancy between the findings of defective *in vivo* and *in vitro* CSR and normal generation of SHM also fits with this hypothesis because CSR and SHM are known to use different pathways for DNA repair. The NHEJ pathway, which repairs DSB, is involved in CSR-DNA repair (Casellas *et al.*, 1998; Manis *et al.*, 1998; Rolink *et al.*, 1996). Nevertheless, a defect in one of the NHEJ components is unlikely to account for this condition because NHEJ proteins are also required for V(D)J recombination of both T- and B-cell receptors. Patients exhibited normal numbers of T and B cells, with functional antigen receptors, unlike other NHEJ-defective patients such as Artemis-deficient patients (Moshous *et al.*, 2001) or Cernunnos-deficient patients (Buck *et al.*, 2006). One may postulate that another DNA repair pathway is involved, as suggested by the observation of normal CSR in SCID mice, despite the lack of DNA-PK activity (Bosma *et al.*, 2002). A defect in one such repair enzyme could be responsible for this Ig-CSR deficiency.

It has been described that CSR-induced DSB in S regions induce DNA repair foci involving phosphorylated histone γ H2AX and the MRE11/hRad50/NBS1 (MRE11, meiotic recombination homologue 11) complex at Ig loci (Petersen *et al.*, 2001). A defective CSR with normal SHM has been reported in H2AX-knockout mice (Petersen *et al.*, 2001) but a deficiency in H2AX has been ruled out in patients by gene sequencing and normal DNA repair foci formation (Durandy, unpublished data). Mutations in the *NBS1* and *MRE11* genes could be excluded because they are responsible for the Nijmegen breakage syndrome (Varon *et al.*, 1998) and ataxia-telangiectasia-like disorder (Stewart *et al.*, 1999), respectively, features of which being not found in the studied condition. Ataxia-telangiectasia mutated (ATM) plays also a role in CSR-DNA repair as shown by the typical Ig-CSR deficiency condition observed in some patients (Weemaes *et al.*, 1984), characterized by abnormal S region junctions, and normal SHM (Pan-Hammarstrom *et al.*, 2003).

The p53-binding protein (53BP1), which participates to the early step of DSB repair by facilitating end joinings, has also been described as being required for CSR and dispensable for SHM (Manis *et al.*, 2004; Ward *et al.*, 2004). However, 53bp1-deleted mice also present with a partial T-cell deficiency (Ward *et al.*, 2003), not found in patients. Nevertheless, the gene encoding 53BP1 was sequenced and found normal in the patients affected with this Ig-CSR deficiency. The DNA damage checkpoint protein 1 (MDC1), which plays a role in genomic stability and DSB repair, has been reported to be involved in CSR, but at a lower magnitude as shown by the only slight CSR defect observed in *mdc1*^{-/-} mice (Lou *et al.*, 2006). MDC1 involvement was also excluded by gene sequencing (Durandy, unpublished data).

Although the MMR enzymes play a role in CSR, at least in mice (Ehrenstein and Neuberger, 1999; Schrader *et al.*, 1999; Stavnezer and Schrader, 2005), it is unlikely that a defect in one of these molecules could account for the phenotype, herein, described because (1) UNG-dependent CSR should be preserved and (2) no other cancers were detected in these patients.

Therefore, we could not up to now find a defect in any molecule known to be directly involved in DNA repair of S regions in this Ig-CSR deficiency, raising the hypothesis that survival signals to switching B cells or responses to the consequences of DNA damage within the germinal centers could be deficient in this condition.

4.2.2. A Defect in Switched B-Cell Differentiation or Survival

A defect in B-cell differentiation could also be responsible for this Ig-CSR deficiency. For example, Bach2, which is a repressor of the expression of the plasma cell differentiation factors Blimp1 and XBP-1, plays a critical role in

CSR and SHM in mice (Muto *et al.*, 2004). Bach2-deficient mice exhibit a CSR defect but a more profound SHM defect than found in patients. Lymph nodes are depleted of germinal centers and CSR-activated B cells do not express *AID* transcripts, both observations not found in the studied condition. Finally, the involvement of Bach2 in this Ig-CSR deficiency has been ruled out by gene sequencing (Durandy, unpublished data).

A defect in survival signals delivered to switched B cells could also explain this phenotype. Molecular interactions are known to be required for B-cell survival, including BAFF interaction with its BCR, BAFF-R. The natural mutant mouse strain A/WySnJ, BAFF-R-knockout mice, and mice treated with the BAFF-neutralizing BAFF-R-Fc protein disclose B-cell depletion and an impaired T-cell-dependent antibody response (Kayagaki *et al.*, 2002; Schiemann *et al.*, 2001; Thompson *et al.*, 2001). The decreased number of CD27+ B cells and the defective IgG and IgA production in patients fit with this model. However, the observed *in vitro* defective CD40-dependent CSR cannot be accounted for by a BAFF-R or BAFF abnormality, though a defect in another unknown factor can, however, be postulated.

Another hypothesis could be an inappropriate response to DNA damage. In other cells than germinal center B cells, DSB activate p53 and p21, resulting in cell cycle arrest and apoptosis. In germinal centers, in contrast, the p53 response to DNA damage is negatively regulated directly by its highly expressed transcriptional regulator Bcl-6, while p21-induced cell cycle arrest is suppressed through interaction of its transcriptional activator Miz1 with Bcl-6. Both of these events enable intense proliferation of B cells undergoing CSR (Phan *et al.*, 2005). In accordance with this observation, Bcl-6-deficient mice are depleted of germinal centers because of a strong B-cell apoptosis (Ye *et al.*, 1997). Such a defect in transcriptional repression of proteins involved in cell cycle arrest induced by DNA damage could also underline this Ig-CSR deficiency. Investigation of this pathway is ongoing. However, this hypothesis does not fit with the normal generation of SHM which also results from a DNA damage, but possibly does not involve similar breaks.

In any case, the molecular definition of these Ig-CSR deficiencies would be essential for a better understanding of the complex mechanisms of CSR, up to now not completely elucidated, and for the delineation of the human diseases, although rare, characterized by a CSR deficiency.

5. Concluding Remarks

The precise description of the different Ig-CSR deficiency conditions is essential for a medical point of view since prognosis, thus follow-up of patients, may differ according to the defect. All Ig-CSR deficiencies are characterized by a

profound CSR defect, but they differ for SHM generation, raising the question of the role of mutated IgM in defense against infections and in inducing or not autoimmunity. Indeed, AID deficiency is most often characterized by a drastic defect in SHM, while SHMs are present with a skewed pattern in UNG deficiency and normal in AID C-terminal deficiency and the other Ig-CSR deficiencies. At first glance, susceptibility to infections appears identical in the four groups, suggesting that mutated IgM may not play a significant role in efficient immunity to bacteria. However, this requires to be looked in details during the intervals patients are not treated by parenteral injections of immunoglobulins. Careful assessment of susceptibility to infections in animal models may provide useful information on the role of (un)mutated IgM antibodies in anti-infectious immunity. AID-deficient mice do survive after a secondary infection by influenza virus, although they present a severe morbidity, indicating that even unmutated IgM antibodies can contribute to control that particular viral infection (Harada *et al.*, 2003). The protective role of IgM, even unmutated, is emphasized by the overall milder clinical phenotype observed in Ig-CSR deficiencies, as compared to agammaglobulinemia, resulting in the fact that Ig-CSR deficiencies are diagnosed much later up to adulthood (Imai *et al.*, 2005; Quartier *et al.*, 2004).

The role of SHM-modulating autoimmunity can be also discussed. Patients affected with an Ig-CSR deficiency due to an intrinsic B-cell defect present with autoimmune manifestations, some of them being clearly related to IgM autoantibodies. Autoimmunity is likely not simply related to IgM serum levels since autoimmune manifestations are significantly less frequent in CD40L deficiency in which IgM levels are equally elevated. Autoimmunity apparently occurs with the same frequency in AID deficiency characterized by a lack of SHM and in other Ig-CSR deficiencies with normal SHM generation. Although IgM autoimmune antibodies themselves have not been checked for SHM, our observation suggests that autoimmunity can be supported by germ line Ig sequences in accordance with occurrence of autoimmunity in AID deficiency.

Occurrence of B-cell lymphomas is another special concern associated with some of the Ig-CSR deficiencies. Such cancers are not intrinsically linked to Ig-CSR deficiency itself since they were not reported in 62 cases of AID deficiency. They are detected in Ig-CSR deficiency associated with a defective DNA repair of either the AID-induced lesion (as observed in UNG-deficient mice, also in humans?) or the UNG/AP-endonuclease-induced DNA breaks (as observed in Ig-CSR deficiency type 4).

The ongoing delineation of inherited Ig-CSR deficiency syndromes is shedding new light on the process of physiological antibody maturation in humans. Natural mutants observed in human immunodeficiencies have, in some cases, been described before generation of the appropriate mouse model.

The genetic definition of the X-linked CD40L deficiency was determined before the generation of CD40L-deficient mice. Both observations provided clear evidence for the essential role played in antibody maturation by the CD40 signaling pathway.

The study of B intrinsic Ig-CSR deficiencies has contributed to a better understanding of the complex mechanisms underlying the CSR and the SHM. The phenotype of AID-deficient patients and mice, which have been concomitantly described, has demonstrated the master role of this newly described B-cell-specific molecule in both events of antibody maturation, the CSR and SHM. Data obtained in humans have contributed to establish that AID acts upstream from the occurrence of the DSB in S regions. The description of an Ig-CSR deficiency caused by UNG deficiency provided strong evidence for a DNA-editing activity for AID, first suggested by *in vitro* data. Additional data have shown that AID could act in antibody maturation not only by its cytidine deaminase activity but as a potential docking protein recruiting cofactors. Study of natural as well as artificially engineered mutants in humans strongly suggests that the C-terminus of AID could bind specific CSR cofactors, involved in DNA repair rather than in AID targeting. In addition, the description that peculiar AID mutants can exert a dominant negative effect fit with the *in vitro* observation of AID homomultimerization. Finally, the study of the up to now unelucidated Ig-CSR deficiencies could also help to resolve some of the issues that remain obscure in the Ig-CSR process. One also should not exclude the description of new phenotypes possibly related to CSR deficiencies, for instance selective Ig isotype deficiencies, up to now not elucidated (Hanson *et al.*, 1988).

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