Defects of class-switch recombination

Luigi D. Notarangelo, MD,[a] Gaetana Lanzi, PhD,[a] Sophie Peron,[b] and Anne Durandy, MD, PhD[b,c] *Brescia, Italy, and Paris, France*

Shaping of the secondary antibody repertoire is generated by means of class-switch recombination (CSR), which replaces IgM with other isotypes, and somatic hypermutation (SHM), which allows production of high-affinity antibodies. However, the molecular mechanisms underlying these important processes have long remained obscure. Immunodeficiency with hyper-IgM comprises a group of genetically heterogeneous defects of CSR variably associated with defects of SHM. The study of these patients has allowed us to recognize that both T-cell–B-cell interaction (resulting in CD40-mediated signaling) and intrinsic B-cell mechanisms are involved in CSR and SHM. Elucidation of the molecular defects underlying these disorders has been essential to better understand the molecular basis of Ig diversification and has offered the opportunity to define the clinical spectrum of these diseases and to prompt more accurate diagnostic and therapeutic approaches. (J Allergy Clin Immunol 2006;117:855-64.)

Key words: *Immunodeficiency with hyper-IgM, class-switch recombination, somatic hypermutation*

Abbreviations used

AID: Activation-induced cytidine deaminase
APOBEC-1: Apolipoprotein B mRNA-editing enzyme, catalytic subunit 1
CD40L: CD40 ligand
CSR: Class-switch recombination
D: Diversity
DSB: Double-strand DNA break
EDA-ID: Hypohidrotic ectodermal dysplasia with immunodeficiency
J: Joining
NEMO: Nuclear factor κB essential modulator
NF: Nuclear factor
S: Switch
SHM: Somatic hypermutation
sIg: Surface Ig
TRAF: TNF receptor–associated factor
UNG: Uracil N-glycosylase
V: Variable

Antibody-mediated immune responses play a critical role in the immune defense against extracellular pathogens (eg, bacteria) and many viruses. The primary antibody repertoire is generated in the fetal liver and in the bone marrow by means of the V(D)J recombination process, which allows genomic rearrangement between variable (V), diversity (D), and joining (J) gene elements of the Ig heavy chain gene and between V and J elements of the Ig light chain genes. This process, which is both antigen and T lymphocyte independent, results in the expression of membrane IgM and IgD molecules (Fig 1, *A*).

In contrast, the secondary antibody repertoire, characterized by the production of Igs of various isotypes with high affinity for antigen, is shaped in secondary lymphoid organs (lymph nodes, tonsils, and spleen), mainly through a T cell– and antigen-dependent process that involves 2 distinct events: class-switch recombination (CSR) and somatic hypermutation (SHM; Fig 1, *A*).[1]

CSR is accomplished by means of DNA recombination between 2 distinct switch (S) regions located upstream of the C_μ and a target C_x region of another isotype; in this process the intervening DNA sequence is deleted and circularized as an episomal fragment. By means of CSR, the μ chain of Igs is replaced by a different C_x heavy chain, thus allowing production of Igs of different isotypes that mediate distinct biologic properties and differ for half-life and tissue distribution. In contrast, CSR leaves the sequence of the V region intact and thus does not affect antibody specificity and affinity.

On the other hand, SHM is the process through which discrete (mostly missense) mutations are introduced into

From [a]the Department of Pediatrics and "Angelo Nocivelli" Institute for Molecular Medicine, University of Brescia; [b]Développement Normal et Pathologique du Système Immunitaire (INSERM U429), Université Paris 5, Hôpital Necker-Enfants Malades, Paris; and [c]Assistance Publique-Hôpital de Paris (AP-HP).

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Reprint requests: Luigi D. Notarangelo, MD, Department of Pediatrics, University of Brescia Spedali Civili, 25123 Brescia, Italy. E-mail: notarang@med.unibs.it.

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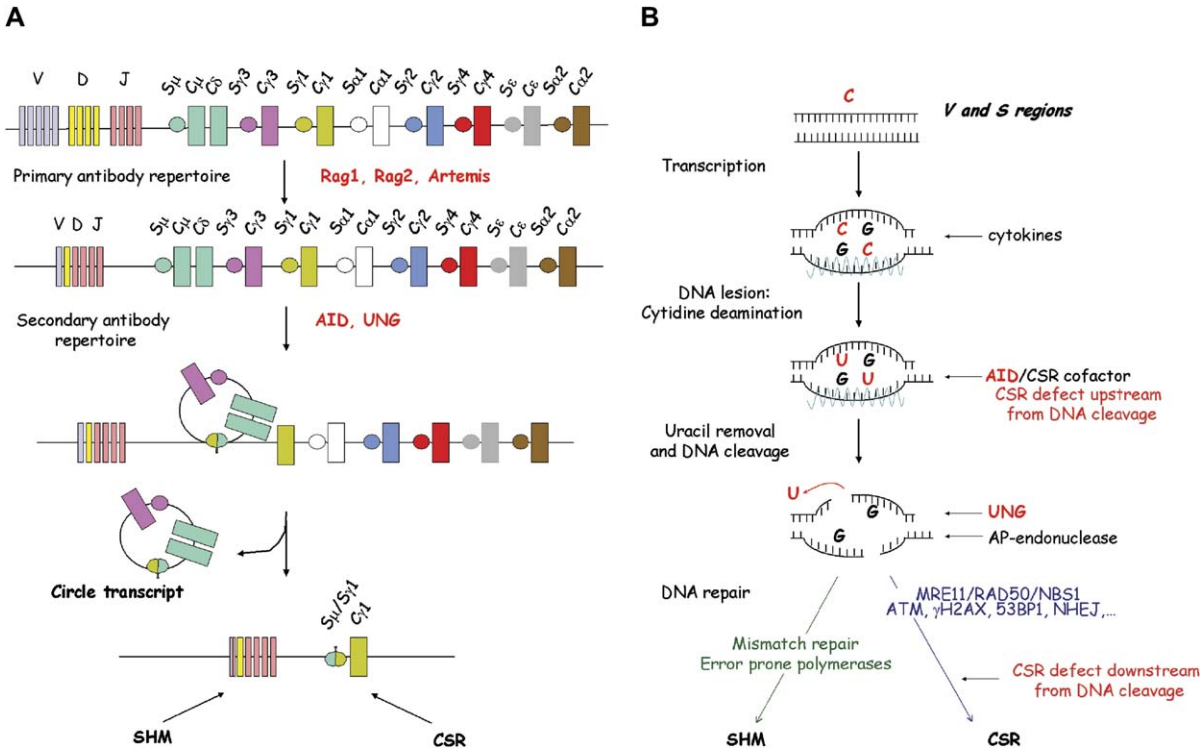

FIG 1. A, Molecular mechanisms involved in generation of the primary and secondary antibody repertoire. **B**, Schematic representation of DNA modifications at S and V Ig gene regions during CSR and SHM. The roles of AID and UNG in this process are shown. Molecular defects involved in CSR defects, variably associated with disturbed SHM, are shown in red.

the V regions of the Igs at high frequency (1×10^{-3} bases per generation). This mechanism allows selection of B cells with a higher affinity for the antigen on interaction with antigen-loaded follicular dendritic cells.

Although both CSR and SHM occur mainly in the germinal center and are often observed in the same B-cell lineage, they are independent processes, so that SHM can occur in IgM-expressing B lymphocytes, and CSR (leading to IgG, IgA, and IgE expression) can occur without changes in affinity for the antigen.

Three successive events are required for CSR and SHM to occur (Fig 1, *B*)[1-3]:

1. *Transcription of target DNA sequences (S and V regions)*. In the S regions this is associated with formation of DNA-RNA hybrids on the template DNA strand, leaving the nontemplate strand accessible to DNA modification and cleavage. Appropriate cytokines and CD40 cross-linking on the surface of B lymphocytes activate the specific I promoter located 5′ to the target C_x gene and induce the synthesis and splicing of so-called germline or sterile transcripts.
2. *DNA cleavage*. For CSR, single-strand DNA break on the nontemplate strand is followed by double-strand DNA breaks (DSBs); during SHM, both single-strand DNA breaks and DSBs are scattered along the target DNA sequence, but the mechanisms involved in generation of the DNA breaks are still unclear.
3. *DNA repair*. During CSR, the histone γH2AX protein is phosphorylated, and several proteins are recruited at the site of DSBs in the S regions, including 53BP1, MRE11, RAD50, and NBS1. Then the nonhomologous end joining DNA repair enzymes (including DNA-PK and the KU proteins) mediate joining of the S_μ and S_x sequences. In contrast, DNA repair during SHM involves intervention of the mismatch repair enzymes and error-prone DNA polymerases η, ζ, and θ.

During the last years, the molecular mechanisms and signals involved in CSR and SHM have been disclosed through the characterization of a group of disorders (collectively named class-switch recombination defects, and previously known as hyper-IgM syndromes) characterized by impaired CSR and variably associated with defective SHM.[4-6] The main molecular and immunologic features of these diseases are summarized in Table I.

HYPER-IgM SYNDROME CAUSED BY DEFECTS OF CD40-MEDIATED SIGNALING

Interaction between T and B lymphocytes on the basis of both cell-to-cell contacts and soluble factors (cytokines) plays a major role in the maturation of antibody responses. Among membrane interactions, binding of CD40 to CD40

TABLE I. Clinical and immunologic features of hyper-IgM syndromes

								Defect of CSR	
	CD40L defect	**CD40 defect**	**XL-EDA-ID**	**AR-AID**	**AID-C ter**	**AID$^{\Delta C}$**	**UNG defect**	**Upstream from DNA cleavage**	**Downstream from DNA cleavage**
Protein affected	CD40L	CD40	NEMO	AID	AID	AID	UNG	?	?
Inheritance	XL	AR	XL	AR	AR	AD	AR	AR	AR
Clinical features									
Bacterial infections	+	+	+	+	+	+	+	+	+
Opportunistic infections	+	+	−	−	−	−	−	−	−
Lymphadenopathy	−	−	−	++	++	++	+	+	+
Autoimmunity	±	±	+	+	+	+	−	−	+
Tumors	+	− (?)*	−	−	−	−	−(?)†	−	+
Immunologic features									
Serum IgG	↓↓	↓↓	Variable	↓↓	↓↓	N − ↓	↓↓	↓↓	↓↓
Serum IgA	↓↓	↓↓	Variable	↓↓	↓↓	N − ↓	↓↓	↓↓	↓↓
Serum IgM	N or ↑	N or ↑	N or ↑	↑↑‡	↑↑‡	↑	↑↑‡	N −↑‡	N −↑‡
CD40-induced CSR	N	Undetected	Variable	Undetected	Undetected	Undetected	Undetected	Undetected	Undetected
CD27$^+$ B cells	↓	↓	↓	N§	N§	N§	N§	N	↓
SHM	↓	↓	Variable	↓↓	N	N	N but biased	N	N

XL, X-linked; *AR*, autosomal recessive; *AID-C ter*, patients with mutations in the C-terminal region of AID; *AID$^{\Delta C}$*, patients with AID C-terminal deletions; *AD*, autosomal dominant; *N*, normal.
*Not observed in the few patients reported thus far.
†Not observed in the few patients with UNG deficiency reported thus far; however, tumors are common in *ung*$^{-/-}$ mice.
‡IgM levels before Ig substitution therapy.
§CD27$^+$ B cells from these patients express sIgM and sIgD only.

ligand (CD40L) is particularly important in promoting survival of B lymphocytes and induction of CSR (Fig 1).

CD40L (also named TNFSF5 or CD154) is a member of the TNF superfamily and is predominantly expressed on activated CD4$^+$ T cells. It consists of 261 amino acids and is encoded by a gene that maps on chromosome X (Xq26-27). The CD40L protein is a type II transmembrane protein and is expressed at the cell surface as a trimer.

In contrast, CD40 is a type I transmembrane molecule of 277 amino acids and is constitutively expressed on the surface of B lymphocytes, dendritic cells, and macrophages but also on some nonimmune cells, such as endothelial and neuronal cells. Engagement of the extracellular region of CD40 by CD40L determines the trimerization of the CD40 receptor, leading to signal transduction.

In particular, cross-linking of CD40 on the surface of B lymphocytes promotes B-cell proliferation, rescues B lymphocytes from apoptosis, induces homotypic cell adhesion and expression of activation molecules, triggers CSR and SHM, and favors generation of long-lived plasma cells. Furthermore, CD154-CD40 interaction is critically required for germinal center formation. On the other hand, cross-linking of CD40 on the surface of monocytes–dendritic cells induces secretion of IL-12 and promotes terminal differentiation of monocyte-derived dendritic cells.

Several signaling pathways are activated after CD40L-CD40 interaction[7,8]; concurrent secretion of cytokines (IL-2, IL-4, and IL-10) released by activated T lymphocytes synergizes with CD40 cross-linking in inducing B-cell activation. Like other members of the TNF receptor superfamily, the cytoplasmic domain of CD40 is devoid of kinase activity and interacts with the TNF receptor–associated factor (TRAF) 2, 3, and 6, which can recruit TRAF-1 and TRAF-5 and form homotrimers or heterotrimers. Integrity of TRAF-2 and TRAF-3 binding sites on the cytoplasmic tail of CD40 is essential for CSR.[9] TRAFs act as adaptor molecules and recruit different kinases, such as phospholipase Cγ, nuclear factor (NF)–κB–inducing kinase, phosphoinositol-3-kinase, the serine-threonine mitogen-activated protein kinases c-Jun N-terminal kinase and p38, and Bruton's tyrosine kinase (BTK). Eventually, this results in activation of the transcription factors activator protein-1 (AP-1) and NF-κB (the latter through both p50 and p52 pathways).[10] In addition, CD40-mediated signaling might also involve TRAF-independent pathways, such as activation of Janus-associated kinases (JAKs) and signal transducers and activators of transcriptions (STATs) molecules.[11] Finally, it has been shown that CD40 on the surface of B lymphocytes interacts with C4 binding protein, the involvement of which might also contribute to B-cell activation.[12] These observations have established a novel interface between complement and B-cell activation and thus link innate with adaptive immunity.

Several genes are expressed as the result of CD40 triggering in B lymphocytes; among them, activation-induced cytidine deaminase *(AICDA)* encodes for activation-induced cytidine deaminase (AID), a molecule critically involved in CSR and SHM (see below).

The critical role played by CD40L-CD40 interaction in B-cell activation and maturation of antibody responses explains why genetic defects that affect expression of these molecules are an important cause of defects in CSR

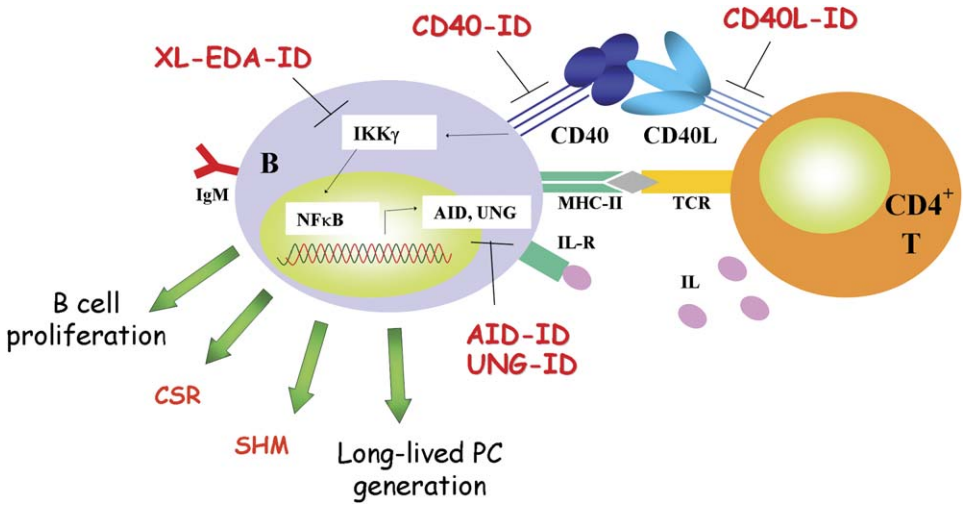

FIG 2. Schematic representation of CD40L-CD40 interaction and its effects on CSR and SHM. Activated $CD4^+$ T cells in the lymph nodes interact with CD40-expressing B cells and secrete ILs that interact with cytokine-specific B-cell receptors *(IL-R)*. CD40-mediated signaling activates the NF-κB signaling pathway and ultimately results in the expression of AID and UNG. Mutations that affect CD40-mediated B-cell activation result in some of the forms of hyper-IgM syndrome. *MHC-II*, Major histocompatibility complex of class II; *TCR*, T-cell receptor; *CD40L-ID*, X-linked immunodeficiency caused by CD40L defect; *CD40-ID*, immunodeficiency caused by CD40 deficiency; *XL-EDA-ID*, X-linked immunodeficiency with ectodermal dystrophy; *AID-ID*, immunodeficiency caused by AID deficiency; *UNG-ID*, immunodeficiency caused by UNG deficiency; *PC*, plasma cell.

and SHM (Fig 2). On the other hand, these defects also compromise monocyte–dendritic cell activation and hence affect T-cell priming. This explains why the immunologic and clinical features of defects of CD40-mediated signaling are broader and more severe than pure antibody deficiencies.

X-linked immunodeficiency with hyper-IgM (CD40L deficiency)

Inherited as an X-linked trait, CD40L deficiency was the first form of hyper-IgM syndrome for which the molecular basis was identified.

Most patients present in infancy with recurrent bacterial or opportunistic infections, including *Pneumocystis jiroveci*–induced pneumonia and diarrhea caused by *Cryptosporidium* species infection.[7,13] Neutropenia is a common finding and might further contribute to susceptibility to bacterial infections. Severe liver–biliary tract disease, progressing to sclerosing cholangitis, occurs in a significant number of patients (with some discrepancy between the European and the United States registries) and is often associated with *Cryptosporidium* species infection. A high proportion of patients (50% in the European series) die before the fourth decade of life, despite substitution treatment with Igs. This has prompted more aggressive forms of treatment on the basis of hematopoietic stem cell transplantation. However, a retrospective analysis of 38 European patients treated with hematopoietic stem cell transplantation has shown that only 22 (58%) of them were cured.[14]

Patients with CD40L deficiency have markedly decreased serum IgG and IgA levels and normal to increased IgM levels. Occasionally, IgA levels might be normal. This is likely due to a CD40L-CD40–independent CSR mechanism, in which B cells are activated by B-lymphocyte stimulator/B-cell activating factor (BLyS/BAFF) and a profileration-inducing ligand (APRIL) molecules (expressed by dendritic cells activated by IFN-α, IFN-γ, or LPS), along with signals provided by cytokines (TGF-β) and B-cell receptor engagement.[15]

Flow cytometry represents the most important screening assay for diagnosis of CD40L deficiency. In most patients, *in vitro* activation of T lymphocytes with phorbol esters and ionomycin fails to induce expression of the CD40L molecule, as revealed by staining with CD40L-specific mAbs or a CD40-Ig chimeric construct. In any case final confirmation of mutations requires molecular analysis. Genomic abnormalities are scattered along the *CD40L* gene (Fig 3).[16] In a minority of patients, milder mutations that allow binding of CD40 (albeit at reduced intensity) are associated with a less severe clinical course, which in a few cases was characterized uniquely by parvovirus B19–related anemia.

B lymphocytes from the patients coexpress surface IgM (sIgM) and sIgD; in most cases the proportion of memory ($CD27^+$) B lymphocytes and the frequency of SHM are reduced. Importantly, defects of CSR and SHM reflect abnormalities of the T-cell compartment, as indicated by the fact that the patients' B cells can be induced to undergo CSR *in vitro* on activation with CD40 agonists and cytokines.[17]

In addition, lymph nodes from CD40L-deficient patients show primary follicles but are devoid of germinal center formation, indicating the importance of CD40L-CD40 interaction for maturation of secondary lymphoid organs.

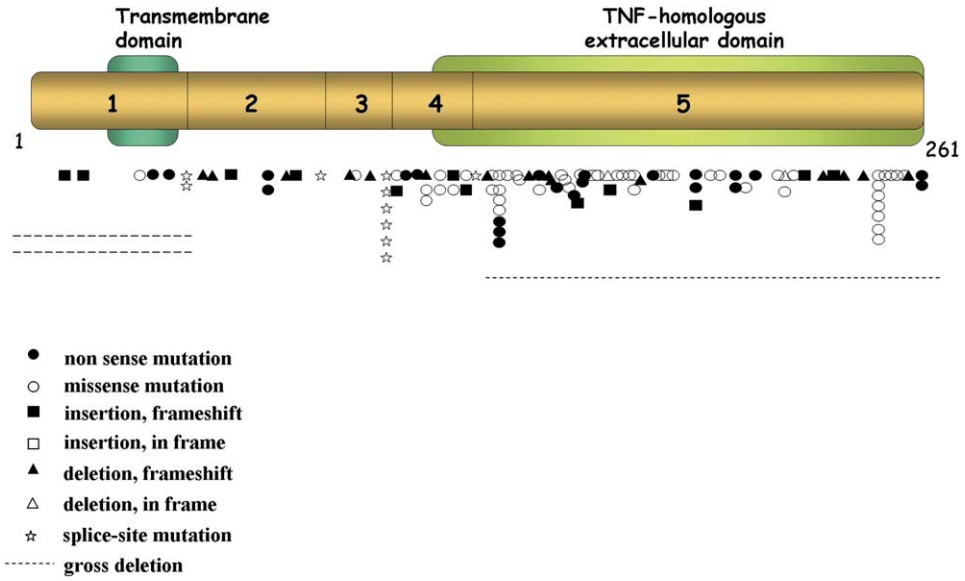

FIG 3. Schematic representation of the CD40L gene and of mutations identified in European patients with X-linked immunodeficiency with hyper-IgM (CD40L deficiency). *Boxes* identify the various exons of the gene.

In patients with CD40L deficiency, impaired expression of CD40L by activated $CD4^+$ T cells also affects monocytes and dendritic cells, with impaired secretion of IL-12 by monocytes, which results in reduced secretion of IFN-γ by T lymphocytes and defective T-cell priming.[18] These functional abnormalities contribute to the susceptibility of CD40L-deficient patients to opportunistic infections.

CD40 deficiency

Up to now, only 4 families have been identified with CD40 deficiency (Notarangelo, unpublished observations; Fig 4).[19,20] In all cases the disease was inherited as an autosomal recessive trait and was diagnosed on the basis of a lack of expression of CD40 on the surface of B lymphocytes and monocytes.

The clinical and immunologic findings of these patients were identical to those reported in CD40L deficiency, with recurrent bacterial and opportunistic (*Pneumocystis* and *Cryptosporidium* species) infections, severe biliary tract disease, and increased mortality. Patients have very low serum IgG and IgA levels and normal to increased IgM levels. The number of memory B cells is reduced, and SHM is impaired. However, at variance with what is reported in CD40L deficiency, B cells from CD40-deficient patients are unable to undergo CSR *in vitro* on activation with CD40 agonists and cytokines.[19] Furthermore, CD40 deficiency causes impaired function of monocyte-derived dendritic cells, with reduced secretion of IL-12, impaired T-cell priming, and diminished release of IFN-γ by cocultured T lymphocytes.[21]

Class-switch recombination defects caused by defective NF-κB activation

Cross-linking of CD40 results in activation of the NF-κB signaling pathway (Fig 2), the critical role of which in CSR and SHM has been disclosed by the recognition of patients affected with hypohidrotic ectodermal dysplasia with immunodeficiency (EDA-ID), which is often characterized by low levels of serum IgG and IgA, normal to increased IgM levels, and impaired antibody responses, particularly to polysaccharide antigens.[5]

In most cases EDA-ID is inherited as an X-linked trait and is caused by hypomorphic mutations in the zinc-finger domain of NF-κB essential modulator (NEMO, also known as IKK-γ), a scaffolding protein that binds to IKK-α and IKK-β, 2 kinase proteins.[22] After CD40 ligation, activation of the IKK complex results in phosphorylation and subsequent ubiquitination of the inhibitors of NF-κB (IκB). This releases the cytoplasmic components of NF-κB that can translocate to the nucleus and drive transcription of several target genes involved in CSR and SHM, including the gene encoding for AID (see below).

The association of ectodermal dysplasia in these patients is accounted for by defective signaling through the ectodysplasin receptor, which is also dependent on NEMO and the NF-κB signaling. On the other hand, hypomorphic mutations outside the zinc-finger domain of NEMO have been associated with osteopetrosis, lymphedema, and increased susceptibility to environmental mycobacteria, whereas Ig levels are often normal in these patients, indicating a critical role of the zinc-finger domain of NEMO for maturation of antibody responses.

Patients with X-linked EDA-ID show a remarkable defect of switched memory (IgD^- $CD27^+$) B cells and an excess of naive B lymphocytes, which coexpress IgM and IgD. The quality of B-cell function appears to differ between patients, likely reflecting heterogeneity of the mutations. Indeed, it has recently been shown that the Ig variable region in B lymphocytes from patients with EDA-ID carrying the same unique *NEMO* mutation is

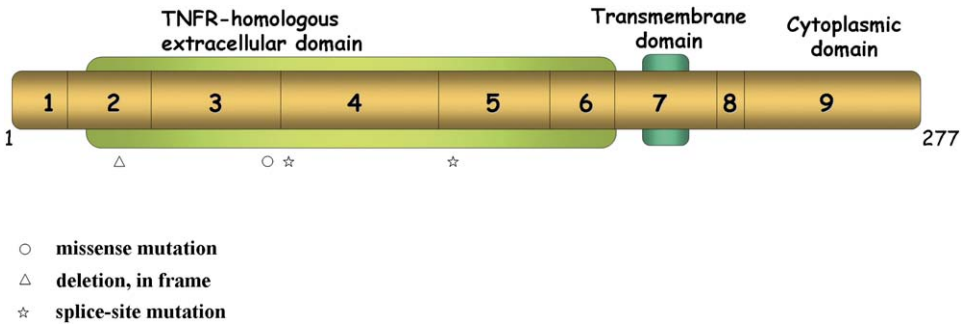

FIG 4. Schematic representation of the CD40 gene and of disease-causing mutations identified in patients with CD40 deficiency. *Boxes* identify the various exons of the gene. *TNFR,* Tumor necrosis factor receptor.

devoid of SHM,[23] although normal SHM generation has been found in others (Durandy, unpublished observations). A similar variability has been observed for CSR on *in vitro* activation of B lymphocytes with CD40 agonists (Durandy, unpublished observations).[22] Even when sterile and AID transcripts were detected, c-Rel activation was found to be severely impaired, and no CSR was observed.[23] Interestingly, NF-κB1/c-Rel knockout mice show a severe impairment of humoral immunity, with markedly reduced serum Ig levels and inability to mount antigen-specific antibody responses.[24]

The observation that AID is normally induced after CD40 ligation in B cells from patients with EDA-ID, even when no CSR is detected, whereas c-Rel activation is impaired, suggests that other possibly c-Rel–dependent factors are strictly required for CSR and SHM. Evaluation of the gene expression profile induced by CD40 cross-linking in B cells from these patients has shown selective deficiency in the expression of some genes, including *LYL1*, *MRE11*, *RAD50*, and *LIG4*, the products of which play a role in CSR.[23] In fact, LYL1 has been implied to be involved in recruitment of the switch recombinase, MRE11 and RAD50 are involved in formation of nuclear foci at DSB S regions, and LIG4 is involved in DNA repair.

B CELL–INTRINSIC DEFECTS OF CSR AND SHM

Although defects in CD40-mediated signaling lead to a broad immune deficiency that involves dendritic cells, monocytes, and T lymphocytes in addition to B cells, other forms of hyper-IgM syndromes are caused by defects intrinsic and selective to B lymphocytes.[25] The identification of the molecular basis of these forms of hyper-IgM syndrome has offered a significant contribution to the elucidation of the mechanisms involved in CSR and SHM (Fig 1, *B*).

The activation-induced cytidine deaminase gene *(AICDA)* was first isolated by means of a subtractive hybridization from a murine B-cell lymphoma line stimulated to undergo CSR *in vitro*.[26] Both in mice and in human subjects, the gene is expressed only in B cells undergoing CSR or SHM. The human *AICDA* gene encodes a 198-amino-acid 24-kd protein that contains a functional cytidine deaminase domain. AID shares homology with apolipoprotein B mRNA-editing enzyme, catalytic subunit 1 (APOBEC-1), an mRNA cytidine deaminase RNA-editing enzyme that catalyzes the production of 2 distinct apolipoprotein B mRNA species. For this activity, APOBEC-1 requires another ubiquitously expressed auxiliary factor.

In addition to the cytidine deaminase domain, AID also contains a putative nuclear localization signal at the N-terminus and a nuclear export signal at the C-terminus. Furthermore, a leucine-rich region at the C-terminus of the protein is likely involved in protein-protein interaction.[27]

Ectopic expression of AID in the presence of a proper substrate (eg, artificial switch constructs or actively transcribed target sequences) is able to induce switch recombination and somatic mutation, indicating that AID is the only B cell–specific component that is necessary and sufficient for both CSR and SHM.[28]

The mechanisms of action of AID are still debated.[1-3] On the basis of its homology with APOBEC-1, it has been originally hypothesized that AID edits an mRNA that encodes a molecule involved in both CSR and SHM. The demonstration that CSR requires *de novo* protein synthesis beyond AID expression is in keeping with this hypothesis.

However, several lines of evidence indicate that AID is a DNA-editing enzyme (Fig 1, *B*). Transfection of AID into *Escherichia coli* results in deamination of DNA deoxycytidine residues into deoxyuridine. Furthermore, it has been demonstrated that AID can induce cytidine deamination on single-stranded DNA in cell-free experimental conditions. As discussed above, transcription of S regions during CSR results in the formation of DNA-RNA hybrids on the template strand, leaving single-stranded nontemplate strand accessible to AID activity onto DNA.

The molecular mechanisms by which AID might work as a DNA-editing enzyme have been recently characterized. It has been demonstrated that AID is phosphorylated by protein kinase A[29] and then interacts with replication protein A, a single-stranded DNA-binding protein. This might enhance AID activity on transcribed double-stranded DNA containing CSR or SHM target sequences.[30]

Deamination of deoxycitidine to deoxyuridine on single-stranded DNA by AID represents a physiologic trigger for base excision repair.

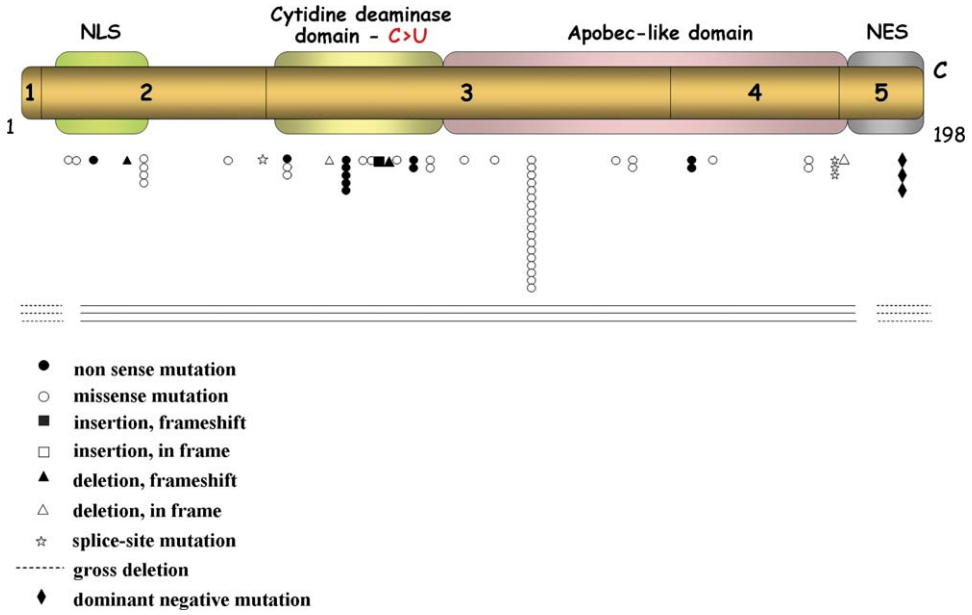

FIG 5. Schematic representation of the AID gene and of disease-causing mutations identified in patients with hyper-IgM syndrome caused by AID deficiency. *Boxes* identify the various exons of the gene. *NLS,* Nuclear localization signal; *NES,* nuclear export signal.

Uracil N-glycosylase (UNG) mediates deglycosylation and removal of deoxyuridine residues.[31] The resulting abasic site is then cleaved by an apyrimidinic endonuclease, like APE1, leading to single-stranded DNA breaks that are processed and repaired (Fig 1, *B*).[32] Alternatively, low-fidelity DNA repair (by error-prone DNA polymerases) at the cleaved end could result in point mutations. This would allow completion of CSR and SHM.[1]

According to this model, UNG deficiency is predicted to impair generation of DNA breaks at S regions, thus affecting CSR. Furthermore, UNG deficiency leads to inability to remove deoxyuridine residues generated by AID and thus causes accumulation of transition at G/C residues (G>A, C>T) as a result of fixation of deoxyuridine/deoxyguanidine lesions on DNA replication. These findings have indeed been observed in UNG-deficient mice.[33] Importantly, discovery of a biased pattern of SHM in UNG–deficient mice and human subjects has given further support to the hypothesis that AID is a DNA-editing enzyme.

In contrast with these observations, an apparent dispensability for UNG activity in CSR has been recently reported[34]; however, the UNG mutants analyzed in this article were not completely devoid of functional activity, thus calling into question the conclusions of this report.

Both in mice and in human subjects, the *UNG* gene has 2 distinct promoters and gives rise to 2 isoforms: UNG1 and UNG2. The former is ubiquitously expressed and localizes in mitochondria, whereas UNG2 is a nuclear protein expressed in actively proliferating cells, including CSR-induced B cells. Importantly, UNG2 has been shown to interact with several proteins involved in base-excision repair, such as replication protein A and proliferating cell nuclear antigen. Additional proteins (XRCC1, DNA ligase, DNA polymerases, and APE1) are also recruited in the UNG2-associated repair complex, which allows completion of CSR and SHM.[35]

The steps at which B cell–intrinsic defects occur along CSR are shown in Fig 1, *B*.

AID deficiency

AID deficiency in human subjects was identified by means of whole-genome linkage analysis in families with autosomal recessive hyper-IgM syndrome, which mapped the locus to chromosome 12p13 in a region containing the *AICDA* gene.[36] A variety of mutations scattered along the gene have been identified in patients with AID deficiency (Fig 5).

In most cases the disease presents in childhood, although late diagnoses are not rare. Patients mainly have recurrent bacterial infections of the upper and lower respiratory tracts. Gastrointestinal infections (including giardiasis, which can cause malabsorption), central nervous system infections, and arthritis have been also reported in 12% to 27% of the patients. Lymphoid hyperplasia (especially of tonsils and cervical lymph nodes) is a prominent feature of the disease and is due to massive enlargement of germinal centers that are filled with actively proliferating (Ki-67$^+$) B cells that coexpress CD38, sIgM, and sIgD, phenotypic markers of germinal center founder cells. Autoimmunity (hemolytic anemia, thrombocytopenia, and hepatitis) affects about 20% of the patients.

Patients with AID deficiency have a profound deficiency (virtual absence) of serum IgG and IgA, whereas IgM levels are normal or increased. Antibody responses

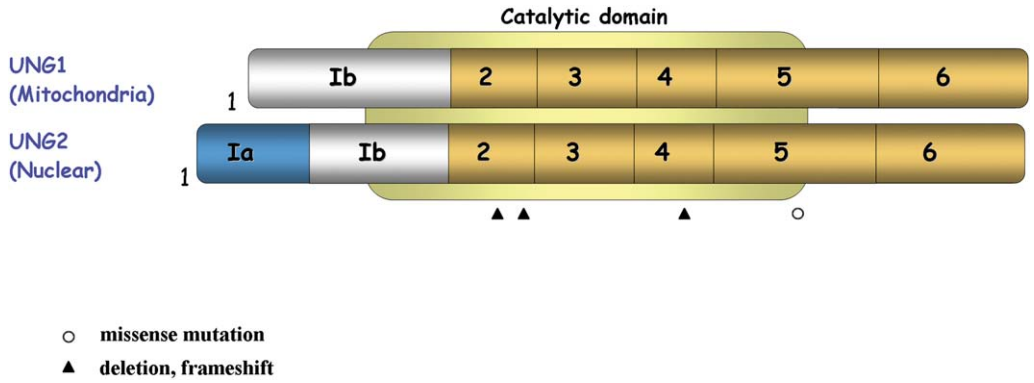

FIG 6. Schematic representation of the UNG gene and of disease-causing mutations identified in patients with hyper-IgM syndrome caused by UNG deficiency. Two different isoforms of the UNG protein (mitochondrial UNG1 and nuclear UNG2) are shown, resulting from use of distinct promoters and alternative splicing.

are restricted to the IgM isotype, without change in affinity after repeating immunization.

The number of circulating T and B cells is normal; however, B cells uniquely express sIgM and sIgD. The proportion of memory ($CD27^+$) B lymphocytes is also normal, but there are no switched ($sIgM^-$) memory B lymphocytes.

Cross-linking of CD40 induces B-cell proliferation; however, addition of anti-CD40 plus IL-4 is unable to promote IgE synthesis. In this system sterile S transcripts are normally generated, but DNA DSBs are not induced in the S regions, indicating that AID acts downstream from transcription of the S region and upstream from DNA cleavage.[37] In keeping with these observations, γH2AX and NBS1 foci are not formed in AID-deficient mice and human subjects.[38]

In addition, sequence analysis of the V regions of $CD27^+$ B lymphocytes from AID-deficient patients has disclosed lack of somatic mutations, indicating that AID is required also for SHM.

Examination of a large cohort of AID-deficient patients has disclosed unique features associated with specific mutations of the *AICDA* gene. In particular, mutations in the C-terminus of the gene result in selective impairment of CSR, leaving SHM intact. This finding suggests that the C-terminus of AID might interact with a protein uniquely involved in CSR.[27] Because mutations in the S_μ regions are normally found in these patients, it is likely that this AID-binding protein plays a role in DNA repair during CSR.

On the other hand, experiments in mice have shown that mutations in the N-terminal region of AID might affect SHM but leave CSR intact, suggesting that different domains of AID interact with cofactors specifically involved in CSR or SHM.[39]

Furthermore, although AID deficiency is usually inherited as an autosomal recessive trait, a specific nonsense mutation in the C-terminus, which results in deletion of the last 9 amino acids, has been shown to be associated with an autosomal dominant pattern of inheritance of AID deficiency.[40] Two nonmutually exclusive hypotheses have been postulated to account for this observation: (1) the deletion affects the nuclear export signal domain of the protein and can lead to nuclear accumulation of the mutant form, which might interfere with normal AID trafficking and function,[41] and (2) it has been shown that CSR requires AID multimerization. It is therefore possible that the 9-amino-acid deleted form of AID is incorporated into these multimers and impairs their activity. According to this hypothesis, the mutant form would exert a dominant negative effect.[27]

UNG deficiency

Up to now, 3 cases of UNG deficiency have been reported in human subjects.[42] In all cases both alleles were mutated. The 4 mutations identified in these patients were located in the catalytic region of the protein and affected both UNG1 and UNG2 expression (Fig 6). In particular, 2 patients carried frame-shift mutations that lead to premature termination, whereas one patient was homozygous for a missense mutation, leading to F251S amino acid substitution. This mutation has been shown to cause mislocalization of the mutant UNG2 protein to the mitochondria rather than to the nucleus.[31]

All 3 patients with UNG deficiency had a history of recurrent infections, and 2 of them showed lymphadenopathy.

The immunologic phenotype of UNG deficiency in human subjects includes a profound defect of CSR, with very low serum IgG and IgA levels, increased IgM levels, and lack of IgG antibody response to vaccine antigens. *In vitro* activation of patients' B lymphocytes with CD40 agonists plus IL-4 fails to induce CSR, and the defect is located downstream from induction of sterile transcripts but upstream from DNA cleavage.

Furthermore, although UNG-deficient patients have a normal number of $CD27^+$ B cells (all of which express sIgM), these cells show a biased pattern of SHM, with accumulation of transitions at C/G residues (whereas both transitions and transversions are detected at A/T residues).

Other genetically undefined, B cell–intrinsic defects of CSR

Most cases of hyper-IgM syndrome caused by intrinsic B-cell defects are not due to AID or UNG deficiency. Although the molecular basis of these cases is still unknown, their clinical and immunologic findings have been well characterized.[5,43,44] In particular, 2 distinct forms have been recognized on the basis of immunologic and molecular findings. In both, the clinical phenotype resembles AID deficiency, with recurrent sinopulmonary and intestinal bacterial infections. Lymphoid hyperplasia, when present (50% of the cases), is milder and is not associated with giant germinal centers. The defect of CSR is also milder because residual serum IgG levels can be detected in some patients. However, there is no evidence of specific IgG production in response to immunization.

In the first of these 2 forms, the CSR defect appears to be located downstream from S region transcription but upstream from DNA cleavage (Fig 1, *B*). Importantly (and at variance with AID and UNG deficiencies), SHM is normal both in frequency and in pattern. The number of $CD27^+$ memory B cells is also normal. It is possible that this form is due to impaired targeting of AID (or other components of the switch recombinase) onto S regions.

From the clinical point of view, these patients have a good prognosis and are not prone to autoimmunity or tumors.

The second form of hyper-IgM syndrome caused by undefined, B cell–intrinsic defects of CSR is characterized by a reduced number of memory B lymphocytes. Because DSBs are normally detected in the S_μ region in CSR-induced B cells, the defect occurs downstream from DNA cleavage (Fig 1, *B*) and could involve a yet unidentified DNA repair protein. Importantly, despite the low number of memory B cells, SHM is present in normal frequency and pattern, indicating that the defect must involve a protein that plays a role in CSR, although not in SHM.

From a clinical point of view, these patients are susceptible to autoimmune manifestations and possibly also to tumors (lymphomas).

CONCLUSIONS

The study of immunodeficiency with hyper-IgM in human subjects has shed light on the complex molecular mechanisms that govern CSR and SHM. In many cases hyper-IgM syndromes have been initially defined in human subjects, indicating once more the importance of primary immune deficiencies as models of defective immune development and maturation. Furthermore, the study of large cohorts of patients has revealed unexpected clinical, immunologic, or genetic findings that have significant implications on the molecular basis of CSR and SHM, as shown for hypomorphic NEMO mutations and for patients with specific AID defects that differently affect CSR and SHM. Therefore the study of naturally occurring primary immune deficiencies has been essential to the advance of immunology.

Although novel exciting findings are awaited from the study of yet genetically undefined forms of hyper-IgM syndrome, identification of distinct molecular defects among the characterized forms of these disorders has enabled collection of clinical data from a large cohort of well-defined patients, thus aiding in diagnosis, assessment of prognosis, and prompting of more accurate forms of treatment.

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