

Molecular mechanisms in allergy and clinical immunology

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The role of activation-induced cytidine deaminase in antibody diversification, immunodeficiency, and B-cell malignancies

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Before exposure to antigen, antibodies with a wide diversity of antigen-binding sites are created by V(D)J rearrangement. After exposure to antigen, further diversification is accomplished by means of somatic hypermutation of the antibody variable region genes and class-switch recombination between the heavy-chain μ constant region and the downstream γ , ϵ , and α constant region. The variable region mutations are responsible for the affinity maturation of the antibody response, whereas class-switch recombination enables the antibodies to be distributed throughout the body and to carry out different effector functions. Both somatic mutation and class switching require an enzyme called activation-induced cytidine deaminase (AID) that converts deoxycytidines to deoxyuracils on single-stranded DNA. Genetic defects of AID in human subjects result in hyper-IgM syndrome type 2. The analysis of both mutant mice and immunodeficient patients has led to a better understanding of the mechanism of action and role of AID in immunity, as well as in the malignant transformation of B cells. (*J Allergy Clin Immunol* 2004;114:726-35.)

Key words: Activation-induced cytidine deaminase, somatic hypermutation, class-switch recombination, immunodeficiency, lymphoma

Abbreviations used

AID:	Activation-induced cytidine deaminase
AP endonuclease:	Apurinic-apyrimidinic endonuclease
APOBEC-1:	Apolipoprotein B mRNA-editing enzyme catalytic polypeptide 1
CSR:	Class-switch recombination
dC:	Deoxycytidine
DLBCL:	Diffuse large B-cell lymphoma
DSB:	Double-stranded DNA break
dU:	Deoxyuracil
GC:	Germinal center
GLT:	Germline transcript
HIGM2:	Hyper-IgM syndrome type 2
MMR:	Mismatch repair
NHEJ:	Nonhomologous end joining
NLS:	Nuclear localization signal
SHM:	Somatic hypermutation
SR:	Switch region
ssDNA:	Single-stranded DNA
UNG:	Uracil N-glycosylase

It has been known for more than 30 years that the affinity of the antibodies to a particular antigen increases

with repeated exposure¹ and that antibody genes undergo somatic hypermutation (SHM),² which could contribute to the affinity maturation of the immune response. It has also been known for many years that the antibody variable (V) region rearranges to downstream constant (C) regions in the Ig heavy-chain locus to mediate class-switch recombination (CSR). Despite the availability of the detailed structure of the heavy-chain gene locus and the sequences of many thousands of mutated antibodies, the detailed biochemical and molecular mechanisms responsible for SHM and CSR were not understood. However, in the last few years, a combination of molecular approaches, reverse genetics in mice, and the analysis of patients with type 2 hyper-IgM syndrome (HIGM2) and other immunodeficiency syndromes have led to a rapid increase in our understanding of the mechanisms responsible for affinity maturation and isotype switching and to a better appreciation of the role of SHM and CSR in health and disease.

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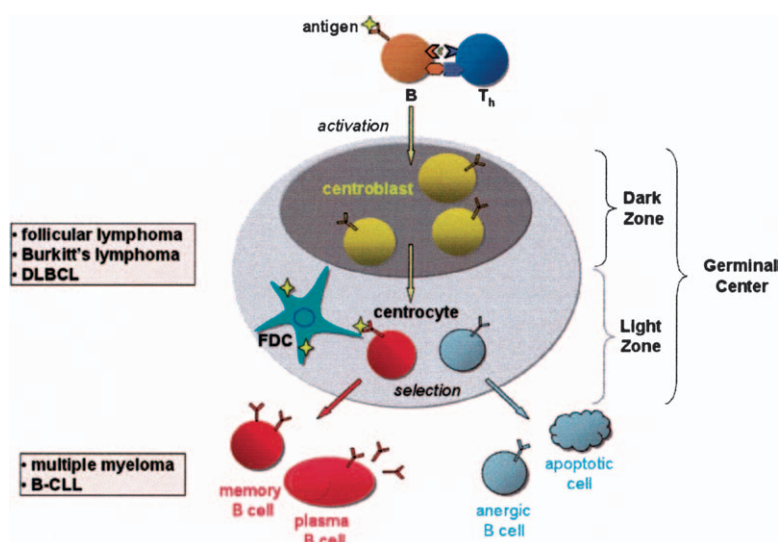


FIG 1. Germinal center reaction. Naive B cells that express surface antibodies specific for a particular antigen are activated by T_H cells, and their clonal progeny migrate to the dark zone of the germinal center in secondary lymphoid organs, where they become centroblasts. Centroblasts express AID, undergo SHM and CSR, and then differentiate into centrocytes. In the light zone of the germinal center, centrocytes interact with the antigen presented by follicular dendritic cells (FDCs). As a result of this interaction, the centrocytes differentiate into plasma or memory B cells. Alternatively, if the surface antibodies have a decreased affinity for the particular antigen or are autoreactive, the progeny of the centrocytes will either undergo apoptosis or become anergic. Lymphomas representing different stages of B-cell development are presented to the left of the B cells from which they are derived. Follicular lymphoma, Burkitt lymphoma, and DLBCL are derived from the germinal center stage; multiple myeloma and B-cell chronic lymphocytic leukemia are derived from the postgerminal center stage.

SHM AND CSR OCCUR DURING THE CENTROBLAST STAGE OF B-CELL DEVELOPMENT

Before exposure to antigen, a large repertoire of B cells expressing antibodies to most, if not all, of the antigens is generated through the rearrangement of germline heavy and light-chain V(D)J gene segments to form mature V region genes that encode the antigen-binding site of the antibody molecule. When an antigen appears that can interact with the antibody made by one or more of the approximately 10^8 new B cells generated every day in the bone marrow, that B cell internalizes the antigen through its surface IgM receptor, degrades the antigen into peptides, and presents some of those peptides on its MHC class II molecules to T_H cells (Fig 1).³ As a result of this interaction, the T and B cells are activated, the B cells proliferate, and their clonal progeny migrate to the dark zone of the germinal center (GC) in secondary lymphoid organs and progress to the centroblast stage of B-cell differentiation (Fig 1).⁴ It is at this centroblast stage of B-cell differentiation that B cells undergo SHM and most of CSR. The GC structure itself appears to be important because genetic defects, such as the inactivation of BCL6, which results in secondary lymphoid organs without GCs, are associated with the loss of affinity maturation but the persistence of isotype switching.^{5,6} Those B cells that, as a result of SHM, express an antibody with increased

binding for the immunizing antigen are positively selected in the light zone of the GC by antigen-presenting cells and T cells. Selected B cells are then stimulated to replicate and differentiate into memory B cells or plasma cells. The cells expressing antibodies with decreased affinity and those making autoantibodies are negatively selected to die or become anergic (Fig 1).⁷

CHARACTERISTICS OF SHM AND CSR

Although SHM and CSR both occur in centroblast B cells, they are distinct and apparently independent processes because there are IgM antibodies with somatic mutations and IgG antibodies with unmutated V regions. Furthermore, these 2 processes appear to be mechanistically quite different: SHM results in single base changes with occasional insertions and deletions, whereas CSR requires recombination. In addition, genetic inactivation in mice and mutations in human subjects of some of the genes required for the nonhomologous end joining (NHEJ) of double-stranded DNA breaks (DSBs) affect CSR but not SHM.⁸ Nevertheless, some patients with autosomal recessive forms of HIGM lack the ability to carry out both SHM and CSR,⁹ suggesting either that one of these is downstream of the other or that they depend on the function of a single gene.

In mice and human subjects the frequency of SHM is as much as a million times higher than the mutation

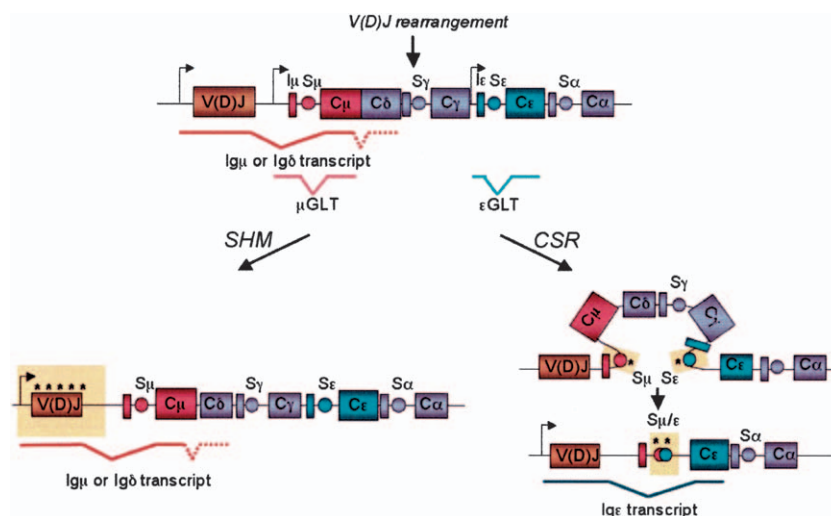


FIG 2. The generation of antibody diversity by SHM and CSR. After successful V(D)J rearrangement, the immunoglobulin heavy-chain (IgH) locus expresses Ig μ or Ig δ mRNA, which comprise the VDJ and the C μ or C δ exons, respectively (top diagram). The V(D)J region undergoes SHM (*) to change the affinity of the antibody variable region (left diagram). If stimulated by specific lymphokines, the B cells express GLTs from the promoters that are located just 5' of I μ and certain downstream I(s), respectively (top diagram). In this example μ and ϵ GLTs are expressed, and S μ and S ϵ recombine, resulting in looping out of the DNA sequence in between. The involved S regions also undergo SHM-like mutations (*). A new chimeric S μ/ϵ region with mutations (*) is formed (right diagram). As a result, the immunoglobulin locus expresses Ig ϵ mRNA encoding IgE protein.

frequency of housekeeping genes,^{10,11} with the result that individual heavy and light-chain V regions from a normal memory B cell that has undergone repeated rounds of SHM will often have 10 to 15 base changes within the approximately 300 bp of each V region. Human B cells that have been trapped in the tonsils can accumulate 40 to 80 base changes within each heavy-chain V region.¹² This is an extraordinary rate of mutation and suggests that SHM is a potentially very dangerous process that must be tightly regulated and efficiently targeted to the Ig genes to avoid the inactivation of essential proteins and the introduction of mutations that lead to malignant transformation.¹³ The mutations introduced by SHM arise about 200 bp downstream from the V region transcriptional start site, occur most frequently over the V region itself, and extend approximately 1.5 kb downstream from the promoter (Fig 2).¹⁴ The initiation of SHM just downstream from the promoter led to the suggestion that transcription is required for and contributes to the mutational process.¹⁵ This has been supported by the finding that SHM and CSR both require transcription, and the rates of SHM and CSR are roughly proportional to the rates of transcription of the V or switch region (SR) genes.^{16,17}

Mutations are observed throughout the rearranged heavy and light-chain V regions and their immediate flanking sequences, but there is preferential targeting to C and G in WRCH motifs (W = A/T, R = A/G, H = T/C/A) on both strands.¹⁸ Transition (purine to purine or pyrimidine to pyrimidine) mutations arise more frequently than transversions (purine to pyrimidine or pyrimidine to purine). However, it appears that in any particular V

region, some potential hot spots are targeted, whereas others are not, even in the absence of selection for higher-affinity antibodies. This suggests that the neighboring sequences or higher-order DNA or chromatin structures might also influence the targeting of mutations.^{19,20} Although C and G in WRCH hot spots are the primary targets of mutation, many mutations also occur in A/T or in C/G that are not in hot spot motifs. Because these non-hot spot mutations were greatly decreased in mice that lacked mismatch repair (MMR) proteins²¹ and in patients with the variant form of xeroderma pigmentosa with mutations in polymerase η ,²² a second phase of SHM was proposed that differs in its biochemistry from the first phase, which only targets C/G in hot spots.²³ SHM is activated in the centroblasts in the GC through the stimulation of the surface Ig with the help of T cells, but the details of this activation process and the exact signal transduction pathways used are not well understood.²⁴

CSR results in the rearrangement of the heavy-chain V region to the C γ , C α , or C ϵ regions and the expression of that V region as an IgG, IgA, or IgE isotype (Fig 2).²⁵ This process is very important because it allows each positively selected antigen-binding site to be distributed throughout the body and body spaces and to pass through the placenta. CSR also allows each of the antigen-binding sites to mediate different combinations of effector functions, such as complement fixation, binding to different Fc receptors, and persistence in the circulation for longer or shorter periods of time. CSR is mediated by an intrachromosomal recombinational event between the SR of the μ constant region (S μ) and one of the downstream SRs (Fig 2).²⁵

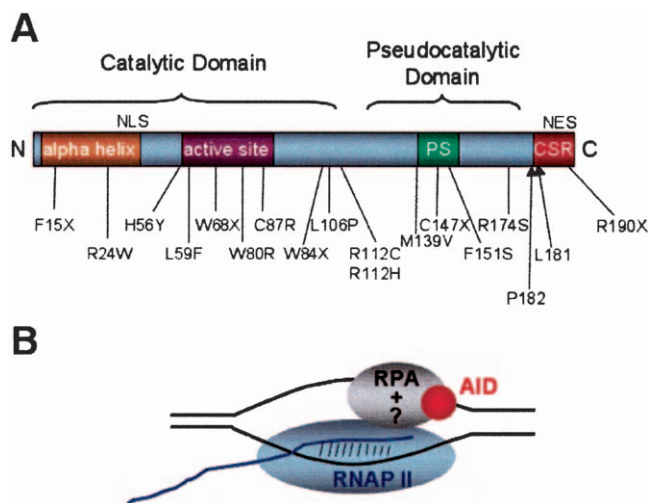


FIG 3. AID and its mode of action. **A**, Schematic representation of the AID protein. PS, Pseudocatalytic site; NES, nuclear export motif. Mutations found in patients with HIGM2 are shown. Arrows indicate insertions at the specified positions. **B**, Model for the mode of action of AID. During transcription, RNA polymerase II (RNAP II) creates a transcription bubble, which generates ssDNA, which serves as a substrate for AID. Replication protein A (RPA) and other unknown factors (?) might help to recruit AID to the V or S regions and to deaminate deoxycytidines on both the template and nontemplate strands of the transcription bubble.

These SRs are located immediately upstream of each of the C regions (except for δ) and contain highly repetitive sequences with G-rich nontemplate strands that are rich in hot spots for mutation.^{26,27} During CSR, the donor S_{μ} that is just upstream of the C_{μ} region and recipient SRs upstream of C_{γ} , C_{α} , or C_{ϵ} undergo mutations (Fig 2).²⁸⁻³⁰ These SR mutations have the same characteristics as V region mutations in that they also arise at high rates, target hot spot motifs, and extend downstream approximately 1.5 kb from the relevant promoters but spare the downstream C region genes.²⁸⁻³⁰ This recombinational process is region specific but not sequence specific.^{25,31} Like SHM, CSR requires transcription, but in this case the transcript is sterile, or germline, which means that it does not encode a protein. These germline transcripts (GLTs) are initiated from a promoter located just 5' to the I exon, elongated through the SRs and the C exons, and spliced to remove the intronic SR mRNA (Fig 2, top panel).²⁵ It remains unclear why the splicing of the transcript is required for CSR.³² CSR is activated in the centroblasts in the GCs through the engagement of surface receptors like CD40 and by lymphokines produced by different subsets of interacting T_H cells. Dendritic cells have also been reported to induce CD40-independent CSR through B-lymphocyte stimulator protein and a proliferation-inducing ligand.³³ Different lymphokines activate the promoters of different S regions, thus instructing the B cells to transcribe a particular downstream GLT, followed by isotype switching to that particular isotype.^{25,31} Significant progress has been made in elucidating the signal transduction pathways and the lymphokines that initiate switching to ϵ and some other isotypes, but it is still unclear how switching to all of the isotypes is differentially regulated.³⁴

ACTIVATION-INDUCED CYTIDINE DEAMINASE INITIATES BOTH SHM AND CSR

As noted above, it had been clear for many years that high rates of mutation and recombination were required for the maturation of the antibody response, but the enzymatic processes and the mechanistic relationship between SHM and CSR were poorly understood. However, in 1999, in the course of seeking new factors that might be involved in CSR, Muramatsu et al³⁵ discovered activation-induced cytidine deaminase (AID) as a differentially expressed gene in a tissue culture cell line when this line was induced to undergo CSR. They showed that AID was selectively expressed in GC B cells, and when they inactivated the AID gene in mice, these mice had very large GCs in their secondary lymphoid organs and made only low-affinity unmutated IgM antibodies because of the inability to carry out SHM and CSR.³⁶ Revy et al³⁷ showed that a subset of patients with hyper-IgM syndrome had biallelic autosomal recessive mutations in the gene that encoded AID and labeled these patients HIGM2 in contrast to HIGM1 with CD40 ligand deficiency and HIGM3 with CD40 deficiency.³⁸

Taken together, these findings suggested that AID is responsible for the mutations in the V region and that AID could also initiate CSR, possibly by producing similar mutations in the SRs. However, the mechanism of action of AID remained unclear.¹³ AID is a small protein (Fig 3), and the gene that encodes it is closely linked and homologous to apolipoprotein B mRNA-editing enzyme catalytic polypeptide 1 (APOBEC-1).³⁵ APOBEC-1 deaminates cytidine 6666 in the apolipoprotein B mRNA to a uracil, resulting in a stop codon so that the edited mRNA

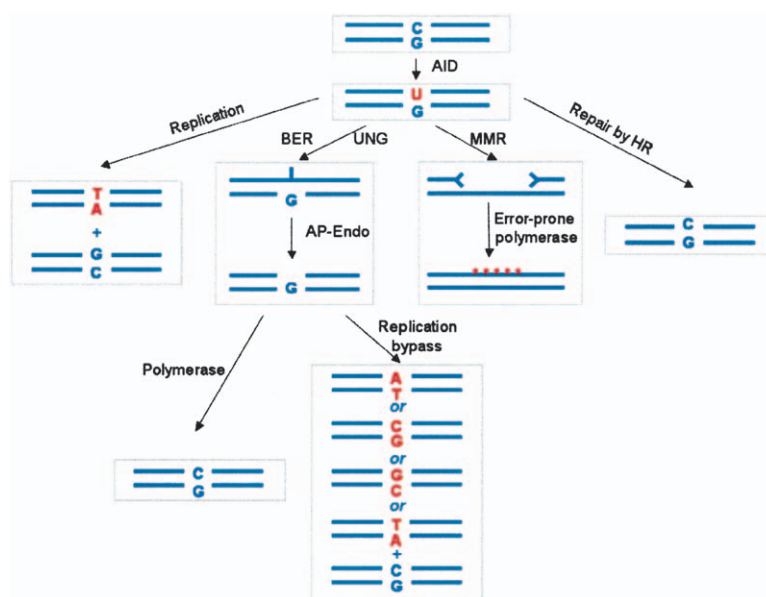


FIG 4. Resolution of U-G mismatch created by AID on DNA. AID deaminates deoxycytidine (C) to deoxyuracil (U) to create U-G mismatches. The mismatch can be repaired by one or more of the 4 pathways illustrated. First, it can be replicated to produce a C to T mutation. Second, it can be repaired by means of base excision repair. The deoxyuracil can be removed by means of DNA UNG to create an abasic site. The abasic site can be converted to a single-stranded nick by apurinic-apyrimidinic endonuclease (AP-Endo), which can either be repaired with error-free replication or bypassed by error-prone polymerases to generate all possible mutations. Third, it can be recognized by MMR proteins. The sequence surrounding the mismatched is excised and replaced with error-prone polymerases, which creates additional mutations of the sequence. Finally, it can be repaired by means of homologous recombination (HR) if the mutations are present in late S and G2 when a sister chromatid is available as a template.

now encodes a truncated protein with a new function.³⁹ The similarities between AID and the family of mRNA-editing enzymes led to the suggestion that AID was acting indirectly by editing the mRNA for an unknown endonuclease and activating the protein so that it could act directly on the V and SR DNA to initiate SHM and CSR.²⁰ The fact that some of the mutations in the patients with HIGM2 were in the deaminase-active site (Fig 3, A) suggested that AID was in fact functioning as a cytidine deaminase. However, this did not formally establish the substrate for this enzyme. Although some still argue that AID is acting indirectly as an mRNA-editing enzyme, it seemed equally possible that AID was acting directly on DNA,⁴⁰ and evidence rapidly accumulated to support this hypothesis. When AID was ectopically expressed in B cells that were not at the centroblast stage of differentiation, in cells of other lineages and in bacteria, it activated the SHM and CSR of reporter genes.⁴¹⁻⁴⁴ These studies indicated that most of the factors involved in SHM and CSR were ubiquitous and highly conserved and that AID was the only B cell-specific factor required for mutation.¹³ Furthermore, it seemed highly unlikely that non-B cells and bacteria contained an mRNA that had to be edited by AID, suggesting that the deoxycytidine (dC) was being deaminated to produce deoxyuracil (dU) in DNA itself.⁴⁴ The uracil can be processed through different pathways, resulting in mutations in the V region, as well as in the SRs (Fig 4). If processed through the base excision repair

pathway, the uracil will be cleaved by DNA uracil N-glycosylase (UNG) to generate an abasic site, which will be further cut by apurinic-apyrimidinic endonuclease (AP endonuclease), resulting in a nick on that strand. Closely spaced nicks on both strands can be processed to form staggered DSBs that are required for CSR.^{8,29,31,45} This hypothesis was supported by the following discoveries: a UNG inhibitor blocked gene conversion, the primary mechanism for creating antibody diversity in chickens, in DT-40 chicken B cells⁴⁶; UNG-deficient mice had a perturbed spectrum of mutation and a decrease in CSR⁴⁷; and human UNG deficiency was associated with defective CSR.⁴⁸

This DNA deamination model for SHM was confirmed biochemically in a cell-free system when it was shown that semipurified AID deaminated dC in single-stranded DNA (ssDNA) but not double-stranded DNA, single- or double-stranded RNA, or DNA-RNA hybrids.⁴⁹ In this system AID targeted hot spots like those targeted in SHM *in vivo*, suggesting that AID itself contained the information needed for the targeting of SHM to hot spots.^{49,50} The best substrate was an ssDNA bubble of about 9 nucleotides that is comparable with a typical transcription bubble created by RNA polymerase II (Fig 3, B). Native AID that was overexpressed and semipurified from 293T cells was also most active on ssDNA.⁵¹ Furthermore, a high-molecular-weight complex was required for its hot spot targeting and maximum activity. This suggests that AID is a

polymer, it is associated with other proteins, or both.⁵¹ In fact, replication protein A, an ssDNA-binding protein, has just been isolated from stimulated mouse splenic B cells as part of an AID-containing protein complex that has transcription-coupled deaminase activity *in vitro* (Fig 3, B).⁵²

Others reported that AID purified from bacteria also acted on ssDNA.^{53,54} The finding that ssDNA is the substrate for AID provides a reasonable explanation for the need for high rates of transcription for SHM and CSR because the higher the rate of transcription, the more transcription bubbles there are for AID to target. It is also possible that transcription factors play additional roles in recruiting and targeting AID, the other proteins that contribute to the mutational process, or both.^{55,56}

Different domains of the AID molecule were initially identified on the basis of the 3-dimensional structure of a bacterial cytidine deaminase and homology with APOBEC1, as well as other mammalian RNA-editing enzymes (Fig 3, A).^{20,57} The amino terminal end of AID contains a bipartite nuclear localization signal (NLS)⁵⁸ and a region with a zinc coordination motif. Homology analysis and site-directed mutagenesis confirm that this domain contains the active catalytic site of the enzyme.²⁰ This is followed by a region that links the catalytic domain to a pseudocatalytic region that is homologous to the catalytic domain but does not seem to play a direct role in catalysis. The C-terminal end of the protein contains a nuclear export motif (nuclear export signal) that has been shown to associate with a nuclear export protein that is involved in the shuttling of AID from the nucleus to the cytoplasm.⁵⁹ Inactivating mutations have been identified at 18 different sites in more than 60 patients with HIGM2 (Fig 3, A).^{9,37,58-62} All of these patients were very susceptible to bacterial infections, especially of the upper respiratory tract, and almost all were treated with intravenous Ig. These clinical findings indicate that each of the domains plays an important role in AID function, but there is no clear genotype-phenotype correlation between the characteristics of disease and location of the mutations.⁹ The role of each of the domains has been confirmed by expressing AID genes containing representative mutations in reporter assays for SHM and CSR.^{58,59,62} The most interesting groups of mutations were at the C-terminal ends of AID (Fig 3, A). AID molecules that lacked the C-terminal 10-17 amino acids or had site-directed mutations could carry out SHM but not CSR.^{59,62} Consistent with the *in vitro* assays, one of the patients with HIGM2 with this mutation was studied and retained the ability to carry out SHM but was deficient in CSR,^{37,59} confirming that the C-terminal domain of AID was required for CSR, but not SHM, *in vivo*. Interestingly, a subset of site-directed mutations located in the N-terminal NLS of AID were recently reported to abolish SHM but not CSR.⁵⁸ It is unclear whether the NLS and nuclear export signal functions contribute in any way to the apparently distinct roles of the N- and C-terminal domains in SHM and CSR. However, the different roles of these regions do support the contention that there must be factors that associate with AID and separate its function in SHM and CSR.^{51,58,59,62}

MODEL FOR ROLE OF AID IN SHM AND CSR

The combination of biochemical, cellular, and clinical findings described above have provided a working model for SHM and CSR that explains most, but not all, of the findings in AID-deficient mice and in patients with HIGM2. This model assumes that the expression of AID is tightly transcriptionally controlled so that it is expressed primarily at the centroblast stage of B-cell differentiation. The regulation of AID expression *in vivo* at either the mRNA or the protein level is not completely understood yet. However, it has been shown that AID can be induced by the stimuli that drive CSR and SHM in mouse and human primary B cells and certain B-cell lines.^{24,35,63} AID is a very basic protein, and therefore it is likely that other factors or posttranslational modifications, such as phosphorylation, facilitate its transport in and out of the nucleus. The mechanisms that target AID to the V regions and SRs are just beginning to be investigated, but its restricted targeting results at least in part from the availability of large amounts of ssDNA in the form of transcription bubbles in the V region and the SRs.⁴⁹ In the mammalian SRs the C-rich template DNA form RNA-DNA hybrids with the nascent G-rich sterile RNA transcripts and potentially create large regions of ssDNA (Fig 3, B).^{26,27} *In vitro* experiments also showed that AID generated many more mutations on the nontemplate strand than on the template strand.^{50,51} However, it is unclear why both strands of the SRs are mutated *in vivo*,⁶⁴ and this reveals that there is much still to be learned about how AID actually works.

High levels of transcription cannot alone explain why AID is targeted to the V and SR of the Ig genes because at least some other highly transcribed genes in B cells are not targeted for SHM.⁶⁵ It has been suggested that selective increases in chromatin accessibility could be responsible for the restriction of AID-induced mutation.⁶⁶ Recent studies show that increases in the acetylation of histone H4 are associated with the targeting of SHM to the V region⁶³; increases in the acetylation of histone H3 are associated with targeted downstream SRs.⁶⁷ In fact, drug-induced global changes in histone acetylation can cause the mistargeting of SHM to the C region and to the part of the V region immediately downstream from the promoter.⁶³ The complete description of chromatin modifications in the V region and SRs should reveal whether particular histone codes actually recruit the different proteins that are involved in SHM and CSR.

Once AID, perhaps with the help of associated molecules, converts dC to dU, dU in the V region and SRs can be changed as follows (Fig 4). It can be replicated to produce a C to T mutation or removed by UNG to create an abasic site. The abasic site can subsequently be converted to a single-stranded nick by AP endonuclease and then repaired by means of base excision repair without generating mutations, or it can be bypassed by error-prone DNA polymerases to generate mutations. It can be recognized by MutS homologue 2–MutS homologue 6 and the complex of other MMR proteins and then excised

TABLE I. Role of AID in SHM and CSR in health and disease

Normal AID function	In germinal centers of secondary lymphoid organs: enhance antibody affinity and effector functions through SHM and CSR; contribute to resistance to some infections. In nasal mucosa: local switching to IgE and SHM in allergic rhinitis. In the intestine: maintain normal flora.
AID deficiency	Human: hyper-IgM syndrome type 2, loss of SHM and CSR. Mouse: Immunodeficiency with loss of SHM and CSR; changes in intestinal lymphoid structure; dysregulation of intestinal flora homeostasis.
Mis-targeting of AID activity in B cells	B-cell malignancies caused by mutations and translocations of non-Ig genes.
AID activity in non-B cells	Transgenic mice have T-cell lymphoma.

and replaced by means of resynthesis with error-prone polymerases that will create additional mutations, including mutations on A-T base pairs. If the mutations are present in late S and G2, they can be repaired by homologous recombination (Fig 4).^{44,68} It is unclear whether these different pathways are coordinated and regulated by other factors or compete with each other in some stochastic way. DSBs have been detected in both SHM and CSR. Although the generation of DSBs in the SRs clearly depends on AID,^{69,70} DSBs in the V region are detected in AID-deficient mice, indicating that they are not the result of AID-induced deamination.^{71,72} In addition, proteins, such as Ku, phosphorylated histone H2A variant, ataxia telangiectasia mutated, DNA-dependent protein kinase catalytic subunit, 53BP1, and probably others that are required to repair DSBs and for efficient CSR, are not required for SHM.⁸ It has also been reported that AID might play a role in processing DSBs that do arise in the V region in cultured B cells.⁷³ Although it is still unclear whether DSBs even occur or are required in SHM, DSBs are required for CSR, and therefore must play different roles in SHM and CSR.^{63,68}

In this model the role of AID is to initiate CSR by introducing dC to dU mutations in single-stranded regions of the SRs, much as it does in the V region (Fig 4). This is supported by characteristics of the mutations and the dependence of CSR on UNG⁴⁸ and MMR.¹³ In addition, it has been suggested that the DSBs in the SRs are the result of closely spaced single-stranded nicks introduced by UNG-AP endonuclease, MMR, or both; these staggered breaks are converted in ways yet to be defined into the DSBs that are required for switch recombination.⁴⁵ These DSBs in the donor S μ and recipient γ , ϵ , or α SRs can be resolved by means of NHEJ or other DNA repair mechanisms.⁸

ROLE OF AID IN SHM AND CSR IN HEALTH AND DISEASE

The current understanding of the role of AID in SHM and CSR in health and disease is summarized in Table I. Patients with HIGM2 syndrome are susceptible to encapsulated bacterial infections, although not to viral or

opportunistic infections.⁹ It was also shown that IgM alone is able to protect AID-deficient mice from influenza virus infection, although there is a delay in the clearance of the organism and significant morbidity compared with that seen in wild-type mice.⁷⁴ The fact that some patients with HIGM2 who retained the ability to carry out SHM but not CSR were still very susceptible to bacterial infections^{37,59} suggests that CSR is more important than SHM in the resistance to microbial infections. Patients with HIGM2 also have an increased incidence of autoimmune and inflammatory disorders.⁹ Abnormalities of AID function probably have an even broader effect than is revealed by the HIGM2 syndrome. For example, mice that are genetically deficient in AID have an increase in the number of nonpathogenic anaerobic bacteria throughout the small intestine. There are also many protruding follicular lymphoid structures caused by hypertrophy of the isolated lymphoid structures that line the small intestine.⁷⁵ On the basis of similar histology seen in patients with common variable immunodeficiency syndrome, the change in intestinal flora suggests that AID-mediated CSR (to produce IgA) and perhaps SHM (to produce higher affinity antibodies) are important in regulating the homeostasis of the intestinal flora⁷⁶ and perhaps the flora of other cavities, such as the sinuses and bronchial tree. Similarly, the local regulation of switching to IgE and SHM in the nasal mucosa might play a role in determining whether allergic rhinitis occurs.⁷⁷

The observations that AID can cause mutations in bacteria and many different cell types make it clear that AID is highly mutagenic and that it is a dangerous molecule. This was confirmed when AID was ubiquitously overexpressed in mice and led to shorter life spans. All the transgenic mice died from T-cell lymphomas.⁷⁸ AID-induced mutations appear to contribute to the high incidence of B-cell malignancies.^{79,80} B-cell malignancies are partly classified by the stage in B-cell differentiation at which they appear to be arrested, and many seem to be associated with the stages of differentiation, where rearrangements and mutations in the Ig gene occur (Fig 1).⁸¹ This association has been attributed to errors in resolution of the DSBs that arise during the course of normal V(D)J rearrangement, CSR, and the single-stranded nicks that occur during SHM.⁸⁰ For example,

most BCL2 translocations in follicular lymphoma involve the J heavy-chain gene segments. The c-MYC gene in many Burkitt lymphomas is translocated to the heavy-chain switch regions, whereas in other Burkitt lymphomas, translocation breakpoints are found in the rearranged V genes.⁷⁹ Such translocations put the proto-oncogenes under the transcriptional control of Ig regulatory elements (promoters or enhancers), leading to their uncontrolled expression and malignant transformation.

Although there is selective targeting of AID to the Ig V region and SRs, other genes that are expressed at comparable levels with that of IgM in centroblast B cells, such as Ig α (mb1) and Ig β (B29), which are required for the membrane expression of IgM, and the proto-oncogenes BCL6, c-MYC, and Fas, undergo SHM-like mutations in normal and malignant B cells, although at lower rates than the V region does.^{80,82} The BCL6 proto-oncogene indicates how such mutations can lead to malignancies, even in the absence of translocations. BCL6 is highly expressed and mutated in normal GC cells^{83,84} and is overexpressed in many diffuse large B-cell lymphomas (DLBCLs).⁸⁵ Although this overexpression is often associated with translocations, in about 16% of the nontranslocated cases, the mutations modify the BCL6 promoter so that the normal downregulation of BCL6 cannot occur.^{86,87} Moreover, the regions targeted by mutations significantly overlap with the major chromosomal breakpoint regions within the oncogenes that were mutated in DLBCL,^{80,85} which suggests that mutations lead to translocations. The c-MYC oncogene is translocated and overexpressed in almost all multiple myelomas, and it also undergoes SHM-like mutations in regions that can lead to its overexpression.⁸⁸ Recently, there have been attempts to correlate the presence of V region mutations, ongoing SHM and CSR, and the expression of AID in various B-cell malignancies with prognosis and therapeutic outcome. Although this would be very useful, there are conflicting observations that require additional studies to resolve.^{89,90} However, these studies have shown that AID can be expressed in malignant B cells that do not seem to have arisen from centroblasts. Although AID expression might be intermittent and occurring in only a small percentage of the malignant cells,⁹¹ it remains unknown whether AID expression is really restricted to only the centroblast stage of differentiation and why AID-induced mutations occur in non-Ig genes. This issue applies to normal B-cell differentiation because V region mutations and class switching have been observed in normal B cells are outside of the GC and do not have centroblast markers.^{92,93} In addition, splice variants of AID have been observed in some of these malignant cells⁹⁴ and in normal B cells activated with lymphokines,²⁴ and therefore it is possible that the AID molecules lacking one of the domains and having partial function are made during the immune response and as part of malignant transformation.

These observations in malignant B cells reveal that there is still much to be learned about how AID expression is regulated and how it functions. They also confirm that

we need to learn about what regulates the targeting of AID. Although there is significant evidence that changes in chromatin structure play a role in the targeting of AID and other mutational factors to the V region and SR,^{63,67} it is unclear whether there are additional cis-acting DNA sequences and binding proteins that contribute to this targeting.³¹ It is also unclear exactly how NHEJ and the other processes downstream of AID that differ in SHM and CSR are selectively recruited and coordinated and why mistargeting of AID to other genes occurs.²¹ Nevertheless, the rapid progress in our understanding of the molecular and biochemical basis of SHM and CSR in the last few years illustrates how the interplay between modern molecular genetics and the careful analysis of patients can advance our knowledge of fundamental biologic processes and clinical syndromes in a very rapid fashion.

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