Progress in Hematology

International Journal of
HEMATOLOGY

The Dark Side of Activation-Induced Cytidine Deaminase: Relationship with Leukemia and Beyond

Kazuo Kinoshita,[a] Taichiro Nonaka[b]

[a]*Evolutionary Medicine, Shiga Medical Center Research Institute, Moriyama;*
[b]*Department of Immunology and Genomic Medicine, Kyoto University Graduate School of Medicine, Kyoto, Japan*

Received January 12, 2006; accepted January 31, 2006

Abstract

Activation-induced cytidine deaminase (AID) is a unique cellular enzyme that can trigger point mutations and chromosomal translocations, both of which potentially disturb normal cellular metabolism and affect cancer initiation and progression. The involvement of AID in the progression of leukemia has been suggested by multiple groups on the basis of observations of the statistical correlation between AID expression and a poor prognosis of B-cell chronic lymphocytic leukemia. The fact that ectopic expression of AID in mice results in tumors of the lung and T-lymphocytes suggests an oncogenic role for AID. The inducible nature of AID expression indicates that AID might be induced and cause oncogenic mutations, even in epithelial tissues, where AID expression is absent or very weak under normal conditions. If AID can be induced in epithelial cells by inflammatory signals, as from B-lymphocytes, it may be involved in various pathologic conditions, including inflammation- and infection-associated cancers, for which the molecular mechanism is largely unknown, despite the clinical significance of these diseases.

Int J Hematol. 2006;83:201-207. doi: 10.1532/IJH97.06011

Key words: AID; Somatic mutation; Chromosomal translocation; Inflammation; Cancer

1. Introduction

Life is a treasure house of mysteries and wonders. How could anyone have imagined that the human genome contains a gene that can mutate the genome itself in response to environmental cues? Perhaps Charles Darwin, and even more so Jean-Baptiste Lamarck, would have been surprised, if they had had a chance to hear about it. This gene encodes an enzyme called activation-induced cytidine deaminase (AID), which was discovered in 1999 in a study of immunoglobulin gene rearrangement [1]. A gene-targeting study in mice [2] and an analysis of human families with an autosomal recessive form of hyper–immunoglobulin M (IgM) syndrome [3] clarified an essential role for AID in immunoglobulin gene diversification, that is, in class-switch recombination (CSR) and somatic hypermutation (SHM), both of which guarantee a repertoire of antibodies diverse enough to protect the body against a variety of pathogens.

Correspondence and reprint requests: Kazuo Kinoshita, MD, PhD, Evolutionary Medicine, Shiga Medical Center Research Institute, 5-4-30, Moriyama, Shiga 524-8524, Japan; 81-77-582-6044; fax: 81-77-582-6041 (e-mail: kkinoshi.shigamed@mac.com).

Recently, AID has been reported to be expressed in a subpopulation of B-cell chronic lymphocytic leukemia (B-CLL) patients, and AID expression has been correlated with an absence of SHM and a poor prognosis of the disease [4-6]. Because AID has the potential to introduce mutations into non-immunoglobulin genes [7-10], AID expression may be involved in the pathogenesis and progression of B-CLL. An extrapolation of this result indicates that AID may be a cause of common malignancies, because AID expression can be detected in non–B-cells. Although the basal level of expression is not high, taking into account the inducible nature of AID in B-cells, AID may be induced by certain stimuli even in non–B-cells. In this review, we summarize the evidence suggesting a link between AID and the evolution of cancer and emphasize that AID may not always be a good thing for people's health.

2. What is AID?

In 1978, Tasuku Honjo and Tohru Kataoka proposed that DNA recombination underlies the class-switch phenomenon of immunoglobulins [11]. Since then, extensive studies have focused on the molecular mechanism of CSR. As a result, a tremendous amount of knowledge has accumulated, starting from the sequence features of recombination-prone regions

and the entire organization of the 200-kb locus of constant region genes of the immunoglobulin heavy chain and continuing to the mechanism of regulation of the recombination target by transcription [12-16]. However, the long and passionate quest for recombinase itself has not been rewarded. When recombination-activating gene 1 (RAG-1) and RAG-2—both of which catalyze another DNA rearrangement of immunoglobulin, variable-(diversity)-joining recombination—were identified one after the other in 1989 and 1990, class-switch investigators must have been overwhelmed by the work of RAG researchers. The subsequent decade of the 1990s was a "RAG time" that flourished with the publication of many important articles.

Another long-unsolved mystery surrounding the antibody was SHM of the variable region [16-18]. This phenomenon was initially proposed in 1966 by Brenner and Milstein with marvelous insight into the molecular mechanism of SHM, was identified at the protein level in 1970 by Weigert and Cohn, and later confirmed at the DNA level in 1981 by Hood and Baltimore. The frequency of mutation in the variable region is almost 6 orders of magnitude greater than the overall frequency of genome mutation of 10^{-9} per generation. Although the involvement of mismatch-repair enzymes had been generally accepted, the key molecule for SHM was totally unknown. For a long time no one could explain why this phenomenon happens.

In 1999, Honjo's group identified a novel gene encoding AID [1]. The AID gene was identified by complementary DNA subtraction. Its expression was induced in cells of a mouse B-cell line, CH12F3-2, that were stimulated to undergo CSR. The pivotal role of AID in CSR was confirmed by a gene-targeting study in mice [2] and by an analysis of a subgroup of patients with hyper-IgM syndrome with an autosomal recessive trait [3]. The fact that not only CSR but also SHM was defective in AID-deficient humans and mice surprised many immunologists. The long-awaited answers to the 2 big questions about CSR and SHM were given simultaneously by the discovery of AID. As is usual for all scientific breakthroughs, however, this finding raised the next level of questions, "How does AID regulate CSR and SHM?"

Detailed analyses of the AID-mediated reaction are appearing all over the world but the entire picture has not yet emerged (at least as of the end of 2005). Because the aim of this review is not to examine the molecular mechanism of AID (see recent reviews [12,13-16,19]), the hot topics on AID function are just briefly mentioned below.

The biggest unsettled issue is over the substrate of AID, whether it is RNA or DNA. When AID was identified in 1999, the protein most homologous to it was an RNA-editing enzyme, Apobec-1 [1]. The requirement of protein synthesis for AID-mediated DNA cleavage in CSR and SHM also supported the RNA-editing model [20-22]. On the other hand, the enzymatic activity of AID in vitro and in *Escherichia coli* suggested the DNA-editing model [23,24]. AID has been reported to also deaminate 5-methylcytosine to yield thymine, in which case repair of the T · G mismatch will result in demethylation or a C-to-T transition [25]. This view was further strengthened by the identification of human Apobec3G/CEM15 (Apobec3 in the mouse), the closest AID homologue in the human genome [26]. Apobec3G has a DNA-editing activity toward human immunodeficiency virus–derived first-strand DNA that suppresses the replicative potential of the virus [27,28]. In addition, the partial dependence of CSR on uracil-DNA *N*-glycosylase (UNG) fit with the DNA-editing model because uracil converted from cytosine on DNA by AID has to be removed by UNG before the DNA is cleaved by apurinic/apyrimidinic endonuclease [29,30]. Despite these studies supporting the DNA-editing model, the fact that even Apobec-1, a bona fide RNA-editing enzyme, can deaminate DNA in vitro [31], whereas it cannot mutate the green fluorescent protein (GFP) transgene in mammalian cells as AID does [32], and the fact that the catalytic (that is, U-removing) activity of UNG is unnecessary for CSR [33] cast doubt on the interpretation of the results described above. For proponents of an RNA-editing function, identification of the RNA target is mandatory. For the DNA-editing alliance, an explanation for the dispensable nature of UNG catalytic activity must be presented. Which of the models is correct remains to be determined, despite the current dominance of believers in the DNA-editing hypothesis.

Three functional domains have been documented in AID. The N-terminal, middle, and C-terminal domains are responsible for SHM, cytidine deaminase, and CSR functions, respectively [34-36]. How these 3 domains are arranged in the 3-dimensional structure has yet to be solved by nuclear magnetic resonance or crystallography studies, which have been hampered by the extreme difficulty in purifying the protein. Separate domains for CSR and SHM suggest the presence of associating cofactors. Identification of the CSR- and SHM-specific cofactors is crucial for the understanding of AID function. AID appears to form a dimer like Apobec-1 and metabolic cytidine deaminases [34], although whether dimer formation has a functional significance is not known. The nuclear-export signal at the C terminus indicates that AID is localized predominantly in the cytoplasm, and AID may shuttle dynamically between the cytoplasm and the nucleus [37,38]. Nuclear localization and/or deamination of single-stranded DNA may be promoted by the recently identified phosphorylation of AID [39-41], the functional significance of which has recently become another source of debate.

3. AID and Cancer

3.1. AID and Leukemia

The mutational status of immunoglobulin heavy chain variable region genes (V_H) and cytogenetic variations [42-44] have been determined to be prognostic markers of B-CLL. B-CLL patients with no V_H mutations have a poorer prognosis than those with mutations. Deletion in 11q, loss of p53, and trisomy 12 are unfavorable cytogenetic markers. Because AID is an essential factor for V_H mutation, it was natural that the 2 groups that examined AID expression in B-CLL came to the same conclusion [4,5]. Oddly enough, a negative correlation was found between AID expression and mutations in V_H. B-CLL cells without SHM do express AID and vice versa (Figure 1). This observation is contrary to the speculation that AID-expressing cells should have

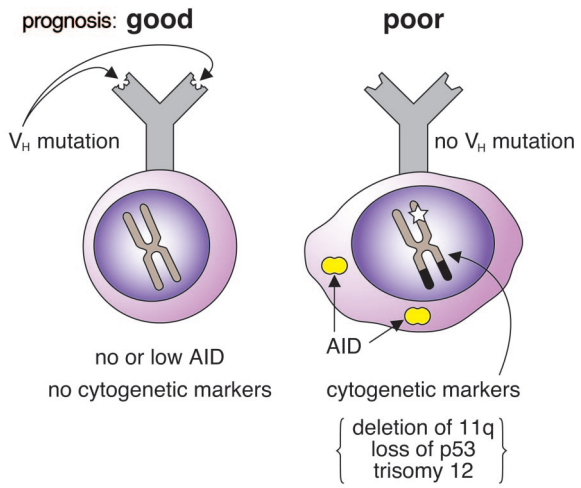

Figure 1. Two prognostic groups of B-cell chronic lymphocytic leukemia differentiated on the basis of somatic hypermutation status, activation-induced cytidine deaminase (AID) expression, and cytogenetic markers. The presence of V_H mutations in immunoglobulin (gray *Y*) is associated with a good prognosis of the disease, low AID expression, and a lack of cytogenetic markers (left). An absence of V_H mutations is associated with a poor prognosis, high AID expression (yellow ovals), and cytogenetic alterations (right). The open star on the *X*-shaped mitotic chromosome represents point mutations or intrachromosomal rearrangement. Black areas on the chromosome represent translocated regions.

more mutations. This observation was confirmed in a study by Heintel and colleagues [6]. They quantitated AID messenger RNA (mRNA) in the peripheral blood of 80 patients with B-CLL and analyzed its statistical correlation with known prognostic markers. These investigators found that not only an absence of SHM but also such cytogenetic markers as deletion of 11q and trisomy 12 were correlated with the expression of AID. The correlation with trisomy 12 is intriguing because the AID gene is located on 12p13 [45]. The level of AID in the peripheral blood was stable over a long period and was not affected by chemotherapy and the white blood cell count. Therefore, the level of AID mRNA in the peripheral blood is a potentially useful marker to predict the prognosis of B-CLL. Quantitation of AID mRNA by real-time polymerase chain reaction (PCR) analysis is more rapid and cheaper than SHM measurement and cytogenetic analysis. Before this method has a clinical application, as investigators have claimed, a long-term cohort study observing the clinical outcomes of many B-CLL patients and a determination of the threshold for AID positivity must be done. One report failed to demonstrate a correlation between AID expression and V_H mutation in B-CLL [46]. This result could have been due to an inappropriate design of their AID primers, which did not span an intron, for their real-time PCR analysis.

A simple correlation does not tell us whether AID expression is the cause or the result of leukemia. However, a causal relationship is suggested from the results of a study of AID transgenic mice in which AID expression was driven by a ubiquitous promoter [9]. AID transgenic mice spontaneously and frequently develop T-cell lymphoma and lung microadenoma. T-cell lymphoma began to be observed 2 months after birth. Half of the mice had died by 8 months, and by 15 months all of the individuals had succumbed to T-cell lymphoma. The onset of T-cell lymphoma tended to be earlier in later generations of the transgenic mice, a result that suggests the accumulation of germline mutations. Lung microadenoma was found to develop much earlier than T-cell lymphoma and was observed in all examined mice older than 3 months. Multiple small foci of adenoma were often observed in the subpleural region, and malignant transformation was occasionally observed in mice older than 1 year. Pathologically, this lung microadenoma was very similar to atypical adenomatous hyperplasia of the lung in humans. The reason that T-lymphocytes and the lung epithelium were the target of cancer formation in AID transgenic mice while AID was expressed ubiquitously is not known. Nonetheless, the fact that aberrant AID expression in mice led to both lymphoid and nonlymphoid tumorigenesis suggests that AID can be a cause of various types of cancer.

SHM, namely mutagenesis by AID, has been considered a phenomenon restricted to V genes of the immunoglobulin heavy chain and light chain. If this is the case, it is unlikely that AID generates mutations in oncogenes, which are, of course, not immunoglobulin genes. A variety of experiments that addressed this targeting specificity revealed that the V region sequence itself does not contribute to SHM targeting and that transcription of the target DNA is required. However, the promoter can be exchanged for other gene promoters, including those driven by RNA polymerase I, and an immunoglobulin enhancer appears to be required. Therefore, transcription and the presence of an immunoglobulin enhancer were thought to play a role in SHM targeting. In 1998, 2 groups reported frequent mutations of Bcl-6 genes in human B-cells, and these mutations were considered to have been generated by the SHM machinery [47,48]. In 2001, Pasqualucci and colleagues reported frequent mutations of several oncogenes, such as PIM1, PAX5, RhoH/TTF, and c-MYC, in B-cell diffuse large cell leukemia (DLCL) of humans [49]. These oncogenes shared properties of mutational pattern and distribution with those of immunoglobulin SHM. The expression level of AID in DLCL was not correlated with the intraclonal heterogeneity of V_H and the Bcl-6 sequence, suggesting a transient expression of AID prior to clinical detection of the disease [50]. In AID transgenic mice that had developed T-cell lymphoma, the genes for T-cell antigen receptor β, c-MYC, PIM1, CD4, and CD5 were mutated [9,10]. In 2 subsequent studies, AID-induced mutations appeared to be dependent on transcription but not on an immunoglobulin enhancer. Yoshikawa and colleagues found that AID expression in NIH3T3 cells, a fibroblast cell line, induced frequent mutation of GFP transgenes [7]. Martin and Scharff reported that AID overexpression in the Ramos B-cell line and Chinese hamster ovary cells induced SHM in immunoglobulin and AID transgenes [8]. From these many studies, it is tempting to speculate that AID can introduce mutations even in non-immunoglobulin genes, although the frequencies may not be as high as in immunoglobulin V genes.

3.2. Oncogenic Role of AID after Virus Infection

3.2.1. Epstein-Barr Virus and AID

Epstein-Barr virus (EBV) is known to cause several forms of cancer in humans. They include Burkitt lymphoma, Hodgkin's disease, nasopharyngeal carcinoma, and gastric and breast cancer [51]. Burkitt lymphoma has a characteristic chromosomal translocation between c-myc and IgH. This translocation does not produce a fusion protein like the Bcr-Abl and PML-RARα fusion genes observed in chronic myelogenous leukemia (Philadelphia chromosome) and acute promyelocytic leukemia, respectively, but brings the immunoglobulin enhancer into the proximity of the c-myc gene, resulting robust c-myc expression and cell cycle progression in B-cells. Recently, mouse c-myc/IgH translocation was reported to be dependent on AID in plasmacytomas developed in interleukin 6 transgenic mice [52]. This finding suggests that the human c-myc/IgH translocation in Burkitt lymphoma may be caused by AID-induced aberrant CSR. If so, how can AID be induced in Burkitt lymphoma? The EBV genome encodes latent membrane protein 1 (LMP1), which hijacks the signaling pathway normally employed via the CD40 molecule on B-cells. LMP1 can transduce signals without ligand engagement, in contrast to CD40 [53]. Because AID expression is induced by CD40 stimulation, it is not surprising that EBV infection and LMP1 induce AID and CSR, as has been reported [54,55]. Therefore, it is quite reasonable to think that AID is induced in EBV-infected B-cells through LMP1.

EBV also infects nasopharyngeal and gastric epithelia. Whether EBV can induce AID in these epithelial cells is unknown. If it can, EBV-positive nasopharyngeal carcinoma and gastric cancers, which constitute 10% of gastric cancers [56], may be initiated by AID. However, c-myc/IgH translocation is unlikely to be detected in epithelial tumors after EBV infection because the immunoglobulin enhancer may not be active in these cells.

3.2.2. Hepatitis C Virus and AID

Hepatitis C virus (HCV) is the leading cause of chronic liver disease and hepatocellular carcinoma in the world. HCV preferentially infects human hepatocytes. However, HCV has also been reported to infect B-lymphocytes through an interaction between HCV envelope protein E2 and CD81 on B-cells [57]. This action may be why HCV infection occasionally triggers non-Hodgkin's B-cell lymphoma. Oligoclonal lymphoproliferative disorders and chromosomal translocation have also been reported to occur frequently in HCV-infected individuals [58,59]. In HCV-associated lymphoma, the immunoglobulin V gene shows frequent SHM and intraclonal diversity. These observations indicate that HCV infection may induce genetic instability in B-cells. The question is how.

To address this issue, Machida and colleagues analyzed mutation frequency in immunoglobulin V, Bcl-6, p53, β-catenin, and β-globin genes in peripheral blood mononuclear cells and B-cell lines with HCV infection [60]. They found a significant increase in mutation frequency in these genes in HCV-infected cells, compared with noninfected cells. They also found that HCV infection in the Raji B-cell line induces the transcription of AID and error-prone polymerases pol ζ and pol ι, all of which can be causes of elevated mutation frequency. In addition, HCV infection induced breaks in double-stranded DNA in V_H and p53 genes. The mutation frequency of V_H in HCV-infected Raji cells was reduced by RNA interference (RNAi) against AID and pol ι but not against pol ζ. In contrast, the mutation frequency of p53 was suppressed by RNAi against pol ζ and pol ι but not against AID, suggesting the presence of AID-dependent and AID-independent pathways for mutagenesis by HCV infection. Although AID may not induce p53 mutation, it may mutate other oncogenes in HCV-infected B-cells.

3.3. AID and Inflammation-Associated Cancer

This story of AID and cancer so far has focused on B-cells. What about other types of cells? Common cancers in adults are of epithelial origin. Can AID be expressed in epithelial cells and induce oncogenic mutations? The fact that AID transgenic mice develop lung tumors that resemble atypical adenomatous hyperplasia in humans, as mentioned above, suggests that AID has the potential to introduce mutations in some oncogenes in the lung epithelium. Evidence hinting at AID expression in non-B cells (that is, expression in organs other than the spleen and lymph nodes) has been reported. For example, AID transcripts have been detected in human kidney and pancreas by the PCR after 36 cycles of amplification [45]. An initial report on AID expression in mice indicated restriction to lymphoid organs [1]. However, mouse oocytes and ovaries express AID [25]. Weak AID expression could be detected in the lung and testis by real-time PCR (approximately 1/10 that of spleen and 1/100 that of mesenteric lymph node), a result that was confirmed even in RAG2-deficient mice (K.K., unpublished data). The detection of AID in RAG2-deficient mice excludes the possibility of AID expression originating from B-lymphocytes in these organs. In dogs, AID transcripts have been observed in the thymus, lung, kidney, colon, and small intestine [61]. In chickens, AID transcripts have been observed in the thymus and testis [62]. In catfish, AID expression has been detected in the intestine, fin, and kidney [63]. This evidence suggests AID is expressed in non–B-cells, although further confirmation by immunohistochemistry or other sensitive and specific tests is needed.

Does such low-level expression of AID induce mutations that drive tumor evolution? AID expression in B-cells is induced by a variety of inflammatory signals, such as CD40L, B-cell–activating factor of the tumor necrosis factor family (BAFF) (also known as B-lymphocyte stimulator), a proliferation-inducing ligand (APRIL), interleukin 4, transforming growth factor β (TGF-β), and lipopolysaccharide (LPS) [1,64]. Therefore, there is a possibility that AID expression is up-regulated in epithelial cells by inflammation and infection. Actually, human primary keratinocytes have been induced to express AID mRNA after TGF-β or LPS treatment (unpublished data). Although there is no experimental support for oncogenic mutations induced by low-level AID expression, it is worthwhile to explore the involvement of

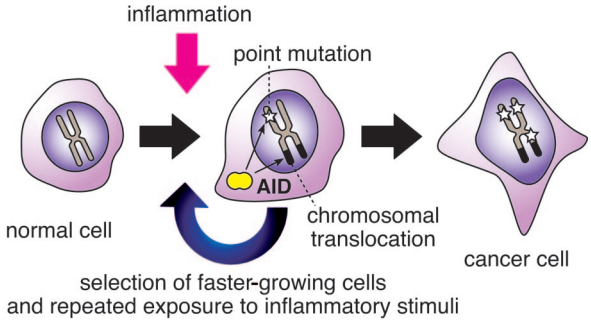

Figure 2. Model of activation-induced cytidine deaminase (AID)-induced tumorigenesis in infection- and inflammation-associated cancer. Stimulation by inflammation due to infection, mechanical and chemical stress, or other stimuli may induce normal lymphoid and epithelial cells (left) to express AID, which induces point mutations and chromosomal translocations (middle). When inflammation is persistent, continuous AID expression may drive successive rounds of genetic changes and selection of proliferating cells, resulting in the evolution of cancerous cells (right).

AID in inflammation- and infection-associated cancer, for which a molecular mechanism is largely unknown [65]. AID may be one of the endogenous mutators responsible for oncogenic mutations in addition to the mutagenic agents that have already been suggested, such as reactive oxygen species and nitrogen oxides (Figure 2).

4. Conclusion

We now know that the human genome harbors a gene for an enzyme that can actively alter genetic information after perception of an environmental cue. Although the AID enzyme is currently understood to be an integral node of the antibody-diversification machinery, its involvement in the progression of leukemia [4-6] and other forms of cancer [9] is just beginning to be discussed. Cancer cells impose enormous stress upon their host and at the same time also experience severe stress, including deficits in nutrition and oxygen and relentless attacks from the immune system and chemotherapeutic agents. Cells may have a strategy to increase genetic diversity to ensure survival of their descendants under stressed conditions. This trait may have been embraced since the era of our unicellular ancestors, because contemporary bacteria also have such mechanisms, including the SOS response and mutagenesis in aging colonies [66]. Such conditional mutability may benefit organisms in a fluctuating environment, as has been suggested by the results of a computer-simulation study [67]. Whether such conditional genetic instability is beneficial to cancer cells and normal cells is not known. If AID is responsible for such genetic plasticity, understanding and inhibition of this activity should contribute to the treatment and prevention of malignancy, a major cause of death in developed countries.

Acknowledgments

This work was supported by a grant-in-aid from the Ministry of Education, Culture, Sports and Technology of Japan and by grants from the Sagawa Cancer Research Foundation and The Ichiro Kanahara Foundation. We wish to thank Masamichi Muramatsu, Hitoshi Nagaoka, Reiko Shinkura, Ilmi Okazaki, Hiroyuki Marusawa, and Sidonia Fagarasan for critical reading of the manuscript and for discussions and Yoko Kitawaki for her excellent technical and secretarial help.

References

1. Muramatsu M, Sankaranand VS, Anant S, et al. Specific expression of activation-induced cytidine deaminase (AID), a novel member of the RNA-editing deaminase family in germinal center B cells. *J Biol Chem*. 1999;274:18470-18476.
2. Muramatsu M, Kinoshita K, Fagarasan S, Yamada S, Shinkai Y, Honjo T. Class switch recombination and hypermutation require activation-induced cytidine deaminase (AID), a potential RNA editing enzyme. *Cell*. 2000;102:553-563.
3. Revy P, Muto T, Levy Y, et al. Activation-induced cytidine deaminase (AID) deficiency causes the autosomal recessive form of the hyper-IgM syndrome (HIGM2). *Cell*. 2000;102:565-575.
4. Oppezzo P, Vuillier F, Vasconcelos Y, et al. Chronic lymphocytic leukemia B cells expressing AID display a dissociation between class switch recombination and somatic hypermutation. *Blood*. 2003;101:4029-4032.
5. McCarthy H, Wierda WG, Barron LL, et al. High expression of activation-induced cytidine deaminase (AID) and splice variants is a distinctive feature of poor-prognosis chronic lymphocytic leukemia. *Blood*. 2003;101:4903-4908.
6. Heintel D, Kroemer E, Kienle D, et al, and the German CLL Study Group. High expression of activation-induced cytidine deaminase (AID) mRNA is associated with unmutated IGV_H gene status and unfavourable cytogenetic aberrations in patients with chronic lymphocytic leukaemia. *Leukemia*. 2004;18:756-762.
7. Yoshikawa K, Okazaki IM, Eto T, et al. AID enzyme-induced hypermutation in an actively transcribed gene in fibroblasts. *Science*. 2002;296:2033-2036.
8. Martin A, Scharff MD. Somatic hypermutation of the AID transgene in B and non-B cells. *Proc Natl Acad Sci U S A*. 2002;99:12304-12308.
9. Okazaki IM, Hiai H, Kakazu N, et al. Constitutive expression of AID leads to tumorigenesis. *J Exp Med*. 2003;197:1173-1181.
10. Kotani A, Okazaki IM, Muramatsu M, et al. A target selection of somatic hypermutations is regulated similarly between T and B cells upon activation-induced cytidine deaminase expression. *Proc Natl Acad Sci U S A*. 2005;102:4506-4511.
11. Honjo T, Kataoka T. Organization of immunoglobulin heavy chain genes and allelic deletion model. *Proc Natl Acad Sci U S A*. 1978;75:2140-2144.
12. Kinoshita K, Honjo T. Linking class-switch recombination with somatic hypermutation. *Nat Rev Mol Cell Biol*. 2001;2:493-503.
13. Honjo T, Kinoshita K, Muramatsu M. Molecular mechanism of class switch recombination: linkage with somatic hypermutation. *Annu Rev Immunol*. 2002;20:165-196.
14. Stavnezer J, Amemiya CT. Evolution of isotype switching. *Semin Immunol*. 2004;16:257-275.
15. Chaudhuri J, Alt FW. Class-switch recombination: interplay of transcription, DNA deamination and DNA repair. *Nat Rev Immunol*. 2004;4:541-552.
16. Maizels N. Immunoglobulin gene diversification. *Annu Rev Genet*. 2005;39:23-46.
17. Kocks C, Rajewsky K. Stable expression and somatic hypermutation of antibody V regions in B-cell developmental pathways. *Annu Rev Immunol*. 1989;7:537-559.
18. Wagner SD, Neuberger MS. Somatic hypermutation of immunoglobulin genes. *Annu Rev Immunol*. 1996;14:441-457.
19. Barreto VM, Ramiro AR, Nussenzweig MC. Activation-induced

deaminase: controversies and open questions. *Trends Immunol*. 2005;26:90-96.
20. Doi T, Kinoshita K, Ikegawa M, Muramatsu M, Honjo T. De novo protein synthesis is required for the activation-induced cytidine deaminase function in class-switch recombination. *Proc Natl Acad Sci U S A*. 2003;100:2634-2638.
21. Begum NA, Kinoshita K, Muramatsu M, Nagaoka H, Shinkura R, Honjo T. De novo protein synthesis is required for activation-induced cytidine deaminase-dependent DNA cleavage in immunoglobulin class switch recombination. *Proc Natl Acad Sci U S A*. 2004;101:13003-13007.
22. Nagaoka H, Ito S, Muramatsu M, Nakata M, Honjo T. DNA cleavage in immunoglobulin somatic hypermutation depends on de novo protein synthesis but not on uracil DNA glycosylase. *Proc Natl Acad Sci U S A*. 2005;102:2022-2027.
23. Di Noia J, Neuberger MS. Altering the pathway of immunoglobulin hypermutation by inhibiting uracil-DNA glycosylase. *Nature*. 2002;419:43-48.
24. Ramiro AR, Stavropoulos P, Jankovic M, Nussenzweig MC. Transcription enhances AID-mediated cytidine deamination by exposing single-stranded DNA on the nontemplate strand. *Nat Immunol*. 2003;4:452-456.
25. Morgan HD, Dean W, Coker HA, Reik W, Petersen-Mahrt SK. Activation-induced cytidine deaminase deaminates 5-methylcytosine in DNA and is expressed in pluripotent tissues: implications for epigenetic reprogramming. *J Biol Chem*. 2004;279:52353-52360.
26. Sheehy AM, Gaddis NC, Choi JD, Malim MH. Isolation of a human gene that inhibits HIV-1 infection and is suppressed by the viral Vif protein. *Nature*. 2002;418:646-650.
27. Lecossier D, Bouchonnet F, Clavel F, Hance AJ. Hypermutation of HIV-1 DNA in the absence of the Vif protein. *Science*. 2003;300:1112.
28. Zhang H, Yang B, Pomerantz RJ, Zhang C, Arunachalam SC, Gao L. The cytidine deaminase CEM15 induces hypermutation in newly synthesized HIV-1 DNA. *Nature*. 2003;424:94-98.
29. Rada C, Williams GT, Nilsen H, Barnes DE, Lindahl T, Neuberger MS. Immunoglobulin isotype switching is inhibited and somatic hypermutation perturbed in UNG-deficient mice. *Curr Biol*. 2002;12:1748-1755.
30. Imai K, Slupphaug G, Lee WI, et al. Human uracil-DNA glycosylase deficiency associated with profoundly impaired immunoglobulin class-switch recombination. *Nat Immunol*. 2003;4:1023-1028.
31. Petersen-Mahrt SK, Neuberger MS. In vitro deamination of cytosine to uracil in single-stranded DNA by apolipoprotein B editing complex catalytic subunit 1 (APOBEC1). *J Biol Chem*. 2003;278:19583-19586.
32. Eto T, Kinoshita K, Yoshikawa K, Muramatsu M, Honjo T. RNA-editing cytidine deaminase Apobec-1 is unable to induce somatic hypermutation in mammalian cells. *Proc Natl Acad Sci U S A*. 2003;100:12895-12898.
33. Begum NA, Kinoshita K, Kakazu N, et al. Uracil DNA glycosylase activity is dispensable for immunoglobulin class switch. *Science*. 2004;305:1160-1163.
34. Ta VT, Nagaoka H, Catalan N, et al. AID mutant analyses indicate requirement for class-switch-specific cofactors. *Nat Immunol*. 2003;4:843-848.
35. Barreto V, Reina-San-Martin B, Ramiro AR, McBride KM, Nussenzweig MC. C-terminal deletion of AID uncouples class switch recombination from somatic hypermutation and gene conversion. *Mol Cell*. 2003;12:501-508.
36. Shinkura R, Ito S, Begum NA, et al. Separate domains of AID are required for somatic hypermutation and class-switch recombination. *Nat Immunol*. 2004;5:707-712.
37. Ito S, Nagaoka H, Shinkura R, et al. Activation-induced cytidine deaminase shuttles between nucleus and cytoplasm like apolipoprotein B mRNA editing catalytic polypeptide 1. *Proc Natl Acad Sci U S A*. 2004;101:1975-1980.
38. McBride KM, Barreto V, Ramiro AR, Stavropoulos P, Nussenzweig MC. Somatic hypermutation is limited by CRM1-dependent nuclear export of activation-induced deaminase. *J Exp Med*. 2004;199:1235-1244.
39. Chaudhuri J, Khuong C, Alt FW. Replication protein A interacts with AID to promote deamination of somatic hypermutation targets. *Nature*. 2004;430:992-998.
40. Basu U, Chaudhuri J, Alpert C, et al. The AID antibody diversification enzyme is regulated by protein kinase A phosphorylation. *Nature*. 2005;438:508-511.
41. Pasqualucci L, Kitaura Y, Gu H, Dalla-Favera R. PKA-mediated phosphorylation regulates the function of activation-induced deaminase (AID) in B cells. *Proc Natl Acad Sci U S A*. 2006;10:395-400.
42. Hamblin TJ, Orchard JA, Ibbotson RE, et al. CD38 expression and immunoglobulin variable region mutations are independent prognostic variables in chronic lymphocytic leukemia, but CD38 expression may vary during the course of the disease. *Blood*. 2002;99:1023-1029.
43. Oscier DG, Gardiner AC, Mould SJ, et al. Multivariate analysis of prognostic factors in CLL: clinical stage, *IGVH* gene mutational status, and loss or mutation of the *p53* gene are independent prognostic factors. *Blood*. 2002;100:1177-1184.
44. Krober A, Seiler T, Benner A, et al. V_H mutation status, CD38 expression level, genomic aberrations, and survival in chronic lymphocytic leukemia. *Blood*. 2002;100:1410-1416.
45. Muto T, Muramatsu M, Taniwaki M, Kinoshita K, Honjo T. Isolation, tissue distribution and chromosomal localization of the human activation-induced cytidine deaminase (*AID*) gene. *Genomics*. 2000;68:85-88.
46. Albesiano E, Messmer BT, Damle RN, Allen SL, Rai KR, Chiorazzi N. Activation-induced cytidine deaminase in chronic lymphocytic leukemia B cells: expression as multiple forms in a dynamic, variably sized fraction of the clone. *Blood*. 2003;102:3333-3339.
47. Shen HM, Peters A, Baron B, Zhu X, Storb U. Mutation of BCL-6 gene in normal B cells by the process of somatic hypermutation of Ig genes. *Science*. 1998;280:1750-1752.
48. Pasqualucci L, Migliazza A, Fracchiolla N, et al. BCL-6 mutations in normal germinal center B cells: evidence of somatic hypermutation acting outside Ig loci. *Proc Natl Acad Sci U S A*. 1998;95:11816-11821.
49. Pasqualucci L, Neumeister P, Goossens T, et al. Hypermutation of multiple proto-oncogenes in B-cell diffuse large-cell lymphomas. *Nature*. 2001;412:341-346.
50. Lossos IS, Levy R, Alizadeh AA. AID is expressed in germinal center B-cell-like and activated B-cell-like diffuse large-cell lymphomas and is not correlated with intraclonal heterogeneity. *Leukemia*. 2004;18:1775-1779.
51. Thompson MP, Kurzrock R. Epstein-Barr virus and cancer. *Clin Cancer Res*. 2004;10:803-821.
52. Ramiro AR, Jankovic M, Eisenreich T, et al. AID is required for c-*myc*/IgH chromosome translocations in vivo. *Cell*. 2004;118:431-438.
53. Uchida J, Yasui T, Takaoka-Shichijo Y, et al. Mimicry of CD40 signals by Epstein-Barr virus LMP1 in B lymphocyte responses. *Science*. 1999;286:300-303.
54. He B, Raab-Traub N, Casali P, Cerutti A. EBV-encoded latent membrane protein 1 cooperates with BAFF/BLyS and APRIL to induce T cell-independent Ig heavy chain class switching. *J Immunol*. 2003;171:5215-5224.
55. Li MJ, Maizels N. Activation and targeting of immunoglobulin switch recombination by activities induced by EBV infection. *J Immunol*. 1999;163:6659-6664.
56. Chang KL, Chen YY, Shibata D, Weiss LM. Description of an in situ hybridization methodology for detection of Epstein-Barr virus RNA in paraffin-embedded tissues, with a survey of normal and neoplastic tissues. *Diagn Mol Pathol*. 1992;1:246-255.
57. Pileri P, Uematsu Y, Campagnoli S, et al. Binding of hepatitis C virus to CD81. *Science*. 1998;282:938-941.
58. Silvestri F, Pipan C, Barillari G, et al. Prevalence of hepatitis C virus

infection in patients with lymphoproliferative disorders. *Blood*. 1996;87:4296-4301.
59. Kitay-Cohen Y, Amiel A, Hilzenrat N, et al. Bcl-2 rearrangement in patients with chronic hepatitis C associated with essential mixed cryoglobulinemia type II. *Blood*. 2000;96:2910-2912.
60. Machida K, Cheng KT, Sung VM, et al. Hepatitis C virus induces a mutator phenotype: enhanced mutations of immunoglobulin and protooncogenes. *Proc Natl Acad Sci U S A*. 2004;101:4262-4267.
61. Ohmori K, Maeda S, Okayama T, Masuda K, Ohno K, Tsujimoto H. Molecular cloning of canine activation-induced cytidine deaminase (AID) cDNA and its expression in normal tissues. *J Vet Med Sci*. 2004;66:739-741.
62. Arakawa H, Hauschild J, Buerstedde JM. Requirement of the activation-induced deaminase (AID) gene for immunoglobulin gene conversion. *Science*. 2002;295:1301-1306.
63. Saunders HL, Magor BG. Cloning and expression of the AID gene in the channel catfish. *Dev Comp Immunol*. 2004;28:657-663.
64. Litinskiy MB, Nardelli B, Hilbert DM, et al. DCs induce CD40-independent immunoglobulin class switching through BLyS and APRIL. *Nat Immunol*. 2002;3:822-829.
65. Coussens LM, Werb Z. Inflammation and cancer. *Nature*. 2002;420: 860-867.
66. Bjedov I, Tenaillon O, Gerard B, et al. Stress-induced mutagenesis in bacteria. *Science*. 2003;300:1404-1409.
67. Travis JM, Travis ER. Mutator dynamics in fluctuating environments. *Proc Biol Sci*. 2002;269:591-597.