Oncogenic events triggered by AID, the adverse effect of antibody diversification

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The generation of an efficient immune response depends on highly refined mechanisms of antibody diversification. Two of these mechanisms, somatic hypermutation (SHM) and class switch recombination (CSR), are initiated by activation-induced cytidine deaminase (AID) upon antigen stimulation of mature B cells. AID deaminates cytosines on the DNA of Ig genes thereby generating a lesion that can be processed into a mutation (SHM) or a DNA double-strand break followed by a recombination reaction (CSR). A number of mechanisms are probably responsible for regulating AID function, such as transcriptional regulation, subcellular localization, post-transcriptional modifications and target specificity, but the issue remains of how unwanted DNA damage is fully prevented. Most lymphocyte neoplasias are originated from mature B cells and harbour hallmark chromosome translocations of lymphomagenic potential, such as the c-myc/IgH translocations found in Burkitt lymphomas. It has been recently shown that such translocations are initiated by AID and that ataxia-telangiectasia mutated, p53 and ARF provide surveillance mechanisms to prevent these aberrations. In addition, evidence is accumulating that AID expression can be induced in B cells independently of the germinal centre environment, such as in response to some viral infections, and occasionally in non-B cells, at least in certain inflammation-associated neoplastic situations. The most recent findings on AID expression and function and their relevance to the generation of oncogenic lesions will be discussed.

Introduction

Adaptive immunity is an exquisite evolutionary development of vertebrates which provides a highly specialized defence barrier against pathogens. The hallmark of adaptive immunity is its extreme specificity for foreign antigens, enabled by antigen receptors of virtually unlimited diversity. Highly diverse antigen receptors are generated during the differentiation of T and B lymphocytes through somatic recombination of their T-cell receptor and immunoglobulin genes, respectively. Germ line antigen receptor genes comprise a collection of variable (V), diverse (D) and joining (J) segments. A combination of V, D and J segment is randomly assembled in each lymphocyte by the site-specific V(D)J recombination reaction that takes place between two switch regions of the IgH locus that will endow the Ig with different effector functions (Figure 1). Both SHM and CSR are initiated by the same B-cell-specific enzymatic activity, activation-induced cytidine deaminase (AID), through the deamination of cytosines on the variable or switch regions of the Ig locus, respectively (Figure 1). This initial lesion on DNA is subsequently processed to allow the fixation of a mutation, in the case of SHM, or a recombination reaction, in the case of CSR.

About 95% of human lymphomas arise from B cells, rather than T cells. Among those, most have their origin in mature—germinal centre or post-germinal centre—B cells. The hallmark of these malignancies is the presence of lymphomagenic chromosomal translocations that involve the Ig locus and a proto-oncogene (reviewed in ref. 1). Evidence has accumulated to prove the involvement of the germinal centre reactions and specifically of AID activity in the generation of lymphoma-associated translocations. Furthermore, newest results show that AID expression might not be absolutely restricted to germinal centre B cells and that this expression could in turn have implications in the development of neoplasias other than B-cell lymphomas.

AID in antibody diversification

AID function. AID was identified by the Honjo lab in 1999 (2) in a search for cDNAs that were specifically expressed in B cells upon CSR activation. Soon after that generation of AID-deficient mice proved that AID is required not only for CSR but also for SHM (3), and that mutations in the AID gene in humans are associated with an immune deficiency called Hyper-IgM syndrome type 2 (4). Moreover, AID expression in fibroblasts transfected with appropriate substrates can reconstitute both SHM and CSR (5,6), thus proving that AID is the only B-cell-specific factor required to trigger the germinal centre reactions.

The actual mechanism of AID activity in promoting these reactions has been the subject of intense debate (reviewed in ref. 7) regarding the nature of its substrate—RNA or DNA. Today, there is compelling genetic and biochemical evidence showing that AID initiates SHM and CSR by deaminating cytosines on the DNA of Ig variable or switch regions, respectively. Cytosine deamination results in the generation of a uracil and therefore turns a DNA C:G pair into a U:G mismatch. This AID-generated U:G mismatch can undergo different fates: (i) it can be replicated over, giving rise to transition mutations at G:C pairs; (ii) it can be recognized by mismatch repair factors

Abbreviations: ATM, ataxia-telangiectasia mutated; Ab-MLV, Abelson murine leukemia virus; AID, activation-induced deaminase; AIDg, AID transgenic; CSR, class switch recombination; DSB, double-strand break; EBNA, Epstein–Barr virus nuclear antigen; EBV, Epstein–Barr virus; HCV, hepatitis C virus; HCC, hepatocellular carcinoma; IgH, immunoglobulin heavy chain; LMP-1, latent membrane protein 1; NF-kB, nuclear factor-kappa B; SHM, somatic hypermutation; ssDNA, single-stranded DNA; UNG, uracil-N-glycosylase; V(D)J, variable, diverse and joining segments.
allowing the generation of either mutations at A:T pairs by error-prone patch repair or a nicked DNA recombination substrate and (iii) uracil can be removed by uracil-N-glycosylase (UNG) to produce an abasic site that enables both transition mutations at G:C pairs and endonuclease cleavage leading to the generation of a DNA double-strand break (DSB) (8). Accordingly, mismatch repair and UNG deficiencies result in shifted mutation patterns towards C:G mutations and transitions at G:C pairs, respectively (8–10). Likewise, CSR is severely decreased in UNG-deficient mice (11) and virtually absent in UNG/C0/MutS Homologue 2/C0 mice (12). The mechanism that prevents AID-induced deamination from being faithfully repaired by UNG and base excision repair remains unknown, but it might entail a tight coordination of UNG activity and passage of the replication fork, as suggested previously (13). In the case of CSR, generation of DSBs at Ig switch regions is an obligate intermediate leading to the recombination reaction. Switch region DSBs have to be recognized, brought together and ligated with the ensuing deletion of the intervening DNA sequence (Figure 1). CSR ligation is carried out by some components of the non-homologous end joining machinery, including the phosphorylated form of histone variant H2AX (20), the ataxia-telangiectasia-mutated (ATM) protein (21), the p53-binding protein 1 and Nijmegen syndrome protein 1 component of the Mre11-Rad50-Nbs1 complex complex (22,23). For a more extensive review on the factors involved in CSR resolution, the reader is referred to references (24,25).

In vitro AID can deaminate cytosines on single-stranded DNA (ssDNA) substrates, whereas double-stranded DNA molecules are refractory to AID activity (26–29). However, transcription renders double-stranded DNA substrates accessible to AID-mediated deamination, both in vivo and in vitro, implying that transcription can—at least transiently—expose DNA on single-strand configuration (26,29–31). Transcription-associated ssDNA exposure can be achieved in the form of transcription bubbles (30,32), R-loops (33,34) or both. Long before the identification of AID as the initiator activity of the germinal centre reactions, transcriptional activity of variable and switch regions was known to be an absolute requirement for SHM and CSR, respectively. Today, it is accepted that this requirement is mechanistically linked to AID function, since one essential role of Ig transcription during SHM and CSR is to provide AID with access to its bona fide ssDNA substrate.

**Regulation of AID function.** A number of mechanisms regulate AID function. First, AID expression is mostly restricted to activated B cells. Induction of AID expression is commonly linked to signalling pathways that mediate germinal centre B-cell activation, such as those mimicked by lipopolysaccharide, IL4, transforming growth factor β or CD40 ligation (2,35,36). Accordingly, Janus kinase-signal transducer
Oncogenic events triggered by AID

Table 1. Antigen independent AID expression

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Stimulus</th>
<th>Signalling pathway</th>
<th>Functional effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral blood naive B cells</td>
<td>EBV infection</td>
<td>LMP-1, NF-κB, BAFF and APRIL</td>
<td>CSR and mutations in BCL-6 and p53 genes</td>
<td>(70,71)</td>
</tr>
<tr>
<td>and B cell lines</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peripheral blood naive B cells</td>
<td>HCV infection</td>
<td>CD81, CD19, CD21 and TNFα</td>
<td>Mutation in the Ig locus, p53 and β-catenin genes</td>
<td>(76,77)</td>
</tr>
<tr>
<td>and B cell lines</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-B cells B cells</td>
<td>Ab-MLV infection</td>
<td>NF-κB</td>
<td>Mutation in the Ig locus</td>
<td>(79)</td>
</tr>
<tr>
<td>Immature bone marrow</td>
<td>NA</td>
<td>BCR and TLR</td>
<td>Somatic mutation and CSR in Ig genes</td>
<td>(38,39)</td>
</tr>
<tr>
<td>Oocytes, embryonic germ cells</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>and embryonic stem cells</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Spermatocytes</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Epithelial breast cancer cell</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>lines</td>
<td></td>
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<tr>
<td>Hepatoma cell lines and</td>
<td>TGFβ, TNFα and</td>
<td>NF-κB</td>
<td>Mutations in p53, c-myc and pi3l genes</td>
<td>(81,85)</td>
</tr>
<tr>
<td>primary human hepatocellular</td>
<td>HCV infection</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>carcinoma</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Gastric epithelial cells</td>
<td>Helicobacter pylori infection</td>
<td>NF-κB</td>
<td>Mutations in p53 gene</td>
<td>(87)</td>
</tr>
</tbody>
</table>

APRIL, proliferation-inducing ligand; BAFF, B-cell-activating factor of the tumour necrosis factor family.

and activator of transcription and nuclear factor-kappa B (NF-κB) pathways seem to be involved in this signalling (35,36). A number of regulatory elements and transcription factors have been described to regulate AID transcription, including the counterbalanced activities of E47 and Id3 and of inhibitory of DNA-binding 2 and Pax5 helix-loop-helix proteins (for a review see ref. 25). Very recently a highly conserved DNA region in the AID gene has been reported essential to allow AID expression in vivo as measured in indicator mouse strains (37). In addition, these mouse strains have provided in vivo evidence that under physiological conditions, AID is not detected in differentiating bone marrow B cells and that AID expression is induced transiently in germinal centres and is then shut down in fully differentiated plasma cells and memory B cells (37). However, AID expression has been reported in immature B cells from bone marrow (38,39), presumably at levels detectable by PCR but not with indicator mouse strains (37); the significance of this observation requires further examination. Second, AID activity can be regulated by controlling the protein subcellular localization. Indeed, most of AID protein resides in the cytoplasm by virtue of a C-terminal nuclear export signal that restricts its presence in the nucleus (reviewed in ref. 25). Finally, AID activity is enhanced upon phosphorylation by protein kinase A (40–42), possibly by facilitating AID interaction with replication protein A and in turn its access to ssDNA (40,43). Although a lot remains to be understood about these regulatory mechanisms, they are certain to play an important role in minimizing the risk of unwanted DNA damage that AID activity might entail. Of special interest to the topic of this review, we will discuss below some cases of unconventional AID expression and its possible implications in normal and malignant physiology.

Specification of AID targeting. One of the most important issues in understanding the pathogenic consequences of AID function relies on deciphering what is the mechanism that targets AID to the Ig loci and not to other genes throughout the genome (for more extensive reviews on the topic the reader is referred to refs 25,44). AID has preference for so-called mutational hotspots defined as a degenerate trinucleotide motif (A or T, A or G, C) both in vivo and in vitro (reviewed in ref. 45). Obviously, the low complexity of this motif cannot account for a major specificity of AID activity based on primary DNA sequence alone. Indeed, Ig sequences can be replaced by heterologous sequences that can still support AID activity (reviewed in ref. 46). As mentioned above, transcriptional activation of the Ig loci is an absolute requirement for AID function. Numerous studies have tried to tackle SHM target specificity on the basis of this feature. Early works showed that removal of cis elements at the Ig locus abolishes SHM; however, if transcription is ensured through heterologous regulatory elements, SHM can be achieved (reviewed in refs 44,46). Moreover, AID is known to target genes other than Ig genes: (i) when over-expressed in non-B cell lines, AID can introduce mutations in transgenes as long as they are transcriptionally active, and the rate of SHM correlates with the transcription rate of the target gene (5); (ii) transcriptionally active transgenes are mutated by AID in lymphoma cell lines (47) regardless of their integration site (48) and (iii) SHM-like mutations are found in a number of non-Ig genes of normal B cells, both murine and human, including BCL6, FAS, B29 and MB1 (reviewed in ref. 49), although at frequencies 50- to 100-fold lower than in Ig genes. However, transcription alone is not sufficient to grant AID targeting, as genes known to be transcribed do not accumulate mutations (50). In addition, experiments of IgL promoter replacement in the hypermutating DT40 chicken B-cell line have shown that a strong RNA polymerase II promoter that yields high transcription levels of the Ig locus is nevertheless unable to support SHM (51). This observation could be in line with the finding that an E box in the Igκ locus enhances SHM without enhancing transcription (52) and with analysis of AID transgenic mice which revealed a common E47-binding motif in the enhancer/promoters of the identified mutated genes (53). In summary, it seems feasible that specification of AID targeting requires a combination of (i) high transcriptional activity and (ii) cis elements that could contribute to enhance AID recruitment to particular genes, either directly or through scaffolding by other protein factors. The identity and number of these cis elements and any putative protein factors are far from being determined. A more defined picture of the mechanisms regulating AID targeting will probably emerge after comprehensive genome-wide screening of mutated genes and SHM permissive loci.

AID in B-cell lymphomas

A vast majority of human lymphoid malignancies derive from B cells rather than T cells, from which most have their origin in germinal centre or post-germinal centre B lymphocytes. A hallmark of mature B-cell lymphomas is the presence of recurrent chromosome translocations that juxtapose one of the Ig loci and a proto-oncogene, such as c-myc in Burkitt lymphoma, BCL1 in mantle lymphoma, BCL6 in diffuse large B cell lymphoma or BCL2 in follicular lymphomas (reviewed in refs 1,54). As a result of the translocation, expression of the proto-oncogene is deregulated, usually by becoming under the
control of Ig regulatory elements. Deregulated proto-oncogene expression has been proved critical for neoplasia development using in vivo models. For instance, transgenic animals expressing c-myc under the control of the IgH enhancer (Eμ,myc) develop B-cell lymphomas (55). Translocations that involve the IgH locus are more common than those involving the IgL locus, and break points usually cluster at switch regions or variable regions. Proto-oncogenes are also known to accumulate mutations in some lymphomas (56), (reviewed in ref. 49), but the relevance of these mutations to lymphomagenesis has not been fully determined. Together, these observations suggested from early on that lymphomagenic chromosome translocations could emerge in mature B cells undergoing CSR or SHM, but only after AID identification as the initiator of these reactions, could this hypothesis be tested experimentally.

AID in chromosome translocations. In mice, both IL6 transgenes and pristane injection promote lymphoid hyperplasia and plasmacytosis that are enriched in cells harbouring c-myc/IgH translocations (57). These c-myc/IgH translocations closely resemble those found in human Burkitt lymphoma. In the absence of AID, c-myc/IgH translocations were absent from IL6 transgenic mice (58). A similar result was obtained by Unniraman et al. (59), who did not detect c-myc/IgH translocations in AID-deficient mice after pristane-induced plasmacytosis. These results indicated that in vivo AID is required for the generation or accumulation of chromosome translocations (for additional discussion on these data the reader is referred to (49). Additional studies allowed to establish that AID-mediated cytosine deamination can trigger c-myc/IgH translocations in vitro in a UNG-dependent fashion, and therefore that the molecular mechanisms that initiate CSR and aberrant chromosome junctions are the same (60).

C-myc/IgH translocations induced upon normal B-cell activation are extremely rare (60,61), which suggests that there might be cellular mechanisms that prevent the generation or spreading of these aberrations and instead drive AID-induced DSBs towards a normal CSR joint. Indeed, ATM seems to be involved in such a surveillance pathway as ATM-deficient B cells accumulate a higher frequency of c-myc/IgH translocations than wild-type cells do. However, the mechanism by which a DSB is recognized by ATM as a translocation intermediate rather than as a CSR substrate remains unknown. Interestingly, frequency of c-myc/IgH translocations increases dramatically in activated B cells that lack p53, suggesting that DSBs that are not resolved into a CSR reaction can be prevented from a translocation event by activation of the p53 pathway through ATM. In addition, ARF-deficient B cells also accumulate a higher frequency of c-myc/IgH translocations, which points to an oncogenic stress response driven by the translocated c-myc that in turn would lead to p53-mediated apoptosis or cellular senescence. Altogether, these results imply that a number of cellular pathways exert protective functions against the appearance of AID-induced chromosome aberrations in B cells. Accordingly, inactivating mutations in p53, ARF and ATM are frequent events in mature B-cell lymphomas (1).

AID in lymphomagenesis. The actual contribution of AID to lymphoma disease development in vivo has been more difficult to study. AID transgenic (AIDtg) mice, where AID expression was driven by a ubiquitous chicken β-actin promoter, developed mainly T-cell lymphomas and lung microadenomas (see below) (62). T-cell lymphomas from AIDtg mice accumulated mutations in their T-cell receptor β genes and in the c-myc proto-oncogene, but no translocations were found (62). A similar result was reported by Rucci et al. (63) in AIDtg mice under the lck T-cell-specific promoter; however, human T-lymphotropic virus promoter failed to promote lymphoma development in spite of similar levels of AID protein being produced. The absence of B-cell lymphomas in mice ubiquitously expressing AID could be due to an earlier onset of T-cell lymphomas. This issue was addressed by the Honjo lab in mice that express AID under a chicken β-actin promoter only when induced through a B-cell-specific deletion by CD19-Cre expression (64). B-cell lymphomas were not observed in B-cell-specific AIDtg mice up to 20 months of age. Intriguingly, although in these mice the AID transgene produced high levels of AID protein, it failed to fully reconstitute CSR and SHM in AID-deficient mice (64), which suggests that in this model, AID activity might be insufficient to promote the accumulation of lymphomagenic lesions. It is feasible that negative regulatory mechanisms are specifically employed by B cells to restrict the availability of AID function (64). In addition, promoter-specific effects seem critical to determine the outcome of AID transgenic expression (63). In summary, enforced AID expression can promote lymphomagenesis, at least under some experimental conditions, but the reasons for the impaired activity of transgenic AID in some mouse models remain unknown.

What is the lymphomagenic potential of endogenous AID? Mouse models to address this question are scarce. IL6 transgenic mice develop lymphoid hyperplasia regardless of the presence of AID, although AID-deficient mice, where c-myc/IgH translocations are absent, show a delayed onset of the disease (58). This subtle effect can be due to the fact that IL6 over-expression—at least in the absence of an immune response—promotes a polyclonal proliferation and therefore does not completely mimic a lymphoma situation. Eμ-myc transgenic mice develop monoclonal pre-B and B-cell lymphomas (55). Kotani et al. (65) have recently reported that lymphoma generation in Eμ-myc is not dependent on AID. However, AID deficiency induced a dramatic shift in the phenotype of the generated lymphomas, from predominantly mature B cell lymphomas—as measured by the surface expression of Ig— in AID-proficient mice, to pre-B cell lymphomas in AID−/− mice. These results indicate that in the Eμ-myc model AID is involved in the generation of mature but not pre-B-cell lymphomas, which is in agreement with AID being expressed at later stages of development. In addition, mutations were found in the Pim1 proto-oncogene of tumours from AID-proficient mice. These results could be interpreted as AID having a dual role in B-cell development: (i) initiation by chromosomal translocations—provided by the Eμ-myc transgene in this model—and (ii) secondary hits by mutagenesis (65).

Novel scenarios for AID expression in B cells. Is deregulated AID expression associated with pro-lymphomagenic situations? AID expression has been reported in germinal centre-derived lymphomas as well as in leukaemias or lymphomas originated from B cells at other stages of differentiation (56,66–69). However, AID expression does not correlate with the accumulation load of particular proto-oncogenes or with the presence of ongoing SHM in Ig variable regions. This is not surprising, since AID expression may have been functional at earlier stages of the disease and then be shut down. In addition, as pointed out earlier, the presence of AID per se may not be warrant of ongoing deamination activity (64).

In addition, induction of AID expression has been reported upon infection with a number of oncogenic viruses. Epstein–Barr virus (EBV) is a gamma-herpesvirus that can promote B-cell transformation and immortalization and is consistently associated with endemic Burkitt lymphoma. Different studies have reported that EBV infection of B cells can induce AID expression (70,71), presumably through one of the EBV gene products, latent membrane protein 1 (LMP-1). LMP-1 cytoplasmic tail is functionally homologous to CD40 and promotes NF-kB nuclear translocation in a ligand-independent manner (reviewed in ref. 72). He et al. (70) showed that LMP-1 expression in lymphoblastoid cells induced AID expression and CSR and promoted the expression of B-cell activation factor of the tumour necrosis factor family and proliferation-inducing ligand, two inducers of T-independent CSR, through NF-kB activation. More recently, AID and error-prone polymerase η expression has been reported after EBV infection of primary B cells from peripheral blood (71). AID and error-prone polymerase η induction was detected after 10 days of infection and was persistent over long periods of culture, concomitant with the accumulation of mutations in the BCL6 and p53 proto-oncogenes and in the β-globin gene (71). These results could provide a mechanism explaining the transforming potential of EBV, by means of deregulating AID expression and promoting lymphomagenic
lesions. In apparent contradiction, early Epstein–Barr virus nuclear antigen 2 (EBNA2), another of the nine gene products coded by EBV, has been shown to down-regulate AID expression in lymphoblastoid cell lines (73). This result is striking as EBNA2 in turn induces LMP-1 expression, suggesting that these two gene products might have counteracting effects, that of EBNA2 being dominant over LMP-1 expression. EBV infection can lead to alternative expression programmes; one of them, the so-called latency III state or ‘growth program’, is observed both in lymphoblastoid cells and in infectious mononucleosis and is associated with EBNA2 expression. In contrast, latency I or ‘survival program’ is typical of Burkitt lymphoma cells and is associated with EBNA1 expression and apparently with counter-selection of EBNA2 expression (reviewed in ref. 72). Therefore, EBV infection can result in diverse and complex genetic programmes that are probably intertwined with the nature, differentiation and activation state of the infected cell. Understanding the implications of this complexity for the final outcome of the infection, and most particularly, for AID-associated transforming potential, will demand further investigation.

Hepatitis C virus (HCV) is a causative agent of chronic hepatitis, liver cirrhosis and hepatocellular carcinoma (HCC). In addition to associated transforming potential, will demand further investigation. For the final outcome of the infection, and most particularly, for AID expression can result in diverse and complex genetic programmes that are probably intertwined with the nature, differentiation and activation state of the infected cell. Understanding the implications of this complexity for the final outcome of the infection, and most particularly, for AID-associated transforming potential, will demand further investigation.

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AID in other neoplastic contexts

We have already discussed that AID expression in B cells can lead to the appearance of DNA lesions, most notably chromosome translocations, underlying the aetiology of B-cell lymphomagenesis. Could AID function be linked to the development of non-lymphoid neoplasias?

Two early evidences support the hypothesis that AID is potentially functional in non-B cells; first, heterologous expression of AID in fibroblasts is sufficient to trigger SHM in transcriptionally active transgenes (5) and second, transgenic mice ubiquitously expressing AID developed T-cell lymphomas and lung microadenomas (62) and, as recently reported, liver tumours in 25% of the cases (81). Therefore, forced AID expression in non-B cells can result in neoplastic development, with particular tissues apparently displaying enhanced susceptibility to malignant transformation.

Interestingly, Morgan et al. (82) reported for the first time detection of AID expression in normal tissues other than B cells, in particular in a number of pluripotent tissues, including oocytes, embryonic germ cells and embryonic stem cells, but not in normal somatic tissues such as liver, brain, kidney or heart. More recently, AID expression has also been reported in spermatocytes (83). Although the physiological meaning of AID expression in germ cells is not yet understood, these observations point to a more loosen regulation of AID expression than previously anticipated.

Only very recently has the issue of AID expression in non-lymphoid tumours been addressed. In one instance, AID expression was reported in epithelial breast cancer cell lines (84), although the pathways leading to this aberrant AID expression needs further investigation. In other set of studies, hepatoma cell lines and primary human HCC samples were analysed. HCC is a primary malignancy that very often appears secondary to chronic hepatitis—after hepatitis B or C infection—or to liver cirrhosis. Kou et al. examined the presence of AID transcripts in a cohort of samples from normal liver, tissues with chronic liver disease and liver tumours. AID transcripts were detected both in liver tumours and in tissues with chronic liver disease as opposed to normal liver tissues, where only minute AID expression was detectable (85). In addition, the same authors found mutations in the p53 gene both from tumour and from surrounding non-tumour tissues of HCC patients. Interestingly, the presence of AID transcripts and protein was not attributable to infiltrating B cells alone but was also detected in hepatocytes (85). These results suggest that AID expression could be induced in non-B cells concomitant with pro-inflammatory situations, such as those seemingly characteristic of chronic liver disease leading to hepatocarcinogenesis. Indeed, AID expression can be induced in human hepatocytes after transforming growth factor β (85) or tumour necrosis factor (81) treatment. In addition, the HCV core protein can also promote AID expression in human hepatocytes. In all cases, AID expression seems dependent on activation of the NF-kB pathway (81), which is in line with other reports implicating NF-kB in the transcriptional activation of AID in B cells (36,86). Importantly, these findings establish a correlation between pro-inflammatory situations, for instance those ensuing chronic viral infection, and aberrant AID expression in non-B cells.

Finally, AID expression was analysed in gastric epithelial cells after Helicobacter pylori infection (87) that causes chronic gastric inflammation and can eventually result in gastric cancer development. H. pylori infection of gastric adenocarcinoma cells cells promoted the appearance of mutations in the p53 gene and induced AID expression in an NF-kB-dependent fashion (87). Interestingly, both effects were achieved only when virulent, cag pathogenicity island-positive, H. pylori strains were used and p53 mutation load was decreased after AID knockdown (87). Moreover, AID protein was detected in 78% of H. pylori-infected human gastric cancer tissues and p53 mutations were found in 86% of H. pylori-positive gastric cancers and 40% of gastritis specimens (87).

These findings pose a completely new and unexpected picture in which AID deregulation could contribute to a wide spectrum of neoplasias (Table I). However, deciphering the scope of this deregulation and its physiological relevance to malignant transformation will have to await the development of suitable in vivo models.

Conclusions and future perspectives

Over the last few years, the identification of AID has allowed a new level of understanding of SHM and CSR, critical reactions for the generation of a highly competent repertoire of antibodies. Concomitant with AID’s role in the normal immune response, evidence is
accumulating showing that its activity can also trigger oncogenic lesions, most notably the generation of pro-lymphomagenic chromosome translocations. Moreover, this deleterious effect might not be restricted to B cells alone but instead could strike other cell types upon deregulated expression of AID, for instance in certain pro-inflammatory situations. This novel scenario will certainly raise an intense research effort that will demand the development of suitable in vivo models where the contribution of AID to neoplastic development can be assessed. At the same time, understanding the involvement of AID in the generation of DNA lesions will require a minute understanding of its activity and regulation. In this regard, a number of key questions still remain unanswered:

- How is AID-mediated cytosine deamination prevented from faithful repair and is instead driven into mutations or DNA DSBs?
- What are the molecular events leading to AID expression and under what circumstances are AID expression deregulated?
- What is the role of post-transcriptional modifications in the regulation of AID function?
- How is AID target specificity achieved? What is the role of transcription and cis regulatory elements in this specificity? When is AID targeting promiscuous and what is the contribution of AID loose specificity to B-cell malignancies?

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