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Review

Viral infection-oxidative stress/DNA damage-aberrant DNA methylation: separate or interrelated events responsible for genetic instability and childhood ALL development?

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ABSTRACT

Acute lymphoblastic leukemia (ALL) is a malignant disorder that originates in a single B- or T-lymphocyte progenitor and is characterized by a range of numeric and structural chromosomal aberrations. Although, so far no clear cause can be found for ALL the most commonly recognized and strongest causal factor is infection. However, an interesting question is how viral infection may be responsible for genetic changes that lead to lymphoid cell transformation. A plausible mechanism by which infection might impact the process of leukemogenesis via genetic alteration is through: oxidative stress/DNA damage which is closely linked with inflammation, aberrant expression of AID/ABOBEC family enzymes which may be responsible for massive mutation introduction and alteration of DNA methylation, leading to changes in the expression of hematopoietic genes. In this review we propose several specific molecular mechanisms which link infection with all the above-mentioned processes. The most likely event which links common virus infection with ALL pathogenesis is aberrant expression of AID/APOBEC. This event may be directly responsible for the introduction of point mutations (as the result of cytosine or 5-methylcytosine deamination and formation of G:U or G:T misspairs) as well as changes in DNA methylation status.

Contents

Abbreviations: AID, activation-induced cytidine deaminase; ALL, acute lymphoblastic leukemia; APOBEC, apolipoprotein B mRNA-editing enzyme catalytic polypeptide-like deaminase BER, base excision repair; 5-hmCyt, 5-hydroxymethylcytosine; 5-hmUra, 5-hydroxymethyluracil; 5-mCyt, 5-methylcytosine; MUTYH, mutY homologue DNA glycosylase; 8-oxoG, 8-oxo-7,8-dihydroguanine; 8-oxodG, 8-oxo-7,8-dihydro-2′-deoxyguanosine; OGG1, 8-oxo-7,8-dihydroguanine DNA glycosylase; ROS, reactive oxygen species; SHM, somatic hypermutations; TDG, thymine DNA glycosylase; TETs, ten-eleven translocation proteins; UNG, uracil DNA glycosylase

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1. Introduction

Acute lymphoblastic leukemia (ALL) is the most common pediatric malignancy, but it can occur in any age group. ALL can develop from any lymphoid cell blocked at a particular stage of development, including primitive cells with multilineage potential [1]. It is a malignant disorder that originates in a single B- or T-lymphocyte progenitor and is characterized by a range of numeric and structural chromosomal aberrations [2]. Essentially most patients with ALL harbor acquired genetic alterations (somatic mutations) that contribute to the increased

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proliferation, prolonged survival, and/or impaired differentiation of the lymphoid hematopoietic progenitors. In the majority, albeit not all, of patients diagnosed with ALL, one or more of these genetic alterations can be detected [3].

However, so far no clear cause can be found for ALL. Therefore, understanding of the events which are involved in ALL development may contribute to possible prevention and/or may open new avenues for therapy of this malignancy. One of the most commonly recognized and strongest causal factors is infection although there is a general understanding that no singular or exclusive cause of the disease exists. It has also been suggested that infection is a secondary event after initiation events linked with chromosomal rearrangements, usually in utero [4].

Several works suggest a role of delayed exposure to common viral infections during infancy in the etiology of childhood ALL [4–6]. Strong support of this hypothesis was delivered by an epidemiological study that examined the relationship between infections and ALL [7] where it was demonstrated that ALL cases had more episodes of infections in infancy, especially those infected in the neonatal period, compared to controls.

However, an interesting question is how viral infection may be responsible for genetic changes that lead to lymphoid cell transformation. A plausible mechanism by which infection might impact the process of leukemogenesis via genetic alteration is through: i/oxidative stress/DNA damage which is closely linked with inflammation and endogenous ROS production; ii/aberrant expression of activation-induced cytidine deaminase (AID)/apolipoprotein B mRNA-editing enzyme catalytic polypeptide-like deaminase (ABOBEC) family enzymes which, in turn may be responsible for massive mutation introduction and; iii/alteration of DNA methylation, leading to changes in the expression of hematopoietic genes. Here we propose several specific molecular mechanisms which link infection with all the above-mentioned processes.

2. Oxidative stress and DNA oxidation in ALL; possible link with inflammation/infection

Normal cellular metabolism appears to be a main source for endogenous reactive oxygen species (ROS). However, various external events, such as infections, car exhaust fumes, cigarette smoke, exposure to ionizing or UVA radiation, can lead to an increase in the generation of ROS (importantly the aforementioned factors are among the "postulated casual exposures" possibly involved in childhood leukemia) (see Ref. [4]). The resulting disturbance of the pro-oxidant/antioxidant balance in favor of the former leads to a condition of oxidative stress, with subsequent oxidation of cellular components, activation of cytoplasmic/nuclear signal transduction pathways, modulation of gene and protein expression and alteration of DNA polymerase activity [8]. DNA is a particularly important target for oxidation, as damage may lead to heritable alterations (for review see Ref. [9]).

The association between inflammation, which is closely linked with infection, and oxidative stress is well documented, with a number of studies of inflammatory conditions or infections reporting elevated levels of 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG, the most widely studied and best recognized marker of oxidatively modified DNA): hepatitis C infection and atopic dermatitis [9,10]. The inflammatory response can lead to the recruitment of activated leukocytes, which may, in turn, give rise to a "respiratory burst" — an increased uptake of oxygen that causes the release of high quantities of ROS, such as superoxide and hydrogen peroxide, with subsequent DNA damage production [4]. In addition to the enhanced formation of ROS inflammation processes lead to the release of $^{\cdot}NO$ that may react with O_2^{-} giving rise to peroxynitrite, which may also be involved in the formation of DNA lesions [11]. It has been estimated that chronic inflammation may be involved in the development of about one-third of all cancer cases worldwide [12].

The consequences of a failure to protect or repair the genome would appear to be manifold and include: the induction of mutations, microsatellite instability, loss of heterozygosity, chromosomal aberrations, altered gene expression and eventually cytostasis, cytotoxicity, or neoplastic growth. It is clear that, depending upon the lesion in question, one consequence of oxidized base lesions persisting in DNA is mutation, and elevated levels of oxidatively induced DNA lesions have been found in many types of malignancies, strongly implicating such damage in the etiology of cancer (for review see Ref. [9]).

Moreover, oxidative mechanisms have been demonstrated to possess a potential role in the initiation, promotion and malignant conversion (progression) stages of carcinogenesis. As a result of elevated ROS, transcription factors, and their corresponding genes, may be permanently activated which, coupled with increased DNA damage, creates a selection pressure for a malignant phenotype seen in cancer [13].

Several studies have demonstrated that oxidative stress, expressed as plasma antioxidant status or lipid peroxidation products, is linked with hematological malignancies including childhood ALL [14]. Moreover, it was shown that at the diagnosis time, the level of oxidatively modified DNA bases in lymphocytes of children with ALL was significantly higher than in children without the disease [15,16] which was associated with decreased antioxidant enzyme level (glutathione peroxidase, catalase and superoxide dismutase). Likewise, urinary excretion of 8-oxodG (a well-recognized biomarker of oxidative stress/DNA damage) was significantly elevated in children with ALL when compared with matched healthy controls [17].

To prevent DNA lesion formation multiple systems exist, and should damage occur, these ensure rapid lesion removal. Interestingly, it was also found that the base excision repair (BER) capacity (expressed as uracil DNA glycosylase (UNG) and endonuclease III efficiency) was decreased in pediatric ALL patients when compared with healthy children [17] and that the risk of the disease was increased as a result of polymorphism of genes which are directly involved in the removal of 8-oxo-7,8-dihydroguanine (8-oxoGua) (OGG1 and MUTYH, the enzymes primary responsible for the removal of 8-oxoGua in human cells) [18]. These polymorphisms may compromise DNA repair.

Summing up this part of our paper, oxidative stress and the events directly linked with its occurrence, namely increased ROS production, elevated level of 8-oxodG and impaired repair of oxidatively modified DNA, documented in children with ALL, all suggest that this condition may contribute to the genetic instability of precursor-B cells which may be linked with the development of the disease. It should also be stressed that our results demonstrate that healthy, full-termed newborns are under severe oxidative stress which is reflected in the elevated level of urinary excretion of 8-oxoGua and 8-oxodG when compared to the values characteristic for adult organisms [19]. This finding is especially intriguing in the context of a model for "infection-derived proliferative stress in the selection of pre-leukemic cells"[4], which assumes that one percent of normal newborns have the *TEL/AML-1* fusion gene in the cells which is present as an expanded clone [20]. Probably, once these selected "pre-leukemic cells" were exposed to cooperating events, like postnatal-enhanced oxidative stress, all these may contribute to clinical leukemia development in predisposed children. Therefore, in the case of selected group of infants, when oxidative stress is expected to be higher (preterm, low weight, documented common infections during infancy) it would be reasonable to consider antioxidant supplementation with additional nutrients rich in antioxidant vitamins C and E.

3. DNA methylation pattern in childhood ALL

5-Methylcytosine (5-mCyt) is the central epigenetic mark and epigenetic marking of the genome is one of the events in the establishment of tissue-specific gene expression which in turn is directly related to cell differentiation and development. However, it is also known that aberrant DNA methylation may play a significant role in cancer development. As a matter of fact one of the most important issues during recent years in the field of oncogenesis has been called "the cancer epigenome", which has a relevance to cancer promotion and progression.

This, in turn, is linked with a plethora of abnormalities which are based on somatic heritable modifications that are not caused by DNA primary sequence changes. Likewise, in several studies investigating a limited panel of genes and/or performed in small patient groups it was observed that the cytosine methylation pattern of the leukemic cell differed when compared to the non-leukemic [21–23].

Moreover, recent studies have found a distinct DNA methylation pattern of childhood ALL, which correlates with the expression of these genes which are central to the initiation and maintenance of lymphocyte transformation [24]. ALL is a heterogeneous disease where patients are divided into risk groups based on the type of genomic lesion. Recently in a genome-wide study it has been found that the different ALL subtype groups, which have a different type of chromosomal aberration in their leukemic cells, may be characterized by a distinct methylation pattern. However, more importantly there is a pattern common to all subtypes. This pattern correlates to gene expression in 65% of the genes which are frequently mutated in ALL. This in turn suggests that this kind of epigenetic change may be directly involved in the completion of the transformation process [24]. Moreover, in the above-mentioned study it was found that the genes affected with severe structural changes frequently exhibited aberrant methylation, which in turn suggests that the changes in methylation pattern may be a necessary event to complete the leukemogenesis.

In a similar study conducted recently with a large group ($n = 764$) of children with ALL and a non-leukemic control group ($n = 137$) [25] a characteristic methylation pattern in all ALL subtypes was found. Interestingly, in an earlier study of these authors quantitative correlation between cytosine methylation and allele specific gene expression of ALL patients was observed [26] which is a kind of support of the Figueroa et al. studies. Since aberrant methylation status was detected in childhood ALL future epigenetic therapeutic approaches will be of interest in the treatment of leukemias [27].

The important questions are: what event(s) may be directly responsible for aberrant methylation in childhood ALL and how is it linked with infection/inflammation? Below some possible mechanisms are presented.

4. Aberrant DNA methylation and Ten Eleven Translocation (TETs) enzymes activity

DNA methylation has been studied as a stable epigenetic modification for decades since a methylation pattern is maintained during replication by the action of DNA methyltransferase 1. However, an increasing body of recent experimental evidence implicates active DNA demethylation, which involves enzymatic oxidation (with TET enzymes) with subsequent formation of 5-hydroxymethylcytosine (5-hmCyt) as the key event in epigenetic reprogramming (reviewed in Ref. [28,29]). Probably, active DNA demethylation may be responsible for aberrant methylation and possibly linked with leukemogenesis. Indeed, hematological malignancies were among the first where aberrant demethylation status was discovered, and the *TET1* gene was initially defined as a fusion partner of the mixed lineage leukemia gene in acute myeloid leukemia [30]. In mouse and human cells TET2 (one of the two other *TET* genes family with the same catalytic activity as TET1) appears to be an important regulator of hematopoietic stem cell development [31]. Moreover, its inactivation leads to a dramatic deregulation of hematopoiesis that in turn triggers blood malignancies (for review see Ref. [32]). It has been shown that *TET2* mutations in acute myeloid leukemia (and other myelodysplastic syndroms) range between 15 and 22% [33]. These mutations resulted in a decrease of 5-hmCyt level and aberrant methylation status (see above), which in turn may lead to the disruption of important hematopoietic functions and results in malignant transformation and possibly ALL formation [32]. Of note is that TET2 mutations have a profound effect on hematopoietic functions in the case of both T-cell and B cell lymphomas [34].

A second plausible scenario for active DNA demethylation involves prior deamination of 5-mCyt to thymine which generates a G:T substrate for engagement of thymine DNA glycosylase (TDG) (or some other G:T glycosylases). Here cytidine deaminases of the AID/APOBEC family (see below) were implicated in the deamination step. It is possible that TDG may act in concert with these deaminases [35]. 5-HmCyt could also be deaminated by AID to yield 5-hydroxymethyluracil (5-hmUra) which in turn may be removed by TDG (review in Ref. [36]). However, this hypothesis has been questioned since it was found that AID/APOBEC family members preferentially deaminate unmodified cytosine and discriminate against 5 substituted cytosine substrates with increasing size [37]. In certain physiological/pathophysiological conditions linked with malignant transformation deaminase enzymes (AID) could play an important accessory role [38]. It is possible that an aberrantly activated deamination route, which can generate a high level of mutagenic G:T or 5-hmUra:G mispairs, may play an important role in oncogenesis (Fig. 1.).

5. Activation-induced cytosine deaminase as a source of DNA lesions

About fifteen years ago it was found that enzymatic deamination of cytosine to uracil in Ig locus in B cell lymphocytes is necessary for antibody diversification after antigen exposure [39]. AID initiates the process that consequently produces substitutions of all four nucleotides, which in turn introduces point mutations into variable regions of Ig genes at a rate almost a million times higher than spontaneous mutations in somatic cells. This high mutation rate is responsible for somatic hypermutation (SHM) of the Ig genes and directly involved in class switch recombination. Generally processing of uracil formed as a result of AID action recruits uracil glycosylase-UNG2 (from BER pathway) and MSH2–MSH6 (from mismatch repair pathway). These enzymes interact with several low fidelity DNA polymerases. It has been proposed that there are two distinct phases of SHM: a first phase which depends on the activity of AID and a second one that depends on the mutagenic activity of the error-prone repair of the AID-induced substitutions [40]. Phase One occurs when UNG2 removes uracil formed after AID action and creates an abasic site (with no coding potential), which in Phase Two can be filled in with any of four bases [41]. Phase Two depends mostly on the error-prone mismatch repair pathway. Instead of repairing the uracil, MSH2–MSH6 proteins attract members of a family of low-fidelity translational DNA polymerases which contribute to nucleotide substitutions at an unprecedented level (for a detail review see ref. [42]).

One of the most intriguing questions is whether the above-mentioned unprecedented level of mutation is selectively targeted to restricted regions of the antibody genes or whether it is possible that other regions of the gene and other than Ig genes that are expressed in activated B cells undergo such a rate of mutation.

Recent studies have demonstrated that AID deaminates cytosine moieties in 25% of the expressed genes of mouse germinal center B cells. However, the mutation rate of these genes is much lower than the Ig loci i.e. for Bcl6 and Cd83 mutation frequencies were 20- and 40-fold lower, respectively, and for all the other genes about 100-fold lower than the reference gene. It was shown that there are two levels of genome protection: i/selective targeting of AID and ii/safety net of high fidelity DNA repair mechanisms which remove uracil generated by AID just like uracil formed by other processes. Deregulation of these processes may lead to cancer development. Of note, as mentioned above, in childhood ALL BER mechanism, which is directly involved in the uracil removal, is compromised.

6. Aberrant AID expression and cancer development

Aberrant AID expression, which can lead to genome-wide mutations in other than Ig genes and/or in non-lymphoid cells, may contribute to harmful genetic changes resulting in cancer. Constitutive AID

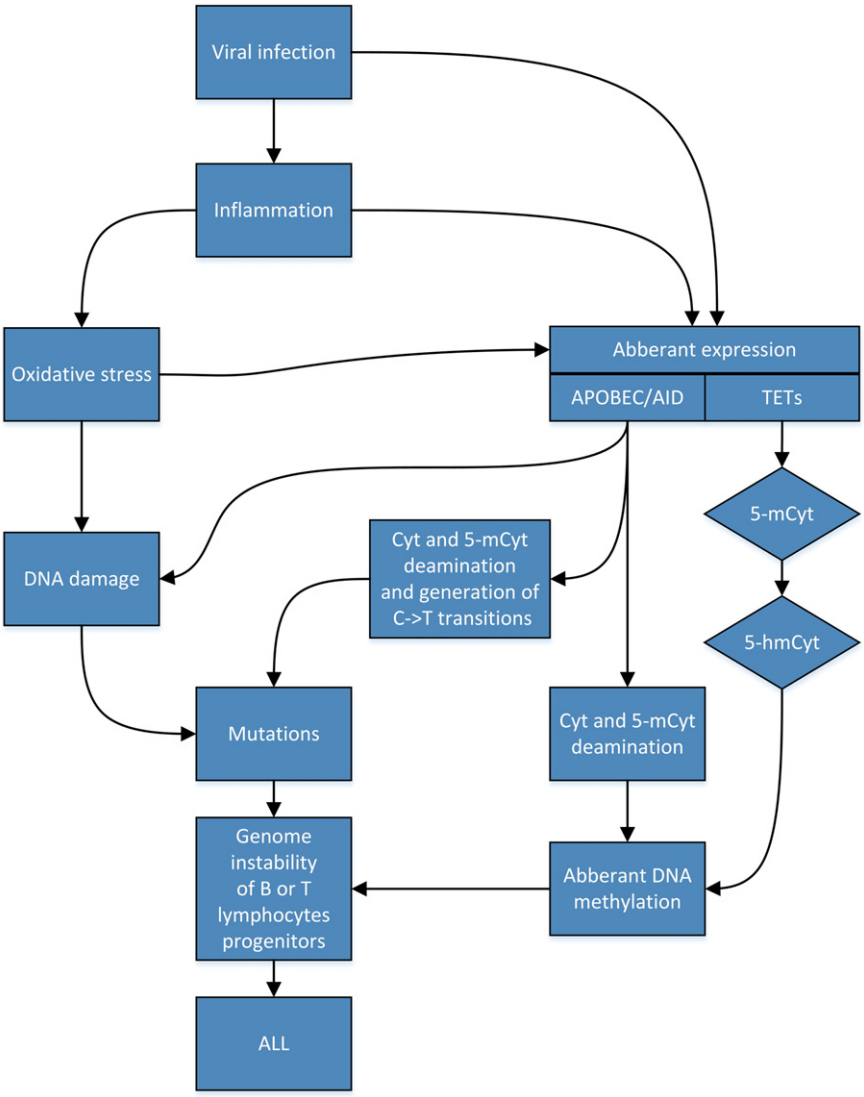

Fig. 1. *Routes of aberrant DNA methylation in childhood ALL development.* Infection triggers inflammation and oxidative stress/DNA damage responsible for point mutations. Since TETs (the enzymes involved in active DNA demethylation — see Section 4) depend on oxoglutarate and oxygen it raises the possibility that during infection/inflammation the enzymes may be aberrantly activated. This, in turn, suggests that inflammation-induced oxidative stress may also be linked with aberrant methylation status. Aberrant expression of AID/APOBEC family of DNA cytosine deaminases, which may be a result of viral infection, results in cytosine to thymine transition mutations. Since these enzymes may also participate in active DNA demethylation process this phenomenon may be linked with aberrant methylation. Altogether the above-described processes may lead to genome instability in prenatally generated pre-leukemic cells and the emergence of ALL.

expression in transgenic mice is responsible for the development of lymphomas [43]. Furthermore, a variety of human tumors have AID-generated mutations in key oncogenes (*MYC*, *PIM1*, *RHOH*, *PAX5*) and the tumor suppressor gene *p53* [44]. Importantly, these genes may play a key role in ALL development [45].

It was found that *Helicobacter pylori* infection is responsible for the aberrant expression of AID. It was suggested that the infection directly activates proinflammatory cytokines via nuclear factor-κB pathway, which in turn might trigger AID expression. This suggests that AID expression, at least in human gastric epithelial cells, is regulated through an activation of nuclear factor-κB pathway. Aberrant AID expression directly leads to p53 mutagenesis in gastric cancer [46].

Aberrant AID expression was also described for human B-cell non-Hodgkin's lymphoma [47], human hepatocellular carcinoma and the surrounding noncancerous liver tissues in patients with underlying chronic hepatitis or liver cirrhosis.

It was suggested that AID expression in chronic myeloid leukemia cells was responsible for the promotion of overall genetic instability by hypermutation of tumor suppressor and DNA repair genes [48].

About 50% of germinal center derived diffuse large B-cell lymphomas is characterized by hypermutations which suggests AID targeted non-Ig genes [49]. Moreover, it has been well documented that AID can occasionally target genes including those frequently mutated in ALL [50]. Deregulated expression of AID was reported in B-cell malignancies including B-lineage acute lymphoblastic leukemia (B-ALL), where it positively correlated with *BCR/ABL-1* rearrangement [51]. Further experimental evidence which strongly suggests the involvement of AID in ALL comes from the model study of *BCR/ABL-1*-driven ALL [52]. In this model, characterized by an increase of genome instability, it was demonstrated that AID expression resulted in a more aggressive phenotype, with a shorter survival time in comparison with AID-deficient leukemia.

The *BCR/ABL-1* rearrangement is the most frequent genetic aberration in adult B-ALL (20-30%), and about 3% of children with ALL [53] and it is likely that AID could be involved in this process, because patients expressing AID have a higher number of alterations compared to "AID-negative" patients [54].

AID belongs to a large family of cytosine deaminases called apolipoprotein B mRNA-editing enzyme catalytic polypeptide-like 3 (APOBEC3)

(for an excellent review see Ref. [55]). APOBEC3 enzymes have a high degree of homology to AID and both of them are single-stranded DNA cytosine deaminases. Moreover, the genes encoded APOBEC3 and AID are positioned in vicinity to each other in the human genome (and in the genomes of most vertebrates) which suggests that both genes have common ancestry [55]. It was demonstrated that upregulated level of APOBEC3 is linked with different cancers while low levels of expression of this protein were characteristic for a variety of normal human tissues [56]. Moreover, aberrant expression of APOBEC is directly linked with specific mutational signature (mostly characterized with C to T transition), which in turn is the most common signature found in cancer genomes including ALL [56–58].

Since APOBEC overexpression may be linked with carcinogenesis it should be some explanation for the potential benefits for its encoding. Interestingly, it has been assumed that this kind of innate immunity may be very important early in life [55].

7. Conclusions

Different subtypes of ALL and the existence of a significant variability of genomic lesions strongly suggest that there are many factors that may be responsible for the genome instability of lymphoblastic cells and the development of this disease. Seemingly many alterations of several genes which are involved in the regulation of hematopoiesis, lymphoid development and active proto-oncogenes are necessary for leukemia induction. Interestingly, in experimental models it has been documented that the cooperation of these alterations is necessary for leukemogenesis [45].

Greaves and colleagues proposed a “two steps” or “two hits” model for ALL where leukemia begins with chromosomal translocation before birth [4]. One of the most common chromosomal aberrations in childhood ALL is t(12;21) *TEL/AML-1* (ETV6-RUNX1) translocation (22–26% of children harbor this aberration). However, it is also known that the above-mentioned aberration is not enough for development of the disease. Therefore, this strongly suggests the requirement for additional secondary (postnatal) genetic events which may be crucial for the development of the disease [59]. Two hypotheses, which implicated infection, possibly viral, were proposed as early as 1988 as a likely causal factor in promoting leukemogenesis [4,5]. Although a rich epidemiologic literature supports both of these hypotheses, very little support for specific molecular mechanisms has been documented.

Here we propose that infection can trigger a series of events which are directly involved in genome instability (depicted in Fig. 1). The most likely event which links common virus infection with ALL pathogenesis is aberrant expression of AID/APOBEC. This event may be directly responsible for the introduction of point mutations (as the result of cytosine or 5-mCyt deamination and formation of G:U or G:T misspairs) as well as changes in DNA methylation status (see above). The development of leukemia clearly requires either chromosomal aberration and/or cooperating mutations. If a role for AID/APOBEC in participating in these events is confirmed, it offers an opportunity for therapeutic approaches like AID activity suppressors to hinder leukemogenic transformation [39,60].

It is also worth mentioning that more than 30 years ago Shlomai and Kornberg, based on their own results, postulated that “a regulated inclusion of uracil leading to periodic breaks in DNA may be a cellular design to facilitate recombination or other metabolic or structural features of advantage to the cell” [61]. However, it is possible that aberrant overexpression of cytosine deaminases, as a result of viral infection, may lead to massive inclusion of uracil which may contribute to genetic instability in prenatally generated pre-leukemic cells and the emergence of overt leukemic ones [4].

Since AID/APOBEC expression is directly involved in immune response our proposal is in line with Greaves' hypothesis that the immune system or rather its response to infection is responsible for childhood ALL [4].

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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