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Potential roles of Activation-Induced cytidine Deaminase in promotion or prevention of autoimmunity in humans

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Abstract

Autoimmune manifestations are paradoxical and frequent complications of primary immunodeficiencies, including T and/or B cell defects. Among pure B cell defects, the Activation-induced cytidine Deaminase (AID)-deficiency, characterized by a complete lack of immunoglobulin class switch recombination and somatic hypermutation, is especially complicated by autoimmune disorders. We summarized in this review the different autoimmune and inflammatory manifestations present in twelve patients out of a cohort of 45 patients. Moreover, we also review the impact of AID mutations on B-cell tolerance and discuss hypotheses that may explain why central and peripheral B-cell tolerance was abnormal in the absence of functional AID. Hence, AID plays an essential role in controlling autoreactive B cells in humans and prevents the development of autoimmune syndromes.

Keywords

Primary immunodeficiency; class switch recombination-deficiency; tolerance

Introduction

Immunoglobulin class switch recombination deficiencies (CSR-D) are rare primary immunodeficiencies with an estimated frequency of about 1 in 200,000 births. These deficiencies also referred to as hyper IgM (HIGM) syndromes are characterized by normal or elevated serum IgM levels and a decrease in (or an absence of) IgG, IgA and IgE (1). Defects in immunoglobulin class switch recombination (CSR) are often associated with faulty generation of somatic hypermutations (SHMs) in the Ig variable (V) region. The molecular identification and analysis of several CSR-D have provided new insights into the

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mechanisms underlying CSR and SHM, both of which are key elements in the maturation of antibody (Ab) responses (2).

Molecular basis of CSR-D

The genetic basis of CSR-D is diverse and is caused by defects in either the CD40L/CD40 pathway essential for B cell activation, germinal center (GC) formation and the induction of CSR, or the enzymes involved in CSR and SHM processes. Investigation of CD40L/CD40 deficiencies has provided evidence for an essential role for CD40 activation pathway in both CSR and SHM (3, 4). CD40L/CD40 interaction is also required for full T lymphocyte/dendritic cell interaction, and CD40L and CD40-deficiencies are characterized by a defect in cellular immunity, leading to susceptibility to opportunistic and viral infections, which are not controlled by immunoglobulin substitution (5, 6). Other CSR-D are caused by an intrinsic B cell defect, affecting the CSR machinery itself. Patients suffer from recurrent bacterial infections but are generally well under immunoglobulin substitution. The most common of the CSR-D due to an intrinsic B cell defect is the autosomal recessive form due to mutations in *AICDA* gene encoding for activation-induced cytidine deaminase (AID). It is characterized by impairment of both CSR and SHM (7), emphasizing AID's master role in Ab maturation. The AID enzyme selectively modifies cytosine (C) residues into uracils (U) leading to the introduction of U:G mismatches on the single-strand DNA of transcribed switch (S) and V regions of the Ig, introducing a DNA lesion (8). AID can also deaminate the non-template strands in transcription bubbles (9) or through its interaction with the RNA exosome (10). However, it is very likely that AID plays an additional role in CSR, as indicated by the phenotype of patients harboring mutations in the C terminal part of AID that affect neither its catalytic activity nor the SHM process but abrogate the CSR (11). Moreover, C terminal mutations located in the nuclear export signal (NES) exert a dominant negative effect, likely because of the increased nuclear localization of truncated AID (12). Other CSR-D due to an intrinsic B cell defect are caused by DNA repair impairment, including the very rare Uracil N-glycosylase (UNG)-deficiency (13). This observation emphasizes the editing activity of AID since UNG removes the uracil residues from DNA leading to an abasic site. In V region, U:G mismatches' repair involves also the MSH2/MSH6 complex (a component of the mismatch repair (MMR) machinery) and error-prone DNA polymerases for accomplishment of SHM (14). In contrast, the CSR process requires DNA double strand breaks for inter switch recombination: the UNG-induced abasic sites are eventually cleaved by apurinic-apyrimidic endonucleases (APEX), already characterized in mice but not in humans so far, that leads ultimately to the formation of single-strand DNA breaks (SSBs) which have to be processed into double-strand breaks (DSBs) (15). The MMR plays a role in the processing of the SSB into DSB in mice (14, 16-18) as in humans (19, 20). Thereafter the DSBs are sensed by several molecules, including the Ataxia Telangiectasia Mutated (ATM) protein, and repaired mostly through the classical, non-homologous end-joining (c-NHEJ) pathway; however, a recently described alternative end-joining pathway can also perform repair based on microhomology (21). Mutations in genes encoding MMR, ATM or NHEJ lead to different but severe phenotypes in which the CSR-D, although sometimes drastic, is only a side effect.

Immunologic features of AID-deficiency

Although AID-deficiency is a very rare primary immunodeficiency, we could collect clinical data from 45 patients we diagnosed as affected by an autosomal recessive (AR) AID-deficiency and compared them to that of CD40L-deficient patients. Because of the rarity of the disease, patients are scattered all along the world and clinical information are sometimes sparse, especially when patients live in a developing country, whose tradition may include consanguineous marriages. All AR AID-deficient patients are characterized by a drastic defect in CSR (normal or increased IgM, lack of detectable IgG and IgA levels in serum). Mutations are scattered all along the gene, with no obvious hot spots of mutations (*figure 1*). SHM when evaluated were found negative except in two patients, both presenting mutations in splice sites in intron 4, leading in one patient to an homozygous in-frame insertion of 31 amino-acids and in the other one to heterozygous deletion of exon 4; this heterozygous change was associated with a missense mutation (F151S) on the second allele. NES is expected to be unaffected and AID catalytic activity was found normal (22), but CSR was defective, further revealing the role of the C terminal portion of AID in CSR in addition to the NES (23).

All AR AID-deficient patients suffer from bacterial infections, affecting mostly the upper respiratory and the gastro-intestinal tracts. No susceptibility to opportunistic infections is reported, which is in sharp contrast with CD40L or CD40-deficiencies as previously reported (4-6).

Lymphocyte numbers are normal in peripheral blood, with normal percentage of T and B cells, including $CD27^+$ B cells. However, no switched IgM^-IgD^- B cells are detected, pinpointing to the complete absence of CSR (7). Strikingly, in this intrinsic B cell defect, $CD4^+/CD8^+$ ratio is <1. This unexpected weak decrease in $CD4^+$ T cells could result from an exhaustion of T cells due to repeated infections before Ig replacement therapy. In addition, in CD40L-deficient patients as well as in AR AID-deficient patients, the number of $CD3^+CD4^+CD25^{hi}CD127^{lo}FOXP3^+$ Tregs was found significantly decreased (24, 25).

A hallmark of the disease is lymphadenopathy affecting 75% of patients (essentially cervical and mesenteric lymph nodes). The enlargement of lymph nodes is so impressive that patients undergo recurrent biopsies. All histological sections reveal the same aspect with a marked follicular hyperplasia, with giant germinal centres (GC) (5 to more than 10 times larger than GC from control reactive lymph nodes), filled with numerous proliferating ($Ki67^+$) GC founder cells ($CD38^+sIgM^+sIgD^+$ B cells). A dark zone and a lighter zone can be distinguished in some patients' follicles on Ki67 staining. However this "light zone" also contains numerous cycling cells and $sIgD^+$ B cells. The mantle zone (with normal B cell phenotype) and inter-follicular areas are present, although reduced in size (*figure 2*). IgM and IgD plasma cells are found in GC and T cell areas, but neither IgG nor IgA plasma cells. The high proliferation frequency of B cells in GC is associated with a dense network of macrophages filled with apoptotic bodies that gave the GC a starry sky appearance. The reason of such lymph node enlargement remains unknown, although hypertrophy of Peyers' patches and of isolated lymphoid follicles in the lamina propria has been related to intestinal bacterial expansion in mice (26). In humans, such a correlation is unclear since

lymphadenopathies can develop even in patients receiving efficient Ig substitution with no detectable infectious episode. Interestingly enough, no giant GC was observed in the lymph node biopsy of the patient with the homozygous splice site mutation in intron 4. Although often impressive, lymphadenopathies are not the most severe complication of AR AID-deficiency.

Autoimmune syndromes often develop in AR AID-deficiency

Besides increased susceptibility to infections, which is the hallmark of the disease, AR AID-deficient patients are prone to develop autoimmune manifestations (27). Autoimmune manifestations can occur before but also under appropriate Ig substitution and can be life-threatening, requiring steroid treatment, anti-CD20 monoclonal antibody and in some cases immunosuppressive agents administration. In our cohort of 45 patients with AR AID-deficiency for whom clinical data are available, autoimmune or inflammatory manifestations were found in 13 patients (29%) *(Table 1)*.

P1 developed hemolytic anemia, thrombocytopenia and an auto-immune hepatitis with several autoantibodies of IgM isotype, including anti-hepatocyte, liver-kidney-microsome, smooth muscle, cardiolipin, erythrocyte (Coomb's test) and platelet antibodies. P2 presented with a thrombocytopenia and a cryoglobulinemia. P3 presents with chronic hepatitis and, though no auto-antibodies were found, its auto-immune origin was supported by a negative infection screen, histological findings and the efficacy of corticosteroids and immunosuppressive therapy. He suffered also from a non infectious arthritis. Three other patients were reported as affected by inflammatory arthritis (P4, P5 and P6). Two patients were diagnosed with systemic lupus erythematosus (SLE): P7 suffers only from a cutaneous SLE with photosensitivity, with no detectable anti-nuclear factor, but presence of rheumatoid factor and anti smooth muscle antibodies and controlled by hydroxychloroquine. P8 presents two severe episodes of SLE with multi organ failure, including the kidney and the central nervous system. Anti-nuclear factors, antibodies to SSA and RNP, anti-cardiolipine of the IgM isotype were found. The severity of the disease leads to treatment with long term azathioprin. P9 developed an inflammatory bowel disease mimicking Crohn's disease and treated by pentasalazin and low dose corticosteroids. P10 developed a chronic destructive, bilateral and symmetrical polyarthritis with the typical and radiological features of rheumatoid arthritis, which was controlled with low dose prednisone. No rheumatoid factor was detected. The biopsy of synovitis revealed a dense inflammatory infiltration (28). P11 had bilateral chronic uveitis, which required corticosteroids and cyclosoprin treatment, suggesting an autoimmune disorder although no autoantibody was found. P12 presented with cold agglutinins, however with no clinical consequences (27). P13 presented with a thrombocytopenia, and anti-cardiolipine autoantibodies.

Among this cohort of 45 patients affected by AR-AID deficiency, 2 patients were harbouring mutations located in the C terminal part of AID resulting in a defect in CSR but without affecting the SHM process and none of them have developed so far autoimmune complications.

Defective central B-cell tolerance checkpoint in AR AID-deficiency

The observation that patients with CSR-D are prone to the development of autoimmune disease suggests that B-cell tolerance is not properly established and/or maintained in these patients (5, 6, 27). In humans, most developing autoreactive B cells generated during random V(D)J joining are removed at 2 discrete checkpoints during early B-cell development (29). First, a central checkpoint in the bone marrow between early immature and immature B cells removed most clones expressing polyreactive and anti-nuclear antibodies. Next, a peripheral checkpoint at the transition between new emigrant and mature naïve B cells further counterselected some autoreactive B cells that may have encountered peripheral autoantigens probably not expressed in the bone marrow environment. Defects in these early B-cell tolerance checkpoints were identified in CSR-D patients (24, 25) (*Figure 3*).

The regulation of central B cell tolerance involves B cell receptor (BCR) signalling pathways that regulate recombination activating gene expression and central tolerance mechanisms such as receptor editing, anergy, and deletion in immature B cells (30), and is mostly controlled by B cell-intrinsic factors. Indeed, we have previously shown that alterations of the BCR signaling pathway in patients lacking functional BTK, or in healthy subjects carrying the R620W PTPN22 risk allele result in a defective central checkpoint and a failure to counter select developing autoreactive and polyreactive B cells (31). Mutations in the Toll-like receptor (TLR) pathway genes encoding molecules such as IRAK-4, MyD88, UNC-93B or Adenosine deaminase (ADA) also lead to defects in proper counter selection, especially towards nucleic acid containing antigens (32, 33).

Central B-cell tolerance was normally established in CD40L-deficient patients, with percentages of new emigrant B cell clones expressing polyreactive B cell receptors similar to what we find in HD ((25) and *figure 3*), suggesting that CD40L, which is not expressed in developing B cells, does not play an important role in the establishment of central B cell tolerance. In contrast, new emigrant/transitional B cells from AR AID-deficient patients express an abnormal immunoglobulin repertoire and a high frequency of polyreactive antibodies, demonstrating that AID is required for the establishment of central B cell tolerance ((24) and *figure 3*). The mechanisms by which AID affects central B cell tolerance are currently unknown but they seem to require AID expression but not induction of SHM or CSR, since all antibodies cloned from AID-deficient and healthy donor transitional B cells were of the IgM isotype and devoid of somatic mutation. Although AID expression was previously believed to be restricted to activated B cells and GCs, we and others have now detected AID transcripts in human and mouse immature B cells, further supporting an intrinsic role for AID during bone marrow B cell development (24, 34-37). In addition, AID-deficient mice also display abnormal central B-cell tolerance, further identifying a major and previously unexpected role for AID in the removal of developing autoreactive B cell clones in both mice and humans (24, 38).

Defective peripheral B-cell tolerance is common to AID- and CD40L-deficiencies

The factors that control the second B-cell tolerance checkpoint, eliminating autoreactive B cells in the periphery before they enter the $CD19^{+}CD10^{-}IgM^{+}CD21^{+}CD27^{-}$ mature naive B-cell compartment, are less well established. In both CD40L- and AID-deficiency, this checkpoint is impaired as evidenced by the significantly higher proportion of mature naïve B cells expressing polyreactive and autoreactive antibodies including anti-nuclear antibodies (ANAs) ((24, 25) and figure 3). Transgenic mouse models have suggested that $CD4^{+}$ T cells may play an important role in the elimination of peripheral autoreactive B cells through MHC class II/T cell receptor; CD40/CD40L and Fas/FasL interactions (39, 40). Interestingly, $CD4^{+}CD25^{hi}CD127^{lo}FOXP3^{+}$ regulatory T cell (Treg) numbers and frequencies are decreased in both CD40L- as in AID deficient patients (24, 25). This correlation may suggest the involvement of switched memory B cells in either the generation or the maintenance of some Treg cells in humans.

In addition, both CD40L- and AID-deficient patients display a 2 to 3 fold increase in B-cell activation factor (BAFF) in their serum (24, 25). BAFF is a critical B cell survival factor that controls the number of peripheral B cells (41). Mice overexpressing BAFF develop autoimmune disorders similar to SLE and Sjögren's syndrome characterized by the production of autoreactive antibodies including rheumatoid factor, anti-DNA and other ANAs (42). However, $AID^{-/-}$ mice do not display increased serum BAFF concentration (38). It remains to be determined whether these differences result from the patients exposure to pathogens or if the higher proportion of switched memory B cells in humans, which express several receptors for BAFF (BAFF-R and TACI) and which are absent in both HIGM syndromes, may account for increased BAFF concentration in both AID- and CD40L-deficient patients.

Increased secretion of autoreactive antibodies in AID-deficient patients

Since unexpected autoimmune manifestations occur in one fourth of the AR-AID-deficient patients, we examined if peripheral B-cell tolerance was further broken by analyzing their sera for autoreactive antibodies. Indeed, in all 13 out of 13 patients tested thus far, although not affected by any clinical autoimmune manifestation, ((24) and unpublished data) serum autoreactive antibodies could be observed. The sera reacted against cell structures of HEp-2 cells, similar to IgM autoreactive antibodies from SLE patients (23). In sharp contrast, none of the CD40L-deficient patients or healthy donors showed autoreactive IgM. We conclude that B-cell tolerance is further breached in AID-deficient patients compared with CD40L-deficient patients, and this breach correlates with the higher frequency and severity of autoimmune manifestations in AR AID-deficient patients (our personal observation).

Concluding remarks

The production of pathogenic IgG autoreactive antibodies have been long linked to the development of autoimmune diseases. It has been clearly demonstrated that these IgG autoreactive antibodies in both mice and humans display somatic hypermutations with

patterns indicating that autoreactive B cell clones were actively selected by self-antigens (43-49). Because AID is responsible for both CSR and SHM, AID may therefore promote the development of autoimmune diseases by allowing the production of mutated autoreactive IgG B cells and autoreactive high affinity antibodies. Indeed, AID-deficiency in MRL/lpr mice abrogates lupus nephritis and a decrease in AID expression delays the development of such pathology (50, 51). Moreover, lupus-prone MRL/faslpr/lpr mice display increased AID expression, further supporting a role for AID in the development of autoimmune conditions in mice (52). However, our study of a large cohort of AID-deficient patients revealed that these patients do suffer from auto-immune or inflammatory disorders, suggesting that AID may also prevent the development of autoimmune syndromes. Indeed, 29 % of the patients from whom we can get a sufficient follow-up present such a complication, most of them early in life (during the first decade). Autoimmunity cannot be only related to increased serum IgM levels since these manifestations are more rare in CD40L-deficiency, in which serum IgM concentrations are also often elevated (5, 6). A correlation between the appearence of autoimmunity and infectious events is unclear in that AR AID-deficient patients on efficient Ig substitution can still develop autoimmune complications. In addition, organ specific auto-immune disease, linked to IgM antibodies to gastric antigens, has been reported in elderly $AID^{-/-}$ mice, independently of infections (53).

Hence, the pathogenesis of auto-immunity in AID-deficiency remains unclear and could be explained by:

1. It could be related to an intrinsic B cell defect. Low levels of AID expression in bone-marrow immature and transitional B cells may play a role in the control of central B-cell tolerance in both mice and humans (24, 38). In the absence of AID, immature and transitional B cells may be more resistant to apoptosis and thus more susceptible to expansion of autoreactive B lymphocytes (24, 38). Besides this role in central tolerance, AID could play also a role in the periphery by controlling mature B cell proliferation: AID has been shown to be involved in B cell apoptosis of mature B cells (54) and AID-deficient mice and humans display giant germinal centres filled of proliferating B cells, suggesting a lack of B cell proliferation control which can lead to the emergence of autoreactive B cells. Generation of tertiary lymphoid organs inside the gastric mucosa in the autoimmune gastric disease of $AID^{-/-}$ mice could also suggest a role of AID in B cell homeostasis. Expansion of autoimmune B cells could also be facilitated by the high levels of BAFF observed in AID-deficient patients' serum. Finally, AID has been shown to deaminate methylated cytidines, and thus might play a role in the epigenetic regulation of gene expression (55-58).
2. A role for T cells is also suspected in AR AID- as well as CD40L-deficient patients. Tregs play a major role in the control of autoimmunity, as shown by Foxp3-deficient mice and humans (59, 60). Decreased numbers of Tregs in both AR AID- and CD40L-deficient patients may therefore directly contribute to the development of autoimmune conditions (24, 25). The autoimmune gastritis described in AID-deficient mice is associated with an infiltration of activated $CD4^+$ T cells, which are able to transfer the gastritis when injected to a T-depleted

recipient (53), emphasizing the role ot autoreactive T cells in organ-specific autoimmunity. One could also hypothesize that T cells are not fully normal in AID-deficiency. Indeed, novel data suggest that AID could be transiently expressed in T cells (61).

3. Both hypothesis are not exclusive since there is a cross-talk between T and B cells for autoimmune disease development. B cells have been shown to contribute to autoimmunity not only by secreting autoantibodies but also through activation of autoreactive T cells by presenting self-antigens (62), Using an antibody-independent autoimmune mouse model, Chan et al. could demonstrate that B cells could be involved in SLE pathogenesis as potential activators of autoreactive T cells and seem to be important for the maintenance of memory T cells (62). Anti-nuclear antibodies from an SLE mouse model require SHM to be generated (43-45, 48, 63). This observation is in apparent contradiction with our AID-deficient patients' cohort in which autoreactive and anti-nuclear antibodies can be identified especially in AR AID-deficient patient 8 who suffer from SLE (Table 1). The 12 AR AID-deficient patients affected by autoimmune and/or inflammatory diseases were all harboring bi-allelic *AICDA* mutations located outside the C terminal part of AID, likely disturbing SHM. Because other patients were receiving immunosuppressive therapy at time of examination, only three out of the 12 (P1, P10 and P12) could be tested for SHM, that was found completely abrogated. These results suggest that IgM autoantibodies even devoid of somatic mutation can lead to tissue damage. Of note, none of the two patients with the splice site mutations in intron 4 (mutations preserving the cytidine deaminase activity and SHM as well as the NES, but modifying the C terminal part of AID leading to complete lack of CSR) present with autoimmunity. In addition, out of the 3 UNG-deficient patients described, one is suffering from severe auto-immune manifestations (AIHA, Sjogren's syndrome), and UNG-deficiency is not associated to defective SHM (although the nucleotide substitution pattern is biased)(13). Thus, our data suggest that the absence of CSR combined or not with a lack of SHM does not prevent the development of autoimmune manifestations in CSR-D patients.

In conclusion, AID appears to be a double-edge sword for the development of autoimmunity: on one hand AID can promote the development of autoimmunity and end-organ damage by promoting the generation of autoreactive mutated IgG B cells specific for self-antigens but on the other hand AID seems also to play an essential role in preventing the development of autoimmune syndromes in both mice and humans by favoring the elimination of developing autoreactive B cells during early B cell differentiation. Further analysis of B-cell tolerance in various primary immunodeficiencies may refine mechanisms leading to autoimmunity and potentially reveal how AID mediated its anti-autoimmune functions. Such approach may not only be important for a better understanding of autoimmunity in humans, but also for the prognosis and accurate follow-up of the patients.

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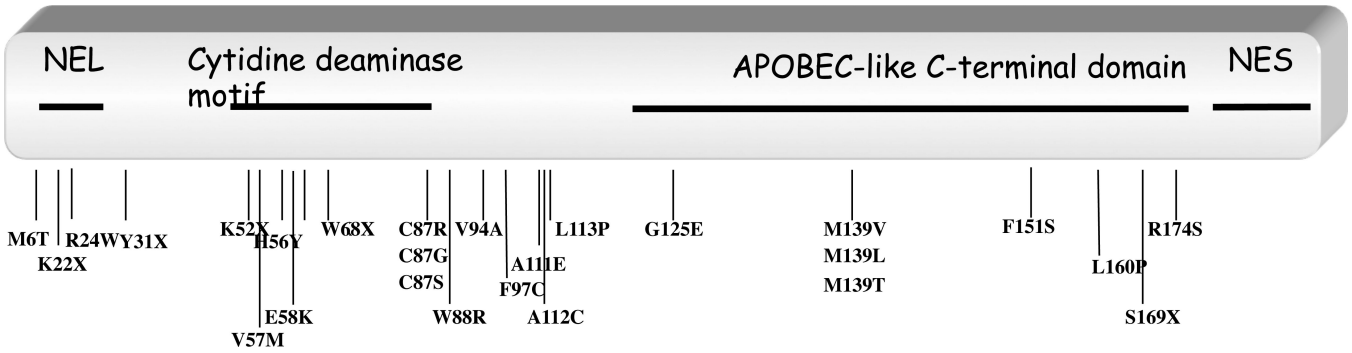

Figure 1.

Schematic representation of *AICDA* gene and localization of mutations observed in 45 AR AID-deficient patients. Deletions are not shown.

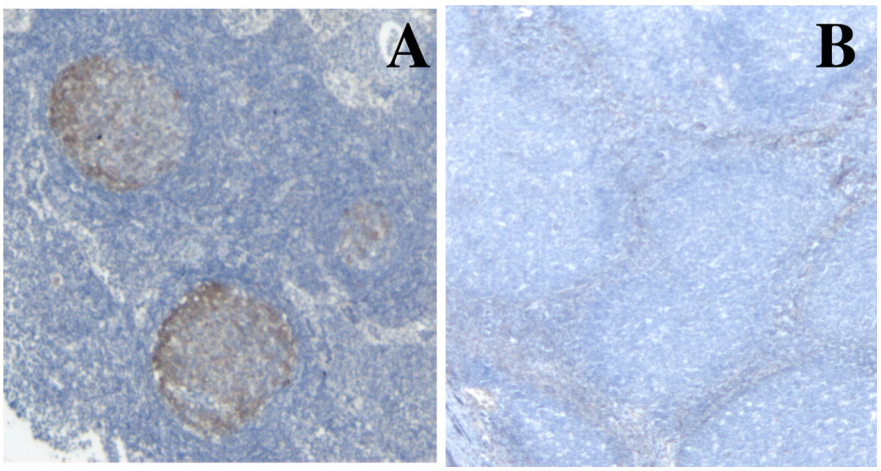

Figure 2.

Germinal centres in a control's reactive lymph node and in an AR AID-deficient patient's lymph node (magnification x25). Hist-immunochemical labeling with an anti-AID monoclonal antibody.

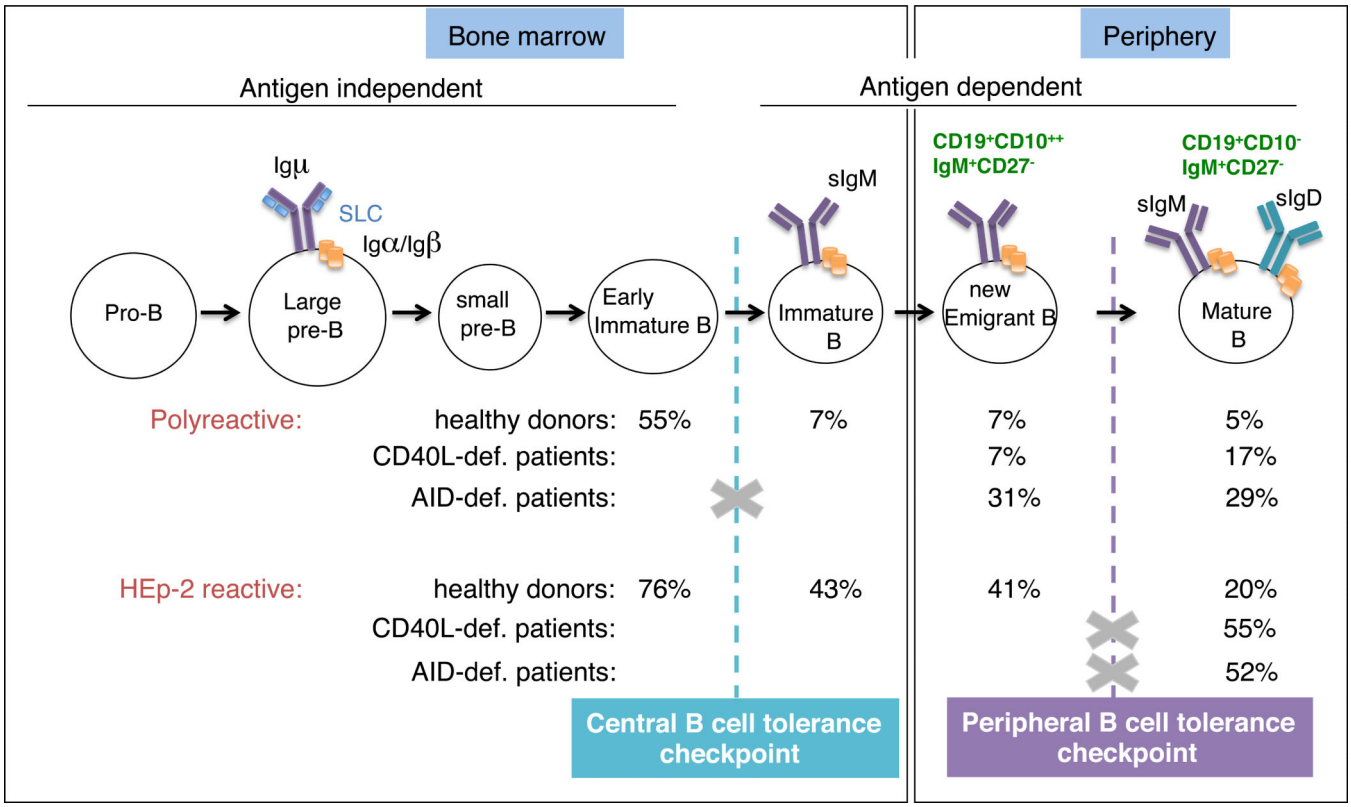

Figure 3.

Central and peripheral B-cell tolerance checkpoints in CD40L- and AR AID-deficient patients. The frequency of B cells expressing polyreactive and HEp-2-reactive antibodies in healthy controls, CD40L- and AID-deficient patients is indicated. Only peripheral B cell fractions were analyzed in the CD40L- and AID-deficient patients. The higher percentage of polyreactive B-cell clones in AID-def. patients versus healthy control new emigrant B cells reveals a defective central B-cell tolerance checkpoint in these patients. The increased frequency of HEp-2 reactive B-cell clones in both CD40L-and AID-deficient patients demonstrates a defective peripheral B-cell tolerance checkpoint in those patients.

Table 1

AIHA: auto-immune hemolytic anemia, ITP: immune thrombocytopenia, Ab: antibodies, LKM: liver-kidney-microsome, AI: auto-immune, SLE: systemic lupus erythematous

Patient	AID mutation	Clinical complications	Associated biological features
P1	H56Y/H56Y	AIHA, ITP, AI hepatitis	IgM Ab: anti LKM, anti smooth muscle, anti liver membrane, anti cardiolipine, anti platelets and erythrocytes
P2	Y31X/Y31X	ITP	cryoglobulinemia
P3	M139V/M139V	AI hepatitis, arthritis	-
P4	M139V/M139V	arthritis	-
P5	F151S/F151S	arthritis	-
P6	L160P/L160P	arthritis	-
P7	V94A/V94A	cutaneous SLE	Rheumatoid factor, anti smooth muscle
P8	F97C/F97C	multi-organ SLE	IgM Ab: anti nuclear factor, anti DNA Ab
P9	W68X/W68X	Crohn's disease	-
P10	deletion/deletion	destructive polyarthritis	-
P11	W68X/W68X	chronic uveitis	-
P12	W68X/deletion	-	cold agglutinins
P13	Exon4 deletion/Exon4 deletion	ITP	anti cardiolipine