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IMMUNE DEFICIENCY AND DYSREGULATION (DP HUSTON AND C KUO, SECTION EDITORS)

Somatic Hypermutation Defects in Common Variable Immune Deficiency

María Belén Almejun[1,2] · **Mercedes Borge**[1,3]

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Abstract Common variable immunodeficiency (CVID) is a heterogeneous disorder characterized by impaired antibody production and recurrent infections. In the last 20 years, several groups have reported that B cells from CVID patients have an impaired somatic hypermutation (SHM). The reported frequency of this defect among CVID patient cohorts is highly variable and so is the methodology used to evaluate this process. Interestingly, the low level of SHM on B cells from CVID patients has been correlated with the presence of infectious and non-infectious complications. In this review, an overview of the studies regarding SHM in CVID patients is presented. We highlight the importance of SHM studies in CVID patients as a clinical tool due to the reported association with clinical complications by several groups. We also considered SHM measurement useful to guide future investigations in order to identify genetic defects involved in the development of the disease.

Keywords CVID · SHM · Clinical complications · CSR · IgV

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✉ María Belén Almejun
mbalmejun@gmail.com

[1] Laboratorio de Inmunología Oncológica, Instituto de Medicina Experimental (IMEX) - Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Academia Nacional de Medicina (ANM), Buenos Aires, Argentina

[2] Departamento de Fisiología, Biología Molecular y Celular, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina

[3] Departamento de Microbiología, Parasitología e Inmunología, Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina

Introduction

Common variable immunodeficiency (CVID) is a heterogeneous syndrome characterized by impaired immunoglobulin (Ig) production [1–3]. The underlying cause of most of CVID cases is unknown. Monogenic causes were only identified in less than 10% of all CVID patients; thus, diagnosis is based on clinical features [4, 5]. The diagnostic criteria for CVID established by the European Society for Immunodeficiency (ESID) include the following: marked decrease of IgG and IgA with or without low IgM levels (measured at least twice; < 2 SD of the normal levels for their age), diagnosis established after the 4th year of life, have excluded secondary causes of hypogammaglobulinemia, no evidence of profound T cell deficiency, poor antibody (Ab) response to vaccines (and/or absent isohemagglutinins) or low switched memory B cells (< 70% of age-related normal value), and at least one of the following: increased susceptibility to infection, autoimmune manifestations, granulomatous disease, unexplained polyclonal lymphoproliferation, affected family member with Ab deficiency (www.esid.org).

CVID is the most prevalent primary Ab deficiency in humans requiring medical attention, with an estimated incidence of 1/25,000 to 1/66,000, and its diagnosis is usually made between 20 and 40 years of age. CVID patients are susceptible to recurrent infections of the respiratory tract and the gastrointestinal system, and they commonly manifest non-infectious complications such as autoimmunity, gastrointestinal disorders, lymphoproliferation, and malignant diseases [1, 6–9]. Both infectious and non-infectious complications significantly contribute to morbidity and mortality in patients with CVID [1, 3, 10, 11].

Interestingly, the main immunologic defect in CVID, the failure of Ig production, is usually associated with normal peripheral B cell counts. Another clinically puzzling

observation in CVID is that serum IgG levels do not always correlate with the incidence and recurrence of bacterial infections [3, 12, 13]. Thus, the decision to treat a patient with intravenously administered immunoglobulin (IVIG) is based on the frequency and severity of recurrent infections rather than on the absolute IgG serum level [14, 15••]. Based on this observation, several groups have focused their studies on the processes involved in the maturation of the Ab response in CVID patients.

During B cell development in the bone marrow, the somatic recombination of the V(D)J segments, encoding the variable regions of the immunoglobulin heavy (IgH) and light (IgL) chains, generates the primary Ab repertoire. After antigen-dependent activation, mature B cells further diversify their Ig genes through class-switched recombination (CSR) and somatic hypermutation (SHM). These two molecular mechanisms are key elements in the maturation of Ab responses.

In the course of CSR, usually B cells change the constant region of their IgH chain through a DNA double strand break-dependent process resulting in the expression of a different isotype and thus in a change of the effector function of the Abs produced. On the other hand, during SHM, B cells diversify the variable region (V(D)J) of the IgH and IgL providing a structural substrate for the selection of Igs with higher affinity for antigen (Ag).

Both CSR and SHM are initiated by activation-induced cytidine deaminase (AID), a DNA-editing enzyme which deaminates cytidine in uridine on single-stranded DNA and is strongly expressed in centroblasts and centrocytes [16, 17]. CSR and SHM predominantly occur in germinal center B cells engaged in a T cell-dependent Ab response, but can also occur in extrafollicular B cells engaged in a T cell-independent Ab response [18, 19].

During SHM, AID deaminates cytidine residues present in the variable regions (IgV) of both IgH and IgL chains. Of note, these deaminations preferentially target specific hotspots, including the RGYW/WRCY motif, where R stands for G or A nucleotides, Y for C or T nucleotides, and W for A or T nucleotides [7]. By this process, AID produces uracile to guanine (U:G) mismatches in the IgV regions that are then processed through specific repair pathways, including base excision repair and mismatch repair (MMR) to convert these mismatches mostly into point mutations. Point mutations induced by AID in the IgV regions mostly generate amino acids replacements in complementarity determining regions (CDR), but do not modify the framework regions (FR), which regulate the structural organization of Ig molecules, changing the conformation of the antigen-binding pocket formed by the V regions of IgH and IgL chains [20, 21] (Fig. 1).

The hypermutation frequency in IgV, of ~ 10^{-3} per bp, is a million times greater than somatic cell mutation frequencies. This high frequency of mutations occurs at the time and place indicated because a large protein complex, mainly identified by ChIP studies, interacts with AID targeting the mutagenic enzyme to the transcriptionally active sites of the IgV regions [22]. Then, B cells with mutated IgV are selected in a process that involves new encounter with Ag and presentation of the Ag in MCHII molecules to follicular helper T cells (Tfh). Tfh cells will then selectively provide survival and maturation signals to those B cells that are more efficient in this capture/presentation process, which most of the times are B cells with higher affinity for the Ag, resulting in affinity maturation of the Abs [23].

The detailed molecular mechanism involved in SHM has been reviewed elsewhere and is not the aim of this review. Here, we will focus on recent advances regarding SHM defects in patients with CVID.

SHM Status in B Cells from CVID Patients

Defects in SHM have been repeatedly reported in CVID patients [15••, 24, 25, 26••, 27•, 28•, 29, 30, 31••]. The percentage of CVID patients harboring SHM defects varies among studies, which can be a consequence of differences in the methodology used to evaluate SHM and/or the intrinsic heterogeneity of this syndrome. The group of Levy Y et al. was the first to evaluate the quality of Ab production by studying the SHM pattern in CVID patients in 1998 [15••]. They assessed SHM through an analysis of the mutational status of sequences from the intronic JH4-JH5 region flanking VH-JH4 rearrangements, which is a region commonly mutated during normal SHM, on purified circulating memory $CD19^{+}IgM^{-}IgD^{-}$ B cells, and the analysis of mutations in V3–23-Cγ transcripts from total peripheral blood mononuclear cells (PBMC). In this study, they found that two of the six CVID patients evaluated had a large proportion of circulating IgG memory B cells harboring germline VH genes.

Two years later, the same group extended the study of SHM in the V3-23-Cγ transcripts to 36 new CVID patients [24]. This analysis allowed them to characterize seven new cases with a dramatic reduction in the frequency of IgV gene somatic mutation, for an aggregate 20% of CVID patient in their cohort with impaired SHM. A similar frequency of CVID patients with hypomutated IgV regions was reported in 2011 by other group using the same methodology [29].

A much greater frequency of CVID patients with SHM defects was reported in 2002 in a six-patient cohort [25]. In this study, the analysis of SHM was carried out by comparing the nucleotide changes causing amino acid changes in VH5-Cμ transcripts respect to the amino acid present in the germlines VH5-Cμ sequences. They found that the SHM was at a very low level in all the patients evaluated. In fact, they found that the SHM status in CVID showed similar levels to those reported for patients with Hyper-IgM syndrome caused by AID mutations.

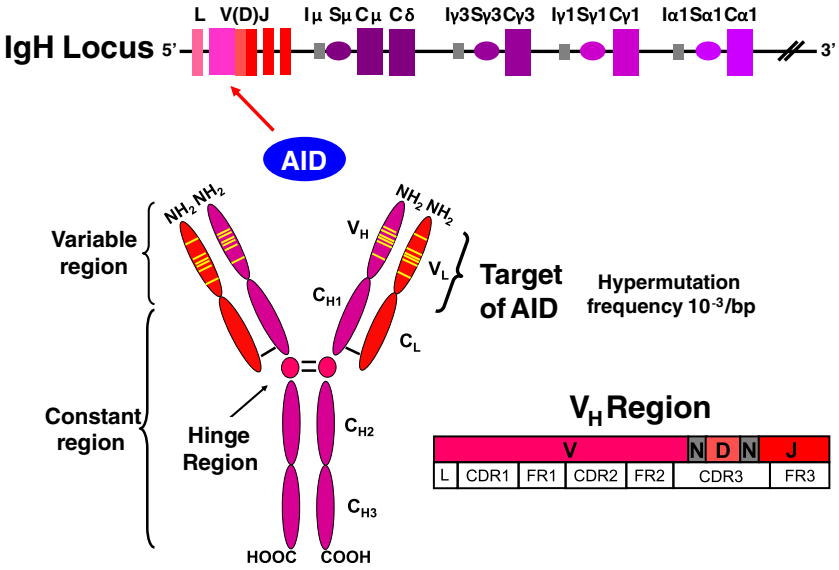

Fig. 1 Schematic representation of SHM process. Top left, AID deaminates cytidine residues present in the IgV of both IgH and IgL locus, giving rise to the SHM mechanism that increases the affinity for the Ag by an error-prone repair pathway. Right below, a scheme of the V region of the IgH containing the variable (V), diversity (D), and joining (J) segments and some insertion of amino acid between junctions (N). Within each V(D)J rearrangement, we distinguish the conserved leader region (L) and hypervariable regions or complementary determining regions (CDRs) in association with conserved zones called framework regions (FR)

The difference in the frequency of CVID patients presenting SHM defects between these two reports (20 vs 100%), could be due to the low number of patients analyzed and/or to the different assay used to measure SHM and also due to the different transcript chosen to evaluate the status of hypermutation. While Levy et al. evaluated the variable region of the γ transcripts derived from total mononuclear cells from peripheral blood, Agematsu and col. observed the variable region of the μ transcripts; in addition the former observed the mutational frequency, while the latter also observed the amino acid changes.

Ab affinity maturation by SHM is not always linked to isotype switching and can also be present in IgV genes of IgM producing B cells [19, 32]. Thus, the analysis of the mutations on the variable regions of a single isotype-specific heavy chain can lead to under- or sub-estimate the levels of SHM in the entire B cell population. Taking this into account, in 2005, the group of Andersen and col. [26••] developed a method to quantify SHM levels on the κ light chain named VκA27-specific restriction enzyme-based hotspot mutation assay (IgκREHMA). This method is based on the fact that almost all (> 90%) of the Igκ transcripts carry SHMs, and that the Vκ gene A27 is the most commonly used light-chain gene (10 to 15% of Vκ transcripts) [33, 34]. VκA27 CDR1 contains a strong hotspot consisting of three overlapping RGYW motifs known to be preferentially targeted by SHM that also contains two overlapping sequences recognized by the restriction endonuclease Fnu4HI that are lost when SHM occurs (Fig.2). By using this novel method, they found that 77% of CVID patients (cohort of 31 patients) presented low levels of SHM. In this study, a greater proportion of patients with CVID with altered SHM were observed compared to the previous study from Levy et al. in which SHM was evaluated only in isotype-switched Abs.

Since 2013, most studies have used the IgκREHMA method to evaluate SHM defects and have reported that a large proportion of the CVID patients from their cohorts have defects in SHM (73 and 96%) [28•, 31••].

Correlation between SHM Status or Memory B Cells and Infectious and Non-infectious Complications

Several reports have attempted to associate SHM levels in B cells from CVID patients with clinical manifestations as infectious and non-infectious complications in order to use SHM levels as a clinical prognostic marker.

In 2005, the work from Andersen et al. showed a significant correlation between high frequency of severe respiratory tract infection (SRTI), which is one of the leading causes of death in CVID patients, and low levels of SHM measured in the VκA27. Interestingly, the mutation levels in each patient remain relatively stable over the years, allowing them to propose the use of SHM status as a prognostic marker for SRTI.

CVID patients also commonly present defects in the memory B cell compartment that might be also contributing to some of the immunological defects in these patients [25, 27•, 35–37]. Warnatz et al. and Piqueras et al. suggested different classifications of CVID patients based on the quantification of memory B cell subpopulations defined by flow cytometry [36, 37]. Piqueras reported an association between

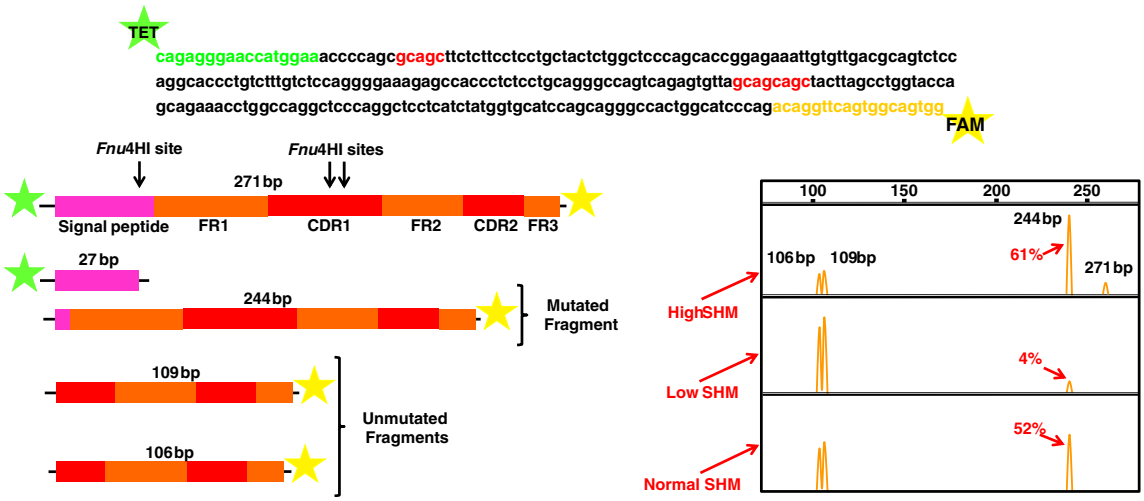

Fig. 2 Schematic representation of IgκREHMA. Above, the VκA27 sequence with the Fnu4HI enzyme sites. Left below, possible products obtained after cutting with FnU4HI the PCR amplification product of VκA27 gene; Right Below, fragment length analysis by using capillary electrophoresis. Peaks of 106 and 109 bp represent the quantity of VκA27 fragments cleaved in one of the two hotspot Fnu4HI restriction sites. The peaks of 244 bp represent the quantity of VκA27 fragments cleaved in the signal peptide Fnu4HI site but not cleaved in the hotspot as a consequence of one or two mutations, eliminating the Fnu4HI sites. The peak of 271 bp represents VκA27 sequences in which cleavage is prevented by mutation in both the hotspot and the signal peptide restriction sites, as well

granulomatous disease, secondary lymphoid proliferation, splenomegaly, and reduced levels of both switched ($IgD^{-}CD27^{+}$) and non-switched ($IgD^{+}CD27^{+}$) memory B cells, while Warnatz found an association between the occurrence of splenomegaly and autoimmune cytopenias and the low frequency of switched memory B cells. Regarding infectious complication, Carsetti et al. found an association between the frequency of recurrent lower respiratory tract infections and low number of IgM memory B cells, but here the SHM of these cells was not evaluated [38].

Combined measurements of memory B cell compartment and SHM in CVID patients might be useful to understand whether SHM abnormalities and memory cell deficiencies are associated with each other and/or which of them has more influence in the course and development of the disease. An important contribution in this aspect was made by the group of Schejbel and col. They performed a combined study of memory B cell populations and SHM [27•] by measuring the mutations in the variable region of each IgG, IgA, and IgM heavy chain by the IgHREHMA method. In this study, they found that deficiency of switched memory B cells is often, but not always, combined with deficiency of SHM; and interestingly, they found that the frequency of SRTI correlated with reduced levels of SHM in IgG-switched B cells and not with the low number of IgG-switched memory B cells. Both, this study and the one from Andersen, support the idea that affinity maturation of Ab is an important clinical measurement that could be associated with increased infection risk even in CVID patients with slightly decreased levels of Abs. Interestingly in 2013, SHM levels were reported to be more stable over the time than the proportion of switched memory B cells in CVID patients, with 28 out of 33 patients with stable SHM levels during the follow-up period, supporting the idea that SHM could be a useful clinical prognostic marker [28•].

SHM levels were also associated with non-infectious complications in pediatric CVID patients [31••] but not in a cohort that included pediatric and non-pediatric CVID patients [28•]. In the pediatric cohort, the authors found that almost all patients, to a greater or lesser extent, had SHM defects and that the presence of severe but not moderate defects in SHM correlated with non-infectious complications. This suggests that the measurement of SHM could be an important clinical tool to discern a subset of CVID patients who are more likely to develop non-infectious complications thus improving early diagnosis and therapy. Importantly, the authors did not find any association between clinical complications and the CSR defects observed in their cohort. Furthermore, even patients with a similar fraction of switched memory B cells than healthy donors showed a marked decrease in SHM frequency, suggesting an intrinsic defect in the SHM machinery at least in some of the pediatric CVID patients.

In Search of Possible Failures of the Hypermutation Machinery in CVID Patients

The molecular defect associated with the impairment of SHM observed in CVID patients has not been elucidated yet.

Many CVID patients, even in the absence of SHM in the IgV regions, have switched memory B cells and present mutations in the 5'BCL-6 intronic sequences, which are targeted by the SHM machinery [24, 39]. Therefore, a defect in AID function is unlikely, while a failure in the targeting of the hypermutation machinery to the IgV sequences, although not evaluated yet, could be suspected at least in some patients.

Defects in the repairing machinery and in the selection of mutated B cells during the SHM process have also been proposed in CVID patients. By analyzing the ratio of replacement to silent mutations (R/S) in the FRs and CDRs regions, an abnormal frequency of silent mutations in the CDR regions was found in some patients, consistent with a defect in the selection processes underlying affinity maturation [29]. In addition, by the analysis of the frequency of transversions and transitions on the IGHV3-23 gene it was shown that, at least a subset of patients has a mutational profile suggestive of defects in the AID-induced mutations repair pathway, comparable to the mutational profiles found in mice deficient in MMR proteins [29].

Some patients with severe defects in SHM also presented failures in the microhomology S junctions repair of CSR [31••], which is reminiscent of defects in the non-homologous end joining repair pathway, suggesting that a defect in a yet uncharacterized molecule involved in both non-homologous end joining and MMR pathways, or in an alternative DNA repair pathway, could be implicated in the SHM defect in some patients.

Finally, T cells are important players during the SHM process, in particular Tfh by the delivery of survival signals to B cells in the germinal center. Thus, defects in this collaboration process could lead to the failure of a successful SHM. Although the diagnosis criteria for CVID patients exclude patients with profound T cell defects in terms of number and proliferation, T cell abnormalities such as a decrease in $CD4^+$ T cell number [40], low proliferative response [1, 41], reduced cytokine production [42, 43], and low number of regulatory T cells [44] have all been described in some patients. Nevertheless, one of the first studies of SHM in CVID showed that T cells from patients with deficiency in SHM in vivo were able to induce SHM in VH genes of the BL2 cell line in vitro [15••]. These findings were indicative of an intrinsic failure in patient's B cells rather than a T cell defect, although abnormalities in the T cell compartment could be contributing to the impaired SHM in some patients.

Concluding Remarks

Overall, SHM defects can be found in a large proportion of patients with CVID and their severity is not surprisingly heterogeneous considering the heterogeneity of the disease among patients. Nevertheless, the study of SHM defects could be helpful to classify patients and define homogeneous subgroups of CVID for functional studies and genetic linkage. The different methods used to evaluate SHM could be adding complexity when the combination of data from different studies is mandatory to draw a conclusion. Hence, the standardization of a reliable method to measure SHM would be useful in order to unify results from different studies and perform conclusion in larger cohort of patients.

The study of SHM in CVID patients can be helpful both in the clinic and in basic research. First, SHM was shown to be associated with infections and non-infectious complications by several groups, and although studies in larger cohort of patients will be needed to confirm the reproducibility of these associations, this could help to perform classifications of patients in this heterogeneous syndrome and to predict the occurrence of these complications. Secondly, the study of the molecules responsible for the SHM defects in CVID patients could help in the future to elucidate the complex mechanisms involved in B cell differentiation pathways. In fact, molecular analysis in samples from patients with Ab-deficiencies has made it possible to describe molecular mechanisms underlying the CSR and SHM pathways. For example, the molecular studies performed in hyper-IgM syndromes led to the identification of deficiencies in CD40, CD40 ligand, AID, and UNG genes as responsible for defects on CSR and/or SHM [45–50], and helped us to understand the essential role of these molecules for both mechanisms. The improved DNA-sequencing technologies (i.e., next-generation sequencing) should enable us to elucidate the possible gene defects underlying the observed changes in the near future.

Compliance with Ethical Standards

Conflict of Interest The authors declare no conflicts of interest relevant to this manuscript.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- •• Of major importance

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