Hyper-IgM syndromes: a model for studying the regulation of class switch recombination and somatic hypermutation generation

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Abstract

Several genetic defects in class switch recombination, which lead to a hyper-IgM syndrome, have been described recently in humans. In addition to the well known role of CD40-ligand-CD40 interaction, these pathologies demonstrate definitively the requirement of CD40-mediated nuclear factor KB activation and the essential role of a recently described molecule, the activation-induced cytidine deaminase in an efficient humoral response, which includes class switch recombination and the production of high-affinity antibodies.

Introduction

The generation of the antibody repertoire requires two successive steps. The first step is antigen- and T-cell-independent, and takes place in the foetal liver and the bone marrow. Immature B-lymphocytes rearrange their immunoglobulin V (variable), D (diversity) and J (joining) gene segments, which produces a functionally integrated VDJ segment that is linked to the μ constant region (Cμ). A primary antibody repertoire, composed of IgM antibodies, is thus generated. The second step is antigen- and T-cell-dependent and takes place in secondary lymphoid organs. After encountering antigen, which they recognize via their membrane IgM, B-cells proliferate and form germinal centres. In this unique anatomic formation, two genetic events lead to the generation of the secondary antibody repertoire. The first event, immunoglobulin class switch recombination (CSR), occurs by a recombination process between two different switch (S) regions that are located upstream of each C region. Replacement of the C region Cμ by a C region of another class of immunoglobulin (Cγ, Cα or Cε) leads to the production of IgG, IgA and IgE molecules which harbour the same V specificity [1]. The second genetic event involves somatic hypermutations (SHMs), i.e. mutations located in the genes that encode the V regions of the immunoglobulin; this step precedes the positive selection of B-cells that harbour a B-cell receptor with high affinity for antigen and the negative selection of B-cells carrying a B-cell receptor with a low affinity for antigen or specific for an auto-antigen. These selection processes occur within the germinal centres in close interaction with follicular dendritic cells [2].
Role of CD40-CD40-ligand (CD40-L) interaction in CSR

The hyper-IgM (HIGM) syndrome that was described first was the X-linked form, which results from mutations in the gene encoding CD40-L (HIGM1 syndrome) [3–7]. CD40-L is expressed by activated helper T-cells and interacts with CD40, which is constitutively expressed on the surface of B-cells, monocytes and dendritic cells. As a consequence of a trans CD40 activation defect, patients' B-cells are unable to proliferate and form germinal centres in lymphoid organs, and to undergo CSR in vivo. The defective IgG and IgA production is responsible for an increased susceptibility to bacterial infections. Impaired CD40-L expression also results in a defective interaction of T-cells with monocytes/dendritic cells, which leads to an abnormal cellular immune response, the consequence of which is a severe susceptibility to opportunistic infections, which cannot be prevented by intravenous immunoglobulin substitution [8].

Interestingly, it has been reported recently that the few IgM+/IgD+-B-cells from HIGM1 patients that express the marker CD27, which is specific for memory B-cells, carry SHMs [9]. The presence of this unswitched, mutated B-cell population suggests that SHMs can occur even in the absence of the classical CD40-L-CD40 interaction, which raises the issue of a second B-cell diversification pathway, the molecular determination of which is still unknown.

A recent report [10] described three patients in two consanguineous families suffering from HIGM3, who had the same susceptibility to bacterial and opportunistic infections as is observed in HIGM1 patients. The lack of CSR was found not to be due to a CD40-L expression deficiency which has been reported in some female patients because of skewing in X chromosome inactivation [11]. The patients' B-cells and monocytes failed to express the CD40 molecules because of two different homozygous mutations in the CD40 gene. However, in contrast with HIGM1 patients, patients' B-cells do not express CD27 molecules and do not harbour SHMs.

Role of CD40-mediated nuclear factor \( \kappa \)B (NF-\( \kappa \)B) activation in CSR

Concomitantly, three groups have recently delineated that the molecular basis of another form of HIGM syndrome associated with X-linked hypohydrotic ectodermal dysplasia is due to missense mutations in the gene for NF-\( \kappa \)B essential modulator [NEMO; also referred to as inhibitor of NF-\( \kappa \)B kinase (IKK\( \gamma \))]) [12–14]. NEMO is involved in the activation and nuclear translocation of NF-\( \kappa \)B [15,16]. All patients present with life-threatening infections, including bacterial, opportunistic and mycobacterial infections. The serum concentrations of immunoglobulins are variable but lower IgG and excessive IgM levels are often noticed. The antibody response, especially to polysaccharide antigens is defective. No data are available concerning SHMs.

All these observations demonstrate the essential role that CD40-L-CD40 interaction and the CD40-induced NF-\( \kappa \)B pathway play in B-cell terminal differentiation, and especially in CSR.

Role of activation-induced cytidine deaminase (AID) in CSR and the generation of SHMs

Another HIGM syndrome, with autosomal recessive inheritance (HIGM2), has been described [17,18]. These patients are affected with a peculiar susceptibility to bacterial infections, but not to opportunistic infections. All patients exhibit markedly diminished serum IgA and IgG levels, with a normal or increased level of serum IgM. B-cells reveal an intrinsic defect since they do not undergo CSR in vivo in the presence of CD40 agonists and appropriate cytokines [18]. In addition, patients' B-cells, although the CD27 marker is expressed normally, exhibit very few if any SHMs. Thus, characteristics of HIGM2 syndrome include not only a lack of CSR, but also a defective generation of SHMs [19].

Another feature of the syndrome is the enlargement of secondary lymphoid organs due to the presence of giant germinal centres filled with proliferating B-lymphocytes that co-express IgM, IgD and CD38, a phenotype described previously as characteristic of the germinal centre founder cell subset [20].

This peculiar HIGM syndrome has been related to deleterious mutations in the gene encoding AID, a molecule that is only expressed in B-cells undergoing CSR and SHM in vivo [21] and in vitro [22].

Interestingly, this protein is structurally related (34% similarity) with the apolipoprotein B (apoB) RNA-editing enzyme (APOBEC-1). RNA editing is widely used to create new functional proteins from the restricted genomes in plants and protozoa [23,24]. An increasing number of mammalian mRNAs are also known to be edited,
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including apoB mRNA, which is edited by APOBEC-1. ApoB mRNA editing involves a site-specific C→U deamination of the first base of a CAA codon encoding Glu\textsuperscript{215b} of apoB100, and produces a UAA in-frame stop codon in apoB48 mRNA. ApoB100 and apoB48 are translation products of the unedited and edited apoB mRNA respectively, and these proteins exert completely different functions with different expression patterns (owing to tissue-specific expression of APOBEC-1). Because of its similarity with APOBEC-1 and its cytidine deaminase activity in vitro, it is likely that AID acts as an actual RNA-editing enzyme; however, its precise role, which appears to be essential in B-cell maturation, as shown by humoral immune deficiency in HIGM2 patients, is unknown at present.

The phenotype of HIGM2 patients suggests that AID exerts its activity on a common substrate that is required for both CSR and SHM. There is some evidence that CSR and SHM, although able to occur independently, could share a part of the genetic alteration machinery, since mutations are frequently found in the S regions [25,26], as well as in the V regions.

It is known that both events require transcription of the target DNA [27,28]; however, it is likely that AID plays a role downstream of the transcription step since sterile germline Iε-Cε transcripts, induced by CD40 agonists and interleukin-4, can be detected in activated B-cells from HIGM2 patients [19]. Several groups have concomitantly demonstrated the occurrence of double-stranded (ds) DNA breaks occurring during the SHM process [29,30], as is strongly suggested in CSR [31]. Some recent data demonstrate that ds DNA breaks occur normally during SHM in AID-deficient B-cells [32]. It has also been shown recently that ds DNA repair proteins (phosphorylated histone 2AX and Nijmegen breakage syndrome protein 1) [33] are not normally recruited for CSR in B-cells after activation in AID\textsuperscript{−/−} mice [34]. Both observations suggest a defect in the very early event of DNA repair, even before the recruitment of histone 2AX and Nbs-1 proteins.

Other proteins involved in DNA repair have been studied largely in the CSR and SHM processes. The role of non-homologous end-joining enzymes (DNA protein kinase and the Ku70–Ku80 recombination complex) has been established in CSR [35,36], whereas they are not required for SHM [37]. The mismatched repair enzymes were also shown to play, at varying levels, a role in CSR and SHM [38–42]. The nucleotide excision repair enzyme, although it has been shown to induce cleavage of S regions in vitro [43], seems to have no actual activity in vivo [44]. Error-prone polymerases, such as Pol-η and Pol-ζ, the requirement for which is already shown in SHM [45–47], could also be involved in DNA repair during CSR, according to the high levels of mutations observed in recombined S regions.

Another hypothesis is to consider AID as a DNA-editing enzyme. As suggested in a recent review, AID might function as a nick-inducer by deaminating of C to form U at the DNA level in V regions and S regions, thereby leading to ds DNA breaks, which therefore become substrates for error-prone DNA repair enzymes [48]. Thus, the description of HIGM2 patients gives evidence for the role of the AID molecule in B-cell terminal differentiation and strongly suggests that SHM and CSR share a, as yet undefined, common mechanism.

Other HIGM syndromes
We have observed patients with a clinical phenotype similar to that observed in HIGM2, including enlarged lymph nodes, but who have normal AID sequence and expression [49]. The immunological study of these patients gives evidence for a defective CSR in vivo and in vitro that leads to the HIGM syndrome. The patients display a normal generation of SHMs, suggesting that a target or a co-factor of AID, which is specific to CSR, is defective. The patients seem to have a milder phenotype, although requiring intravenous immunoglobulin substitution, an observation which can be linked to the protective role of mutated, high-affinity immunoglobulins, even of the IgM isotype.

Conclusion
The delineation of the molecular basis of the different HIGM syndromes in humans have strongly enlightened our knowledge concerning the mechanisms involved in B-cell terminal differentiation necessary for efficient humoral immune response.

References