

Terminal defects of B lymphocyte differentiation

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In humans several abnormalities can occur during terminal B cell differentiation, leading to primary humoral immunodeficiencies. A recent study provided evidence of a qualitative defect of the affinity antibody maturation in some patients affected with common variable immunodeficiency syndrome, the molecular basis of which remains unknown. Several genetic defects in class switch recombination leading to a hyper-IgM syndrome have recently been delineated. Besides the well-known role of CD40–CD40 ligand interaction, they definitively demonstrate the requirement of CD40-mediated nuclear factor κ B activation and the essential role of a newly described molecule, the activation-induced cytidine deaminase, in B cell terminal differentiation. *Curr Opin Allergy Clin Immunol* 1:519–524. © 2001 Lippincott Williams & Wilkins.

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Abbreviations

AID	activation-induced cytidine deaminase
apoB	apolipoprotein B
BCR	B cell receptor
CSR	class switch recombination
CVID	common variable immunodeficiency
HIGM	hyper-IgM syndrome
NF-κB	nuclear factor κ B
SHM	somatic hypermutation

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Introduction

The generation of the antibody repertoire is achieved by two successive steps. The first step, which is antigen and T cell independent, takes place in the primary lymphoid organs, the fetal liver and the bone marrow. Immature B lymphocytes rearrange their immunoglobulin V (variable), D (diversity) and J (joining) gene segments producing a functionally integrated VDJ segment linked to the μ constant region ($C\mu$), leading to the generation of the primary antibody repertoire of the IgM isotype. V(D)J recombination is tightly regulated during lymphocyte differentiation by transcriptional regulation of the lymphoid-specific recombinase genes encoding Rag-1 and Rag-2 proteins. Recently, a non-lymphoid-specific new protein called Artemis has been described as also being involved in this recombination process [1••].

The generation of the secondary repertoire is antigen and T cell dependent and takes place in the secondary lymphoid organs, the spleen, lymph nodes and tonsils. Upon encountering antigens, mature B lymphocytes are activated, proliferate and form germinal centres. In this unique anatomical formation and in close contact with T cells, two different types of somatic DNA modifications occur, class switch recombination (CSR) and somatic hypermutation (SHM) generation:

Immunoglobulin CSR occurs by a recombination process between two different switch (S) regions each located upstream of the constant region and a deletion of the intervening DNA. Replacement of the constant region $C\mu$ by a constant region of another isotype ($C\gamma$, $C\alpha$ or $C\epsilon$) ensues and leads to the production of IgG, IgA or IgE without changing antigen specificity. Signal transduction through the BCR, CD40 and cytokine receptors induces two important events: selection of a target S region and induction or activation of CSR recombinase. The repair step of CSR is mediated by the non-homologous end-joining repair system widely and constitutively expressed [2••].

SHM generation introduces point missense mutations (more rarely deletions or insertions) in the Ig V genes and their proximate flanks; this step precedes the positive selection of B cells expressing a B cell receptor (BCR) with high affinity for antigen and the negative selection of B cells carrying a BCR with low affinity for antigen or specific for an autoantigen. This selection process is achieved by interaction with antigen-presenting follicular dendritic cells [3].

Not all IgG or IgA carry SHM, whereas some IgM have SHM, indicating that neither CSR nor SHM is a prerequisite of the other. However, both require close T cell–B cell cooperation, the best known constituting a molecular pathway consisting of the interaction between CD40 ligand transiently expressed on activated helper T cells and CD40 molecules constitutively carried by B cells. Accessory molecules have also been shown to play a role in this cellular interaction, such as CD28 and a newly described molecule, ICOS, expressed on activated T cells and their counterparts on B cells, respectively, CD80/86 (B7 molecules) and B7-related protein [4••–6••,7,8].

Terminal defects of antibody formation in humans lead to primary humoral immunodeficiencies, characterized by an increased susceptibility to recurrent bacterial infections requiring intravenous Ig substitution.

Common variable immunodeficiency

Common variable immunodeficiency (CVID) is the most common symptomatic primary antibody deficiency syndrome. This entity probably represents a heterogeneous group of different syndromes, all characterized by decreased levels of serum IgM, IgG and usually IgA. Susceptibility genes for CVID within the MHC class II and III loci have been reported, but the molecular defects associated with CVID are up to now unknown [9–11]. Some evidence has been presented for immunological abnormalities, including impaired T helper types 1 and 2 cytokine production and the defective expression of CD40 ligand on activated helper T cells [12,13]. A recent study described in a subset of patients a qualitative defect in affinity antibody maturation, as shown by significantly reduced SHM generation. This defect is suggested to be a consequence of an intrinsic B cell abnormality [14,15•].

Hyper-IgM syndromes

Another humoral immunodeficiency, the hyper-IgM syndrome (HIGM), is characterized by a normal or increased serum level of IgM, contrasting with very low (even null) levels of IgG and IgA, strongly suggesting a CSR defect. This syndrome appears to be heterogeneous, and the recent molecular delineation of different forms has enlightened our knowledge of physiological B cell terminal differentiation in humans.

Hyper-IgM syndrome caused by defects in CD40–CD40 ligand interaction

The first described HIGM was the X-linked form (HIGM1) caused by mutations in the gene encoding CD40 ligand, a protein transiently expressed on activated helper T cells, able to interact with CD40 molecules constitutively expressed on B cells/monocytic–dendritic cells [16–20]. 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*. However, B cells are intrinsically normal and can be normally induced to produce IgG, IgA and IgE when activated *in vitro* by CD40 agonists and appropriate cytokines [21]. Impaired CD40 ligand expression on activated helper T cells also results in a defective T and monocyte/dendritic cell–epithelial cell interaction, leading to an abnormal cellular immune response, the consequence of which is a severe susceptibility to opportunistic infections, which cannot be prevented by Ig substitution [22].

Interestingly, it has recently been reported that some IgM+/IgD+ B cells from HIGM1 patients can express the CD27 marker described as specific to memory B cells, and carry SHM [23•]. The presence of this unswitched, mutated B cell population suggests that SHM can occur in the absence of classical CD40 ligand–CD40 interaction, and suggests a second B cell diversification pathway, the molecular delineation of which is still unknown. This peculiar B cell subset has similarities with murine marginal zone B cells, which are able to produce mutated IgM antibodies to some polysaccharidic antigens in a T-independent way [24••]. Mice deficient in Pyk.2 tyrosine kinase lack marginal zone B cells (probably because of a defective response to chemokines and the homing of B cells to the marginal zone) and display impaired antibody production to thymo-independent antigen [25••].

A recent report [26••] described three patients in two consanguineous families suffering from an HIGM (HIGM3) with the same susceptibility to bacterial and opportunistic infections as observed in HIGM1. The lack of CSR was found not to be secondary to a CD40 ligand expression deficiency which could be due in female patients to a skewing in X chromosome lyonization, a previously reported feature [27]. The patients' B cells and monocytes failed to express the CD40 molecules because of two different homozygous missense mutations. The phenotype of patients affected with HIGM1 or HIGM3 is identical to those described in CD40 ligand $-/-$ or CD40 $-/-$ mice, demonstrating the absolute requirement of CD40 ligand–CD40 interaction in class switch recombination.

Hyper-IgM syndrome caused by a defect in CD40-induced nuclear factor κ B activation

Concomitantly, three groups have recently delineated the molecular basis of another form of X-linked HIGM associated with hypohydrotic ectodermal dysplasia. All patients present with life-threatening infections, including bacterial, opportunistic and mycobacterial infections. The serum concentrations of Ig appear to be variable, but hypo-IgG and hyper-IgM is often noticed. Antibody

response, especially to polysaccharidic antigens, when assessed, is found to be defective. The B cell abnormality is related to an impaired nuclear factor κ B (NF- κ B) signaling after CD40 activation, secondary to missense mutations in the zinc finger domain of the NF- κ B essential modulator gene (also called NEMO or IKK γ) [28••–30••].

NEMO serves as a scaffolding protein that binds to two kinase proteins, IKK1 and IKK2, necessary for NF- κ B activation. After CD40 stimulation, these kinases are activated, leading to the phosphorylation and the degradation of the NF- κ B inhibitory protein (I- κ B) and thus to the nuclear translocation of NF- κ B components [31–33]. The B cell defect observed in X-linked HIGM associated with hypohydrotic ectodermal dysplasia patients similar to the phenotype observed in mice deficient in both p50 and p52 components of NF- κ B provides evidence for the role of NF- κ B in CD40-mediated B cell activation and Ig switching [34]. The ectodermal dysplasia that is a main feature of this syndrome can also be related to the defect of NEMO because the receptor DL, expressed on ectodermal-derived tissues, belongs to the TNF receptor superfamily (as does CD40), and has been shown to activate NF- κ B, through the IKK1/2 NEMO complex [30••].

Hyper-IgM syndrome caused by a defect in activation-induced cytidine deaminase

Another HIGM with autosomal recessive inheritance (HIGM2) has been described previously [35–37]. These patients are affected with a peculiar sensibility to bacterial infections, but not to opportunistic infections. *CD40L* and *CD40* gene sequencing and membrane molecule expression are normal. In contrast to what is observed in CD40 ligand-deficient patients, B cells present an intrinsic defect because they do not undergo *in vitro* class switch recombination in the presence of CD40 agonists plus appropriate interleukines. In addition to the CSR defect, patients disclose defective SHM generation, although B cells do normally express the CD27 marker. Enlargement of the secondary lymphoid organs is often noticed, secondary to giant germinal centres filled with proliferating IgM⁺ IgD⁺ CD38⁺ B cells.

Analysis of pedigrees strongly suggested an autosomal recessive inheritance. A genetic mapping was undertaken by studying the segregation of polymorphic microsatellite markers in consanguineous or multiplex families. The genome was randomly screened, and the disease segregation was shown to be compatible with the telomeric region of the short arm of chromosome 12 in region p13, with a multi-point lod-score of 10.45 [38••]. Recombination analysis defined the critical genetic interval encompassing a 4.5 cM region, in which a gene

coding for a new molecule, the activation-induced cytidine deaminase (AID), has recently been localized [39]. Sequencing of the *AID* gene showed deleterious mutations in all patients from these families, delineating the molecular basis of HIGM2. The phenotype of *AID*^{–/–} mice has been shown to be identical to that observed in HIGM2 patients, associating the three main features, i.e. defective CSR, defective generation of SHM and the presence of giant germinal centres in secondary lymphoid organs [40••].

The murine AID was firstly identified and the gene cloned by subtractive hybridization between murine lymphoma CH12F3-2 B cells induced or not to undergo CSR *in vitro*. The AID protein has been shown to be only expressed in B cells undergoing CSR *in vitro* (after stimulation with soluble CD40 ligand or lipopolysaccharide with appropriate cytokines) and in germinal centre B cells [41]. Interestingly, this protein is structurally related (34% homology) with the apolipoprotein B (apoB) RNA-editing enzyme APOBEC-1. RNA editing is widely used to create new functional proteins from the restricted genome in plants and protozoa [42,43]. An increasing number of mammalian messenger RNA are also known to be edited, including apoB mRNA by APOBEC-1. ApoB mRNA editing involves a site-specific C to U deamination of the first base of a CAA codon encoding glutamine at residue 2158 in 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. APOBEC-1 requires an auxiliary factor (ACF) for site-specific RNA editing of apoB mRNA; this auxiliary factor is widely expressed, even in organs that do not express APOBEC-1 [44].

AID has been shown to contain an active site for cytidine deamination, and to exert an actual cytidine deaminase activity *in vitro* [41]. It also contains a leucine-rich region located in the C terminus part of the protein, which is probably important for protein–protein interaction. The human counterpart of the murine AID exhibits a strong homology (96%), especially in the cytidine deaminase domain (100%) [39]. The concomitant description of HIGM2 patients and *AID*^{–/–} mice demonstrate the essential role of AID in B cell terminal differentiation, including CSR and the generation of SHM.

The AID deficiency does not appear to affect the maturation of B cells because B cells exhibit a phenotype of mature B cells, and are able to be activated through the BCR and form germinal centres. Because of homology with APOBEC-1 and its functional activity demonstrated *in vitro*, it is likely that AID acts as an actual RNA editing enzyme either on a shared substrate

required for both CSR and SHM or on two different templates specific for each event, its specificity being determined by additional co-factors. There is some evidence that CSR and SHM, although able to occur independently, could share a part of the genetic alteration machinery because, for example, mutations are frequently found in the S regions [45,46], suggesting a common mechanism. It is known that both require transcription of the target DNA [47,48]. However, it is likely that AID plays a role downstream of the transcription step because sterile germline Ie-Ce transcripts induced by CD40 agonists and IL-4 can be detected in B cells from HIGM2 patients and from AID-deficient mice [38•,40•]. Recently, several groups have concomitantly demonstrated the occurrence of double-strand DNA breaks occurring during the SHM process [49•,50•,51] as strongly suggested in CSR [52,53]. These observations suggest the role for a specific common endonuclease, the RNA of which might be a good candidate for editing by AID.

The role of non-homologous end-joining enzymes (DNA-PK and Ku70-Ku80 recombination complex) has been established in CSR, whereas they are not required for SHM [54•]. The mismatched repair enzymes were also shown to play at variable levels a role in CSR and SHM [55–59]. The nucleotide excision repair enzyme induces the cleavage of Ig S regions, at least *in vitro* [60•], but its involvement in SHM is not demonstrated. Error-prone polymerases, including Pol ζ or Pol η , the requirement for which is suggested in SHM [61•–63•], could also be involved in DNA repair during CSR.

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 deamination at the DNA level of C to U in V genes and S regions, leading to double-strand breaks in DNA, thus becoming substrates for error-prone DNA repair enzymes [64•].

The concomitant description of HIGM2 patients and AID-deficient mice thus gives evidence for the role of the AID molecule in B cell terminal differentiation, and strongly suggests that SHM and CSR share a yet undefined common mechanism.

Other hyper-IgM syndromes

Some HIGM cannot be related to any of the molecular defects described above. A phenotype identical to the one observed in HIGM2 patients (associating defective CSR and SHM generation) strongly suggests a defect in the target of AID itself or a necessary co-factor in these patients. In some other patients, the observation of a defective CSR with normal SHM generation argues in favour of a defective co-factor specific for CSR. However, as the majority of these patients have no

family history, it is possible that these disorders result from a combination of susceptibility genes and environmental factors [65].

Conclusion

The recent delineation of the molecular basis of some HIGM enlightened our knowledge of normal B cell terminal differentiation in humans. The description of the NEMO deficiency as the cause of HIGM associated with hypohydrotic ectodermal dysplasia provides strong evidence for the role of NF- κ B activation in the CD40 signalling pathway in B cells.

The delineation of a new molecule, AID, clearly indicates its role in the two major events necessary for the generation of the secondary antibody repertoire, the class switch recombination and the generation of somatic hypermutations, and strongly suggests that these two processes share the same mechanisms.

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