

The Hyper IgM Syndromes

Nashmia Qamar · Ramsay L. Fuleihan

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Abstract The hyper IgM syndromes are a group of rare inherited immune deficiency disorders characterized by impairment of immunoglobulin isotype switching resulting from defects in the CD40 ligand/CD40 signaling pathway. X-linked forms of hyper IgM are caused by defects in the CD40 ligand gene or NF- κ B essential modulator, while autosomal recessive forms of hyper IgM are caused by defects in CD40 or downstream signaling molecules including activation-induced cytidine deaminase, uracil N glycosylase or postmeiotic segregation increased 2. The loss of interaction between CD40 and its ligand results in an impairment of T cell function, of B cell differentiation and of monocyte function while only B cell differentiation appears to be affected in defects of signaling molecules downstream of CD40 with the exception of defects of the NF- κ B complex, which mediates signaling via multiple receptor pathways. With many genetic defects in the hyper IgM syndrome identified, it is possible to diagnose patients definitively, to perform genetic screening, and to delineate the clinical manifestations of the different diseases in this syndrome. Stem cell transplantation is an available therapeutic option for defects that result in a combined immunodeficiency while antibody replacement appears sufficient for the strictly humoral immunodeficiencies.

Keywords Hyper IgM syndrome · Immunoglobulin isotype switching · Opportunistic infection · *Pneumocystis jiroveci* · *Cryptosporidium* · sclerosing cholangitis · CD40 ligand · CD40 · Ectodermal dysplasia with immunodeficiency · Switched memory B cells · Registry · Immunoglobulin

N. Qamar · R. L. Fuleihan
Division of Allergy and Immunology, Ann & Robert H. Lurie
Children's Hospital of Chicago, Northwestern University Feinberg
School of Medicine, Chicago, IL, USA

R. L. Fuleihan (✉)
Division of Allergy and Immunology, Ann & Robert H. Lurie
Children's Hospital of Chicago, 225 E. Chicago Avenue, Chicago,
IL 60611, USA
e-mail: r-fuleihan@northwestern.edu

replacement therapy · Stem cell transplantation · Gene therapy

Introduction

The hyper IgM (HIGM) syndromes are a group of rare inherited immune deficiency disorders characterized by low or absent serum levels of IgG, IgA, and IgE with normal or elevated serum IgM [1]. Affected patients are susceptible to recurrent sino-pulmonary infections, opportunistic infections, neutropenia, autoimmune disease, and cancer [1], particularly of the liver, pancreas or biliary tree [2]. The disease was first described in 1961 independently by Rosen and by Burtin [3–5]. The defect in HIGM results in a failure of immunoglobulin isotype switching in B cells from IgM/IgD to IgG, IgA, or IgE, which requires contact-dependent help from T cells [6]. Twenty years ago, we and four other groups independently discovered that X-linked HIGM (X-HIGM) results from defects in the gene for CD40 ligand [7–12], which is expressed transiently on the surface of activated T cells [13]. Subsequently, several other gene defects have been identified affecting the CD40 signaling pathway including CD40 and enzymes required for generation or repair of double strand DNA breaks during immunoglobulin isotype switching [14]. Some of these enzymes are also involved in somatic hypermutation, a mechanism by which B cells generate antibody of higher affinity, and therefore, some of the causes of HIGM are associated with defective somatic hypermutation. In addition, defects in NF- κ B signaling, which is involved in the CD40 signaling pathway, result in X-linked ectodermal dysplasia with immunodeficiency that may present with a HIGM phenotype [15]. There are secondary forms of HIGM [1] that will not be discussed in this review.

Molecular Pathology

HIGM results from defects in the CD40 ligand/CD40 signaling pathway leading to impairment of immunoglobulin

isotype switching in B cells. Patients with X-HIGM have a defect in the CD40 ligand gene, which consists of five exons [16] and is located on the X chromosome at Xq26.3–Xq27.1 [17]. The product of the CD40 ligand gene is a 39-kDa type II membrane glycoprotein that is homologous to tumor necrosis factor (TNF)- α and is expressed transiently on the surface of activated CD4⁺ T cells and can also be expressed on CD8⁺ T cells, B cells, mast cells, basophils, eosinophils, and platelets [18]. CD40, the receptor for CD40 ligand, is a 50-kDa member of the TNF receptor family of proteins that is found on B cells, monocytes, dendritic cells, epithelial cells, endothelial cells, fibroblasts, and some cancer cells [18]. The interaction between CD40 ligand on T cells and CD40 on antigen-presenting cells plays a very important role in immunity and the failure of this interaction is responsible for the immune deficiency in HIGM. Indeed, patients with an autosomal recessive form of HIGM (AR-HIGM), with both males and females affected, have normal CD40 ligand but have a defect in the CD40 signaling pathway in B cells [19, 20] that leads to immunoglobulin isotype switching including defects in CD40 itself [21] or in enzymes involved in generation or repair of DNA breaks during immunoglobulin isotype switching such as activation induced cytidine deaminase (AID) [22, 23], uracil-N-glycosylase (UNG) [24], postmeiotic segregation increased 2 (PMS2) [25], ataxia telangiectasia mutated (ATM) [26] and the gene encoding Nibrin [27], which is defective in Nijmegen breakage syndrome (*NBS1*). Furthermore, gene targeting of either CD40 [28, 29] or its ligand [30, 31] in mice results in a phenotype that resembles the human disease X-HIGM. Therefore, HIGM is caused by defective signaling via CD40 because of the failure to express CD40 ligand, failure of CD40 ligand to bind to CD40 or as a result of a defect in the CD40 signaling pathway.

The various reported defective forms of CD40 ligand include single amino acid substitutions, truncations, in-frame deletions, or out-of-frame deletions and most of the mutated CD40 ligand proteins do not bind to CD40 [32, 33]. Some of the mutated CD40 ligand proteins can be expressed on the surface of T cells and are detectable by monoclonal or by polyclonal antibodies [11, 12, 34]. Because the genetic defect is not lethal to T cells, carriers have random inactivation of the X chromosome in their T cells [35, 36] with no apparent effect on immune function except in the event of severe skewing which may be associated with a mild HIGM phenotype [37]. The inability of mutated CD40 ligand protein to bind to CD40 interrupts the interaction between CD4 T cells and B cells and prevents immunoglobulin isotype switching as well as all the other consequences of this interaction including T cell costimulation and memory B cell development (Fig. 1).

An autosomal recessive form of HIGM (AR-HIGM) affecting males and females equally with a similar phenotype to X-HIGM results from defects in CD40 [38]. Affected

patients are susceptible to opportunistic infections, neutropenia, and sclerosing cholangitis. The mutations found in four patients from three families with CD40 deficiency included a single amino acid change in a highly conserved area of an extracellular cysteine-rich domain, a 6-bp deletion from exon 4 and a cryptic splice site resulting in skipping of exon 5. The absence of any signaling via CD40 interrupts immunoglobulin isotype switching as well as B cell memory formation and T cell costimulation (Fig. 1).

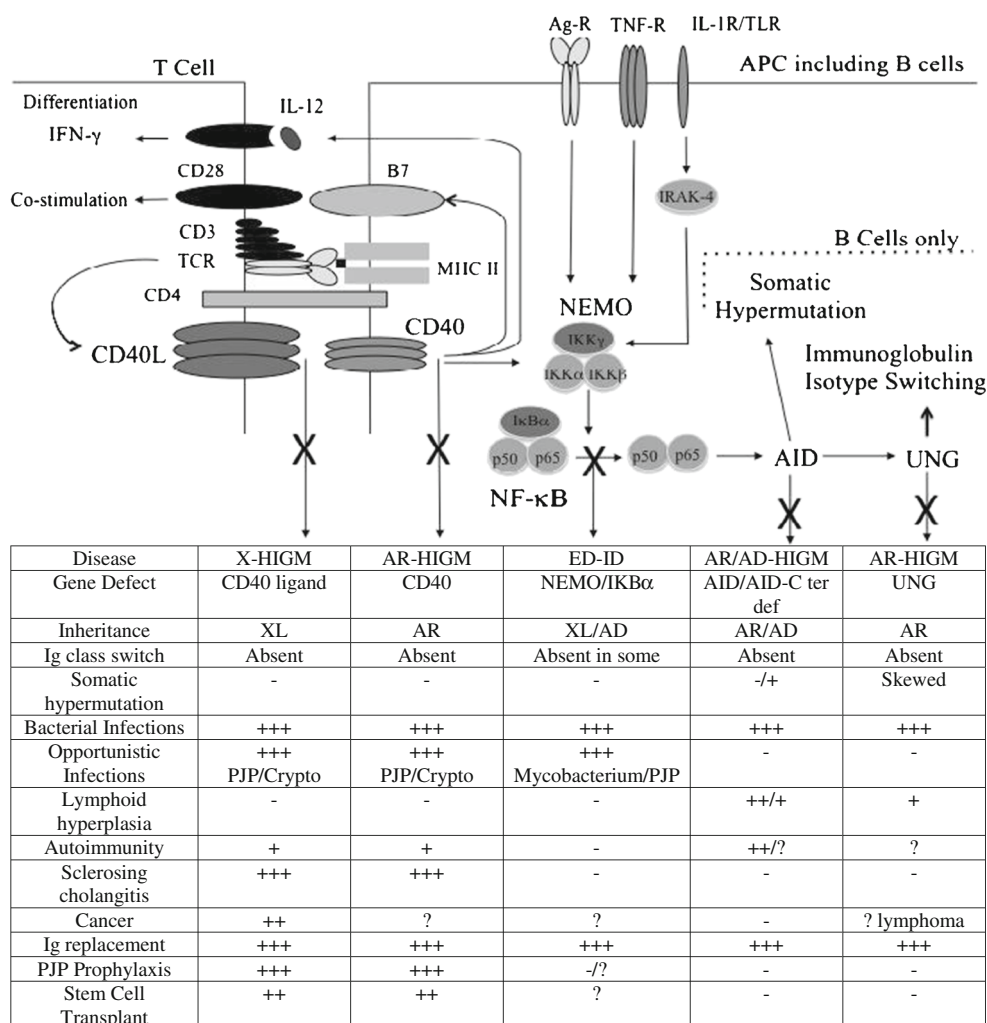
The most common identified cause of AR-HIGM results from defects in the *AICDA* gene encoding for AID [22], an enzyme required for immunoglobulin isotype switching and somatic hypermutation in B cells [39]. AID is a 198 amino acid protein member of the APOBEC family of cytosine/cytidine deaminases. Expression of AID is upregulated in response to CD40 signaling and it initiates immunoglobulin isotype switching by deaminating deoxycytosine in the switch regions of the immunoglobulin heavy chain genes generating deoxyuracils in both DNA strands [40]. UNG then removes deoxyuracils from DNA and initiates the DNA repair pathway [41]. Defects in UNG also result in AR-HIGM syndrome [24]. The clinical picture is similar to that of AID deficiency, including defective class switch recombination, lymphoid hyperplasia and recurrent bacterial infections.

Most cases of mutations in the *AID* gene cause HIGM syndrome in an autosomal recessive manner. However, more recently seven patients have been described with an autosomal dominant form involving a heterozygous mutation in the C terminal end of the molecule, a region involved in nuclear egress [42]. The latter patients have shown defective class switch recombination but intact somatic hypermutation.

Defective immunoglobulin isotype switching leading to a HIGM phenotype has also been found in patients with mutations in enzymes involved in DNA mismatch repair including PMS2 protein [25], which is associated with the development of gastrointestinal adenocarcinomas [43], *ATM* [26] and *NBS1* [27], the genes causing ataxia-telangiectasia and Nijmegen breakage syndrome, respectively. Some patients with ataxia-telangiectasia develop a HIGM phenotype but other patients with mutations in either of these three genes may have reduced but not absent levels of IgG and IgA. Unlike mutations in *NBS1*, mutations in *PMS-2* and *ATM* do not affect somatic hypermutation [25, 44].

Defects in NF- κ B signaling, which is involved in the CD40 signaling pathway, can also cause a HIGM phenotype. Hypomorphic defects in the *IKBKG* gene encoding for the NF- κ B essential modulator (NEMO, IKK-gamma) results in X-linked ectodermal dysplasia with immunodeficiency characterized by a HIGM phenotype in boys [45–47] and gain of function mutations in *NFKBIA*, the gene encoding for IKB α , part of the NF- κ B inhibitory complex result in the same phenotype in boys and girls [48–50]. Because of the widespread expression of NF- κ B and its involvement in multiple

Fig. 1 Schematic representation of the interaction of a CD4 T cell with an antigen-presenting cell (APC) including B cells. CD40 ligand is expressed on the surface of the CD4 T cell after stimulation via the TCR/CD3 complex recognizing peptide antigen/major histocompatibility complex (MHC) class II molecule. CD40 ligand engages CD40 on the B cell/APC leading to expression of costimulatory molecules and cytokine secretion (e.g., IL-12) delivering costimulatory and differentiation (e.g., IFN- γ) signals to the T cell, respectively. In addition, CD40 signaling activates NF- κ B via NEMO leading to activation of AID, UNG and DNA repair enzymes (not shown) in B cells only and resulting in immunoglobulin isotype switching and somatic hypermutation. The level of the gene defect in the different forms of HIGM is shown with an $\times$ and the characteristic features of the disease are listed below in table format. Ag-R antigen receptor, TNF-R tumor necrosis factor family receptor, TLR toll like receptor



signaling pathways including antigen receptors, toll like receptors, the interleukin-1 receptor family and other members of the TNF-receptor family, the immunodeficiency in this condition is more severe than an antibody deficiency resulting from defective immunoglobulin isotype switching, affects the innate and adaptive immune systems and is associated with an enteropathy that can complicate the outcome of stem cell therapy [51].

Clinical and Laboratory Manifestations

The clinical manifestations of HIGM are variable and, in part, depend on the defect in the CD40 ligand/CD40 signaling pathway (Fig. 1). Defects in CD40 ligand (X-HIGM) and CD40 interrupt the interaction between CD4 T cells and B cells (as well as other antigen presenting cells) resulting in a combined immunodeficiency with defective immunoglobulin isotype switching and susceptibility to opportunistic infections [38, 52]. In contrast, defects in the immunoglobulin isotype switching enzymes AID and UNG result in a primarily

antibody deficiency, lymphoid hyperplasia and a higher propensity for autoimmune disease [23, 24, 53]. The other consequences of the interaction of CD40 with its ligand such as T cell costimulation are not affected and the patients do not suffer from opportunistic infections. Defects in the NF- κ B signaling pathway result in ectodermal dysplasia and HIGM with recurrent infections including susceptibility to mycobacterial infections and their course can be complicated by an enteropathy because the defect affects all cells that depend on NF- κ B signaling including gut epithelial cells [15].

The majority of patients with X-HIGM (CD40 ligand defects) develop recurrent sino-pulmonary infections in the first 2 years of life [33, 54] (Table 1). Some patients present to medical attention with *Pneumocystis (carinii) jiroveci* pneumonia during their first year of life, while other patients may not present until adolescence with a parvoviral-induced aplastic anemia [55, 56]. Some patients also suffer from recurrent or persistent neutropenia which may be associated with stomatitis [33, 54]. Patients with X-HIGM are also susceptible to infections with *Histoplasma capsulatum* and with *Cryptosporidium parvum*; the latter is associated with chronic diarrhea and

Table 1 Clinical manifestations in X-HIGM

Clinical manifestation	N (%)
Pneumonia	110 (81)
<i>Pneumocystis jiroveci</i>	56 (41)
Upper respiratory tract infection	88 (65)
Neutropenia	88 (65)
Chronic	52 (38)
Intermittent/cyclic	36 (27)
Anemia	30 (22)
Diarrhea	55 (41)
Oral ulcers	49 (36)
Sepsis	18 (13)
Hepatitis	16 (12)
Sclerosing cholangitis	16 (12)
CNS infection	16 (12)
Tumors	5 (4)

Combined US/European registries, 135 patients in total

sclerosing cholangitis, which may predispose patients to tumors of the liver, pancreas or biliary tree [2]. Cholangitis with elevation of liver enzymes develops with time and is more common in older patients with X-HIGM. A few patients have been reported to develop a fatal and rapidly proliferating neuroendocrine carcinoma [57–59].

Registries for X-HIGM and other primary immune deficiency diseases have been established in Europe by the European Society for Immunodeficiencies and by the Immune Deficiency Foundation and the National Institute of Allergy and Infectious Diseases in the United States of America. A report by the European group describes the clinical manifestations and immune function in 56 patients, who met the criteria for X-HIGM [54]. Infection was the most common clinical manifestation with respiratory tract infections being the most frequent among them. Gastrointestinal disease was the second most common clinical manifestation occurring in over half of the patients and included diarrhea, oral ulcers associated with neutropenia, sclerosing cholangitis associated with cryptosporidiosis, hepatitis, and liver cirrhosis with hepatic failure or severe cholestasis requiring liver transplantation. Hematologic abnormalities included neutropenia and anemia. Thirteen of the 56 X-HIGM patients in this report [2] had died between 9 months and 23 years of age (Table 2). The causes of death included infection, liver failure, and cancer. The oldest living patient was 23 years old.

Similarly, a North American cohort involving 79 patients from 60 families [33] described clinical features of patients with X-HIGM with the most common manifestations including sino-pulmonary infections (pneumonia 81 %, upper respiratory infections 49 %, sinusitis 43 %, recurrent otitis 43 %) and chronic diarrhea (34 %) (Table 1). Also described but less frequently occurring were CNS infections (14 %),

Table 2 Causes of death in X-HIGM

Cause	Age at death	N
Infection		
Pneumonia and sepsis		
<i>P. jiroveci</i> and <i>S. aureus</i>	9 months	1
Pneumonia		
<i>P. jiroveci</i>	9, 14 months	2
<i>Cytomegalovirus</i>	10 years	1
Disseminated Infection		
<i>Cytomegalovirus</i>	20 years	1
<i>M. tuberculosis</i>	15 years	1
Peritonitis	3.5 years	1
Encephalitis		
<i>Echovirus</i>	3 years	1
<i>Cytomegalovirus</i>	12, 16 years	2
Liver failure		
Sclerosing cholangitis	11, 39 years	2
Sclerosing cholangitis post liver transplant	13 years	1
Post liver transplant	14 years	1
Cirrhosis	14, 18 years	2
Hemolytic uremic syndrome	25 years	1
Cancer		
Hepatocellular carcinoma	5, 16, 24, years	3
Hepatocellular carcinoma and Primitive	23 years	1
Neuroectodermal tumor of the colon		

Combined US/European registries, 135 patients in total

sepsis (13 %), cellulitis (13 %), hepatitis (9 %), and osteomyelitis (1 %). In addition to the opportunistic infections described in the European cohort, other opportunistic infections described in the US cohort included Bartonella, herpes family viruses, *Cryptosporidium*, *Cryptococcus*, *Candida*, and Histoplasma. Eight patients had died between the ages of 9 months and 39 years from infection, cancer or liver failure (Table 2). Findings from both these cohorts emphasize that X-HIGM is a severe immune deficiency with significant morbidity and mortality.

The laboratory findings in X-HIGM are not sufficient for making a definitive diagnosis. Low or undetectable serum IgG, IgA and IgE levels with normal or elevated serum IgM in a boy is suggestive of X-HIGM. Patients with X-HIGM produce specific antibodies in response to vaccination, however, the antibodies are restricted to the IgM isotype and they do not exhibit memory responses to repeated immunizations [60]. X-HIGM patients have a normal number of mature B cells but absent switched memory B cells [61], and have normal numbers of T cells and T cell subsets with normal T cell proliferation to mitogens [1, 54], but possibly impaired T cell proliferation to antigens. A group of patients with X-HIGM had decreased T cell proliferation to one or more

antigens [62]. However, in the report from the European registry, ten out of the 16 patients appeared to have normal T cell proliferation to antigens [54] suggesting that antigen-induced T cell proliferation may not be defective in all patients with X-HIGM. Activated CD4⁺ T cells [13] and platelets [63] from patients with X-HIGM may not express CD40 ligand on their surface. However, cell surface expression of CD40 ligand on activated T cells or on platelets is not a reliable diagnostic tool in all patients. Some of the mutations in CD40 ligand result in defective proteins that may be detectable by monoclonal or polyclonal antibodies [11, 12, 34], or less frequently, by soluble CD40 [34]. Furthermore, some patients with common variable immunodeficiency have decreased or absent CD40 ligand expression [64]. Therefore, a definitive diagnosis of X-HIGM in a male patient with hypogammaglobulinemia may require documentation of a mutation in the CD40 ligand gene, especially if the patient does not have maternal uncles or cousins with a confirmed diagnosis of X-HIGM. The Pan American Group for Immunodeficiency and the European Society for Immunodeficiencies have published criteria for the diagnosis of many primary immune deficiency diseases including X-HIGM [65].

CD40 deficiency presents with clinical manifestations that are very similar to X-HIGM including a susceptibility to opportunistic infections and association with neutropenia [38] (Fig. 1). Few patients have been identified and reported to date and thus the full spectrum of the clinical manifestations of CD40 deficiency may not be appreciated until larger numbers of patients are identified and reported. CD40 deficiency should be suspected in any female patient presenting with a classical picture of X-HIGM or any male patient with a X-HIGM phenotype but normal expression of CD40 ligand as determined by staining with soluble CD40 in addition to monoclonal antibody. To date, all patients with CD40 deficiency had no detectable CD40 on the surface of their B cells [38].

The clinical manifestations of patients with defects in AID and UNG differ from the clinical manifestations of X-HIGM (Fig. 1). While serum immunoglobulin levels and a susceptibility to sino-pulmonary infections are similar between the two diseases, opportunistic infections and neutropenia occur much less frequently in these patients [23, 24, 53]. In addition, patients with AR-HIGM resulting from mutations in the *AID* or *UNG* genes have lymph node hyperplasia caused by the presence of giant germinal centers. In contrast, patients with X-HIGM have an inability to generate germinal centers [66]. Six of 22 patients with AID defects had autoimmunity or inflammation: diabetes mellitus, polyarthritis, autoimmune hepatitis, hemolytic anemia, immune thrombocytopenia, Crohn's disease and chronic uveitis [53]. In the report on AD-HIGM from mutations in the C terminal end of the *AID* gene [42], four of the seven patients had recurrent infections but none exhibited autoimmunity. Identification of more

patients with AR-HIGM will help delineate the clinical manifestations of these disorders and may help discover other gene defects that may be responsible for HIGM.

Patients with defects in NEMO demonstrate a range of phenotypic diversity with susceptibility to infection present in almost all patients but ecodermal dysplasia being absent in about a quarter of the patients [15]. Hypogammaglobulinemia was present in over half the patients but only 15 % had a HIGM phenotype. Interestingly, about 37 % of the patients had an elevated serum IgA level. Natural killer cell function was deficient in all patients tested and a majority of patients had specific antibody deficiency. Infections included pyogenic bacterial infections, mycobacterial infections, *P. jiroveci* and DNA viral infections. Disease was complicated by autoimmune or inflammatory disorders in about a quarter of the patients. At the time of the study, 36 % of the patients had died, indicating that this is another very serious and life-threatening condition. NEMO defects should be considered and investigated in every male patient presenting with a severe infection, a HIGM phenotype and normal expression of CD40 ligand.

The clinical manifestations of other causes of a HIGM phenotype including PMS2, ATM and NBS1 are associated with a susceptibility to infection from the resultant antibody deficiency as well as features typical of the underlying disease. Patients with PMS2 have reduced but not absent IgG levels and may develop gastrointestinal adenocarcinoma [43]. In ataxia telangiectasia, ataxia may not be recognized until the child starts walking and telangiectasia may not be present in the first few years of life. However, alpha-fetoprotein levels are usually elevated [67] and the patients have hypersensitivity to X-irradiation. Patients with Nijmegen breakage syndrome also show hypersensitivity to X-irradiation, but have microcephaly and do not have elevated alpha-fetoprotein levels [27]. The HIGM phenotype of low or absent IgG and IgA with normal or elevated IgM can be found in other immunodeficiency diseases including X-linked agammaglobulinemia, common variable immunodeficiency [32] and as recently reported, in association with a hypomorphic mutation in the *RAG2* gene in a patient whose relative had Omenn's syndrome and carried the same mutation in *RAG2* [68]. While some forms of common variable immunodeficiency with a HIGM phenotype may be difficult to differentiate from HIGM with an unknown gene defect, agammaglobulinemia is associated with very low or absent B cells and the patient with a *RAG2* mutation had lymphopenia with nearly absent B cells and poor T cell proliferation to mitogens. Nevertheless, it is evident that there is significant clinical heterogeneity in many immune deficiency diseases including HIGM, and that when a definitive diagnosis cannot be confirmed, other causes of HIGM should be investigated and known causes of HIGM should be considered in patients who may have some but not all the features HIGM.

Pathogenesis of HIGM

Failure of CD40 signaling upon engagement/or lack of engagement by CD40 ligand is the underlying cause of HIGM [69]. CD40 ligand is expressed on the surface of T cells after activation via the T cell receptor/CD3 complex (Fig. 1). Mutations and deletions in the CD40 ligand gene in X-HIGM result in a defective CD40 ligand protein, which, if expressed on the surface, does not activate CD40 normally. Defective expression of CD40 ligand because of abnormalities in the promoter region [70] or in T cell signal transduction would also be expected to result in X-HIGM. There are a few patients who have no detectable CD40 ligand on the surface of activated T cells but have no mutation in the CD40 ligand mRNA sequence [8] or who lack any detectable CD40 ligand mRNA (unpublished observations) who may have a defect in CD40 ligand transcription. Patients with AR-HIGM have a defect in CD40 signaling and thus, although CD40 ligand is expressed normally and binds to CD40, the defect in CD40 signaling leads to a similar immune defect as in X-HIGM. A complete lack of signaling by CD40 in all CD40-expressing cells results in an almost identical immune defect as in X-HIGM (Fig. 1). However, most patients with AR-HIGM do not appear to have the T cell defects and susceptibility to opportunistic infections found in X-HIGM. Indeed, the patients with defects in the AID or UNG genes have an immune deficiency that is limited to the failure of immunoglobulin isotype switching and somatic hypermutation [14], and appear to have normal, or at least sufficient CD40 signaling in monocytes and dendritic cells [71]. In addition, these patients differ from patients with X-HIGM in that they have lymphoid hyperplasia secondary to large germinal centers instead of an inability to develop germinal centers [22–24, 53].

Failure of interaction between CD40 and its ligand results in a defect in B cell differentiation which is reflected in the patients' serum immunoglobulin levels and in their response to immunization. In HIGM, mature B cells neither undergo immunoglobulin isotype switching nor exhibit memory responses [60]. There is a lack of development of memory B cells, specifically switched memory B cells [61], which is consistent with studies in mice showing that generation of memory B cells is dependent on the interaction of CD40 with CD40 ligand [18] and with in vitro studies demonstrating that persistence of CD40 engagement by CD40 ligand results in memory B cell development [72]. Patients with X-HIGM [66], but not patients with AR-HIGM, also lack germinal centers in their secondary lymphoid organs indicating that germinal center formation does not require the AID gene but results from other CD40-mediated signaling events.

The susceptibility to opportunistic infections in X-HIGM but not in AR-HIGM suggests that T cell immunity is affected in X-HIGM. Indeed, T cell priming and differentiation in X-HIGM may be defective. In contrast to T cells from age-

matched controls, T cells from patients with X-HIGM were found to have a lower frequency of the memory cells as determined by the CD45RO isoform, had decreased secretion of the Th₁ cytokine interferon (IFN)- γ , and were incapable of inducing IL-12 secretion by antigen-presenting cells [73]. In vitro infection of peripheral blood mononuclear cells from X-HIGM patients with *Toxoplasma gondii* also failed to secrete IFN- γ and to stimulate IL-12 secretion, which was corrected by incubation with soluble CD40 ligand *in vitro* [74]. Priming of naïve CD4⁺ T cells appears to be dependent on the interaction between CD40 ligand and CD40, which stimulates the up-regulation of B7-1/B7-2 on antigen presenting cells that, in turn, deliver a costimulatory signal back to the naïve T cell via CD28 [75] (Fig. 1). In addition, priming of CD8⁺ T cells by dendritic cells requires interaction between CD40 ligand on a CD4⁺ T cell and the dendritic cell [76], suggesting that defects in CD40 ligand may also contribute to a defect in priming of CD8⁺ T cells. Furthermore, differentiation of CD4⁺ T cells into Th₁ helper cells requires IL-12 secretion by antigen-presenting cells, which is also dependent on the interaction between CD40 and its ligand [77]. The susceptibility to opportunistic infections may be secondary to a failure of T cell priming and differentiation, or may be due to the absence of a direct CD40 ligand-mediated signal to the infected cells. Protection of severe combined immunodeficient mice from *P. (carinii) jiroveci* infection by reconstitution with human T cells was abrogated by antibody to CD40 ligand [38]. CD4⁺ T cells were able to protect against *P. (carinii) jiroveci* in the absence of B cells suggesting that CD40 ligand on T cells was interacting with CD40 on cells other than B cells, such as monocytes. Furthermore, infection by *C. parvum* results in chronic diarrhea and is associated with the development of sclerosing cholangitis and tumors of the pancreas, liver and biliary tree [2]. This is thought to result from a failure to clear *C. parvum* infection of bile duct epithelium. Engagement of CD40 on *C. parvum*-infected HepG2 cells resulted in the induction of apoptosis and in a reduction in the number of *C. parvum*-infected cells suggesting that interaction between CD40 ligand on T cells and CD40 on infected bile duct epithelium is necessary for clearing *C. parvum* [2]. However, IL-12 can protect immune competent and immune deficient mice from *C. parvum* [78] indicating that the failure of IL-12 secretion by antigen presenting cells may also be responsible for the susceptibility of X-HIGM patients to *C. parvum*.

The association of autoimmunity with HIGM remains to be elucidated. Neutropenia is common in X-HIGM and CD40 deficiency but has not been associated with the presence of anti-neutrophil antibodies. The pathogenesis of the neutropenia is unknown but it does respond to G-CSF [79]. There were no significant autoimmune complications reported in the X-HIGM registries from Europe [54] or the US [33] except for a few patients (<5 %) who had anemia and thrombocytopenia in the US registry with no mention of

the presence of autoantibodies. However, six of 22 patients with AID defects had autoimmunity or inflammation: diabetes mellitus, polyarthritis, autoimmune hepatitis, hemolytic anemia, immune thrombocytopenia, Crohn's disease and chronic uveitis [53], indicating that the AR-HIGM resulting from defects in AID and possibly UNG may have a higher susceptibility to autoimmune diseases than X-HIGM or CD40 deficiency.

The potential mechanisms for the development of autoimmunity in HIGM is the requirement for CD40 ligand for negative selection in the thymus as has been shown in mice [80]. However, CD40L is also required for effector function in cellular and antibody-mediated autoimmunity [81]. Therefore, there may be no clinically significant autoimmunity in X-HIGM or CD40 deficiency. Central and peripheral B cell tolerance were examined in patients with X-HIGM and AID defects. The results showed that there was no effect of CD40 ligand deficiency on central B cell tolerance because the antibody characteristics of new emigrant B cells was similar to controls [82]. However, mature naïve B cells had a high percentage of autoantibodies including anti-nuclear antibodies (ANA) suggesting an important role for CD40 ligand in peripheral B cell tolerance. In contrast, new emigrant B cells and mature naïve B cells from AID-deficient patients had a high frequency of autoantibodies indicating a role for AID in both central and peripheral B cell tolerance [83]. Furthermore, anti-nuclear IgM antibodies were detected in the serum of all AID-deficient patients. In addition, CD40 ligand and AID-deficient patients had reduced regulatory T cells and increased serum levels of BAFF, which contribute to the failure of peripheral B cell tolerance [82, 83]. Together, these results are consistent with the reports of autoimmunity in a small percentage of CD40 ligand-deficient patients and in about 25 % of AID-deficient patients and suggest that autoimmunity may be more likely in AID-deficient patients. Investigation of autoimmunity and reporting of autoimmune disease in registries as well as in the literature will be important to understand the impact of these complications on HIGM patients.

The reasons for the increased susceptibility of X-HIGM and CD40-deficient patients to cancer is not well known. The development of cancer of the liver, pancreas and biliary tree in X-HIGM appears to be associated with chronic inflammation and infection with *C. parvum* [2]. X-HIGM patients are also susceptible to a rapidly progressive and poorly differentiated neuroendocrine carcinoma [57–59] (and unpublished observation), the pathogenesis of which is unknown. It is presumed that the combined immunodeficiency in X-HIGM contributes to the susceptibility to cancer and is likely similar in patients with CD40 deficiency. Longer follow-up and identification of larger numbers of patients with all forms of AR-HIGM will be needed to determine if there is an increased incidence of cancer in these patients. Of

interest is the role of UNG in removing Uracil from DNA, which is part of the process of generation of double-strand breaks in immunoglobulin isotype switching, but also necessary for removal of oxidized cytosines after exposure to ionizing radiation, which may predispose to the development of some cancers [84]. UNG-deficient mice develop lymphoma late in their life [85]. There are no reports of cancer in UNG-deficient HIGM patients, but long-term follow-up of these patients will be necessary to determine if there is an increased risk of cancer.

Therapy of HIGM

The antibody deficiency in all forms of HIGM requires immunoglobulin (Ig) replacement with intravenous or subcutaneous immunoglobulin, which reduces the frequency and severity of infections but does not prevent lymphoproliferative disease [1], sclerosing cholangitis or malignancies [2, 57–59]. Whether Ig replacement therapy can prevent autoimmune disease in defects in AID and UNG remains to be determined. Infections should be treated aggressively with specific anti-microbial therapy and patients with X-HIGM or CD40 deficiency need *Pneumocystis (carinii) jiroveci* prophylaxis with oral trimethoprim sulfamethoxazole or pentamidine, IV or inhaled. The length of prophylaxis is not known as the incidence of *Pneumocystis (carinii) jiroveci* pneumonia appears to decrease with age. However, *P. (carinii) jiroveci* pneumonia can occur in young adults with X-HIGM after stopping antibiotic prophylaxis (unpublished observations). Neutropenia can be treated successfully with G-CSF [79].

Stem cell transplantation can cure X-HIGM [86] and HIGM due to CD40 deficiency [87–89] as well as the associated neutropenia [90], cholangiopathy [91] and in combination with a liver transplant for liver failure [92], but may be feasible only when an HLA-matched sibling or closely matched unrelated donor is available. A report from Europe on stem cell transplantation for 38 X-HIGM patients showed that 58 % of the patients were cured, but 32 % died of infection-related complications with half of the patients having severe cryptosporidiosis and half-developing Grade 2–4 graft-vs.-host disease [93]. Long-term studies on the benefit and risk of stem cell transplantation vs. Ig replacement therapy and *P. (carinii) jiroveci* prophylaxis needs to be done to determine the best course of action for a particular patient or to identify patients at higher risk for life-threatening complications and thus may benefit from stem cell transplantation.

Therapy with CD40 agonists has been attempted using a soluble form of CD40 ligand in one study [94] and an agonistic antibody to CD40 in the other [95]. In the first study, recombinant CD40 ligand was not able to rescue the defect in specific antibody synthesis but improved T cell antigen responses were demonstrated during therapy as well as

improved secretion of IFN- γ and TNF- α and changes in the size and architecture of lymph nodes. Agonistic antibody to CD40 was tested in two patients with cryptosporidiosis, which was well tolerated but failed to cure the disease or induce specific antibody synthesis. Frequent dosing in one patient reduced oocyte shedding in the stool and also improved IFN- γ secretion by T cells. The CD40 receptor was rapidly internalized following binding with the agonistic antibody, which might explain the limited effectiveness of this type of therapy. Evaluation of therapy with CD40 agonists is important even if it does not correct the immunodeficiency, because it may help suppress the infectious complications of the disease while the patient is undergoing definitive therapy with hematopoietic stem cell transplantation.

Identification of the genetic defect has made carrier detection and prenatal diagnosis possible [96], and may lead to a genetic therapy of X-HIGM. The success of gene therapy in the treatment of X-linked severe combined immune deficiency disease (X-SCID) and adenosine deaminase deficient SCID is encouraging for the treatment of other immune deficiency diseases [97–100]. However, the development of leukemia in some of the patients with X-SCID who received gene therapy is concerning [101, 102]. In addition, gene therapy of X-HIGM will be more complicated than that of X-linked severe combined immune deficiency because expression of the CD40 ligand gene is highly regulated and current methods of gene transfer result in constitutive gene expression. Attempts to transfer the CD40 ligand gene by a recombinant retrovirus in CD40 ligand-deficient mice corrected humoral immunity but resulted in thymic lymphoproliferative disease [103]. In addition, aberrant expression of CD40 ligand is associated with the autoimmune disease systemic lupus erythematosus [104]. Furthermore, many of the defective forms of CD40 ligand in X-HIGM associate with normal CD40 ligand [105–107] and may behave as dominant negative mutations that inhibit the function of the transferred gene. Therefore, gene therapy of X-HIGM may require correction of the mutation in the endogenous gene. Advances in the delivery of genetic material to hematopoietic stem cells and a better understanding of the regulation of CD40 ligand gene expression and of its role in the immune response is necessary before considering gene therapy for X-HIGM. Towards that end, CD40 ligand expression was corrected in CD40 ligand deficient mice using pre-mRNA trans-splicing [108]. A lentivirus trans-splicer encoding exons 2–5 of the normal CD40 ligand gene was used to infect CD40 ligand-deficient bone marrow cells that were then administered to CD40 ligand-deficient mice. The vector will splice in the normal exons 2–5 when the endogenous CD40 ligand gene is transcribed, thereby maintaining normal regulation. Recipient mice of the transduced bone marrow cells had corrected CD40 ligand expression in CD4 T cells after CD3 stimulation and corrected antigen-specific IgG₁ responses to immunization with keyhole limpet hemocyanin. In addition, *P.*

(*carinii*) *jiroveci* infection was attenuated and there was no evidence of lymphoproliferative disease for over 1 year. Other methods of endogenous gene correction are being examined. Improved efficiency of gene correction with maintenance of normal regulation may make gene therapy a possibility for X-HIGM. Until then, improved methods for stem cell transplantation with reduced side effects will be critical for providing patients with a long-term cure.

Conclusion

HIGM is a severe immune deficiency resulting from the failure of signaling via the CD40 ligand/CD40 pathway, which is critical for immunoglobulin isotype switching, somatic hypermutation, B cell memory, germinal center formation, and T cell priming and function. Complete absence of CD40 signaling in X-HIGM resulting from a defect in CD40 ligand or CD40 leads to a profound immune deficiency involving both the humoral and cellular arms of the immune system. Defects in NF- κ B also result in ectodermal dysplasia and a severe immunodeficiency that may present as HIGM but involves the innate and adaptive immune systems and interrupts several critical signaling pathways important for immunity. Defects in AID and in UNG in AR-HIGM result in an immune deficiency that is limited to immunoglobulin isotype switching and somatic hypermutation in B cells and does not appear to affect T cell function. Defects in DNA repair enzymes such as PMS2, ATM and Nibrin may also present with HIGM in addition to their characteristic clinical phenotype. The HIGM syndrome emphasizes the benefit of unraveling the molecular basis of primary immune deficiency diseases, which serve as experiments of nature that demonstrate the role of the defective genes in the human immune system. A better understanding of the pathogenesis of HIGM and of the function and regulation of expression of the affected genes, as well as advances in bone marrow transplantation or methods for gene transfer/correction may lead to novel and definitive therapeutic options for affected patients.

References

1. Notarangelo LD, Duse M, Ugazio AG (1992) Immunodeficiency with hyper-IgM (HIM). *Immunod Rev* 3:101–122
2. Hayward AR, Levy J, Facchetti F et al (1997) Cholangiopathy and tumors of the pancreas, liver, and biliary tree in boys with X-linked immunodeficiency with hyper-IgM. *J Immunol* 158:977–983
3. Rosen FS, Kevy S, Merler E, Janeway CA, Gitlin D (1961) Recurrent Bacterial infections and dysgammaglobulinemia: deficiency of γ S gammaglobulins in the presence of elevated 19S gamma-globulins. Report of two cases. *Pediatrics* 28:182–195

4. Burtin P (1961) Un exemple d'agammaglobulinémie atypique (un cas de grande hypogammaglobulinémie avec augmentation de la β 2-macroglobuline). *Rev Franc Étud Clin Biol* 6:286–289
5. Ochs HD, Hitzig WH (2012) History of primary immunodeficiency diseases. *Curr Opin Allergy Clin Immunol* 12:577–587
6. Fuleihan R, Ramesh N, Geha RS (1993) Role of CD40-CD40-ligand interaction in Ig-isotype switching. *Curr Opin Immunol* 5:963–967
7. Fuleihan R, Ramesh N, Loh R et al (1993) Defective expression of the CD40 ligand in X chromosome-linked immunoglobulin deficiency with normal or elevated IgM. *Proc Natl Acad Sci U S A* 90:2170–2173
8. Allen RC, Armitage RJ, Conley ME et al (1993) CD40 ligand gene defects responsible for X-linked hyper-IgM syndrome [see comments]. *Science* 259:990–993
9. Aruffo A, Farrington M, Hollenbaugh D et al (1993) The CD40 ligand, gp39, is defective in activated T cells from patients with X-linked hyper-IgM syndrome. *Cell* 72:291–300
10. DiSanto JP, Bonnefoy JY, Gauchat JF, Fischer A, de Saint Basile G (1993) CD40 ligand mutations in x-linked immunodeficiency with hyper-IgM [see comments]. *Nature* 361:541–543
11. Korthauer U, Graf D, Mages HW et al (1993) Defective expression of T-cell CD40 ligand causes X-linked immunodeficiency with hyper-IgM [see comments]. *Nature* 361:539–541
12. Ramesh N, Fuleihan R, Ramesh V et al (1993) Deletions in the ligand for CD40 in X-linked immunoglobulin deficiency with normal or elevated IgM (HIGMX-1). *Int Immunol* 5:769–773
13. Fuleihan R, Ramesh N, Horner A et al (1994) Cyclosporin A inhibits CD40 ligand expression in T lymphocytes. *J Clin Invest* 93:1315–1320
14. Davies EG, Thrasher AJ (2010) Update on the hyper immunoglobulin M syndromes. *Br J Haematol* 149:167–180
15. Hanson EP, Monaco-Shawver L, Solt LA et al (2008) Hypomorphic nuclear factor-kappaB essential modulator mutation database and reconstitution system identifies phenotypic and immunologic diversity. *J Allergy Clin Immunol* 122(1169–1177):e1116
16. Villa A, Notarangelo LD, Di Santo JP et al (1994) Organization of the human CD40L gene: implications for molecular defects in X chromosome-linked hyper-IgM syndrome and prenatal diagnosis. *Proc Natl Acad Sci U S A* 91:2110–2114
17. Graf D, Korthauer U, Mages HW, Senger G, Kroczeck RA (1992) Cloning of TRAP, a ligand for CD40 on human T cells. *Eur J Immunol* 22:3191–3194
18. Vogel LA, Noelle RJ (1998) CD40 and its crucial role as a member of the TNFR family. *Semin Immunol* 10:435–442
19. Conley ME, Larche M, Bonagura VR et al (1994) Hyper IgM syndrome associated with defective CD40-mediated B cell activation [see comments]. *J Clin Invest* 94:1404–1409
20. Durandy A, Hivroz C, Mazerolles F et al (1997) Abnormal CD40-mediated activation pathway in B lymphocytes from patients with hyper-IgM syndrome and normal CD40 ligand expression. *J Immunol* 158:2576–2584
21. Ferrari S, Giliani S, Insalaco A et al (2001) Mutations of CD40 gene cause an autosomal recessive form of immunodeficiency with hyper IgM. *Proc Natl Acad Sci U S A* 98:12614–12619
22. Revy P, Muto T, Levy Y et al (2000) Activation-induced cytidine deaminase (AID) deficiency causes the autosomal recessive form of the Hyper-IgM syndrome (HIGM2) [see comments]. *Cell* 102:565–575
23. Minegishi Y, Lavoie A, Cunningham-Rundles C et al (2000) Mutations in activation-induced cytidine deaminase in patients with hyper IgM syndrome. *Clin Immunol* 97:203–210
24. Imai K, Slupphaug G, Lee WI et al (2003) Human uracil-DNA glycosylase deficiency associated with profoundly impaired immunoglobulin class-switch recombination. *Nat Immunol* 4:1023–1028
25. Peron S, Metin A, Gardes P et al (2008) Human PMS2 deficiency is associated with impaired immunoglobulin class switch recombination. *J Exp Med* 205:2465–2472
26. Noordzij JG, Wulffraat NM, Haraldsson A et al (2009) Ataxia-telangiectasia patients presenting with hyper-IgM syndrome. *Arch Dis Child* 94:448–449
27. Piatosa B, van der Burg M, Siewiera K et al (2012) The defect in humoral immunity in patients with Nijmegen breakage syndrome is explained by defects in peripheral B lymphocyte maturation. *Cytometry A : J Int Soc Analytical Cytology* 81:835–842
28. Castigli E, Alt F, Davidson L et al (1994) CD40 deficient mice generated by RAG-2 deficient blastocyst complementation. *Proc Natl Acad Sci U S A* 91:12135–12139
29. Kawabe T, Naka T, Yoshida K et al (1994) The immune responses in CD40-deficient mice: impaired immunoglobulin class switching and germinal center formation. *Immunity* 1:167–178
30. Renshaw BR, Fanslow WCr, Armitage RJ et al (1994) Humoral immune responses in CD40 ligand-deficient mice. *J Exp Med* 180:1889–1900
31. Xu J, Foy TM, Laman JD et al (1994) Mice deficient for the CD40 ligand. *Immunity* 1:423–431
32. Lee WI, Torgerson TR, Schumacher MJ, Yel L, Zhu Q, Ochs HD (2005) Molecular analysis of a large cohort of patients with the hyper immunoglobulin M (IgM) syndrome. *Blood* 105:1881–1890
33. Winkelstein JA, Marino MC, Ochs H et al (2003) The X-linked hyper-IgM syndrome: clinical and immunologic features of 79 patients. *Medicine* 82:373–384
34. Seyama K, Nonoyama S, Gangsaas I et al (1998) Mutations of the CD40 ligand gene and its effect on CD40 ligand expression in patients with X-linked hyper IgM syndrome [see comments]. *Blood* 92:2421–2434
35. Callard RE, Smith SH, Herbert J et al (1994) CD40 ligand (CD40L) expression and B cell function in agammaglobulinemia with normal or elevated levels of IgM (HIM). Comparison of X-linked, autosomal recessive, and non-X-linked forms of the disease, and obligate carriers. *J Immunol* 153:3295–3306
36. Hollenbaugh D, Wu LH, Ochs HD et al (1994) The random inactivation of the X chromosome carrying the defective gene responsible for X-linked hyper IgM syndrome (X-HIM) in female carriers of HIGM1 [see comments]. *J Clin Invest* 94:616–622
37. de Saint Basile G, Tabone MD, Durandy A, Phan F, Fischer A, Le Deist F (1999) CD40 ligand expression deficiency in a female carrier of the X-linked hyper-IgM syndrome as a result of X chromosome lyonization. *Eur J Immunol* 29:367–373
38. Lougaris V, Badolato R, Ferrari S, Plebani A (2005) Hyper immunoglobulin M syndrome due to CD40 deficiency: clinical, molecular, and immunological features. *Immunol Rev* 203:48–66
39. Muramatsu M, Kinoshita K, Fagarasan S, Yamada S, Shinkai Y, Honjo T (2000) Class switch recombination and hypermutation require activation-induced cytidine deaminase (AID), a potential RNA editing enzyme [see comments]. *Cell* 102:553–563
40. Chaudhuri J, Tian M, Khuong C, Chua K, Pinaud E, Alt FW (2003) Transcription-targeted DNA deamination by the AID antibody diversification enzyme. *Nature* 422:726–730
41. Notarangelo LD, Lanzi G, Toniati P, Giliani S (2007) Immunodeficiencies due to defects of class-switch recombination. *Immunol Res* 38:68–77
42. Imai K, Zhu Y, Revy P et al (2005) Analysis of class switch recombination and somatic hypermutation in patients affected with autosomal dominant hyper-IgM syndrome type 2. *Clin Immunol* 115:277–285
43. Gologan A, Sepulveda AR (2005) Microsatellite instability and DNA mismatch repair deficiency testing in hereditary and sporadic gastrointestinal cancers. *Clin Lab Med* 25:179–196
44. Pan-Hammarstrom Q, Dai S, Zhao Y et al (2003) ATM is not required in somatic hypermutation of VH, but is involved in the

- introduction of mutations in the switch mu region. *J Immunol* 170:3707–3716
45. Zonana J, Elder ME, Schneider LC et al (2000) A novel X-linked disorder of immune deficiency and hypohidrotic ectodermal dysplasia is allelic to incontinentia pigmenti and due to mutations in IKK-gamma (NEMO). *Am J Hum Genet* 67:1555–1562
 46. Doffinger R, Smahi A, Bessia C et al (2001) X-linked anhidrotic ectodermal dysplasia with immunodeficiency is caused by impaired NF-kappaB signaling. *Nat Genet* 27:277–285
 47. Jain A, Ma CA, Liu S, Brown M, Cohen J, Strober W (2001) Specific missense mutations in NEMO result in hyper-IgM syndrome with hypohidrotic ectodermal dysplasia. *Nat Immunol* 2:223–228
 48. Courtois G, Smahi A, Reichenbach J et al (2003) A hypermorphic IkappaBalpha mutation is associated with autosomal dominant anhidrotic ectodermal dysplasia and T cell immunodeficiency. *J Clin Invest* 112:1108–1115
 49. McDonald DR, Mooster JL, Reddy M, Bawle E, Secord E, Geha RS (2007) Heterozygous N-terminal deletion of IkappaBalpha results in functional nuclear factor kappaB haploinsufficiency, ectodermal dysplasia, and immune deficiency. *J Allergy Clin Immunol* 120:900–907
 50. Lopez-Granados E, Keenan JE, Kinney MC et al (2008) A novel mutation in NFKBIA/IKBA results in a degradation-resistant N-truncated protein and is associated with ectodermal dysplasia with immunodeficiency. *Hum Mutat* 29:861–868
 51. Pai SY, Levy O, Jabara HH et al (2008) Allogeneic transplantation successfully corrects immune defects, but not susceptibility to colitis, in a patient with nuclear factor-kappaB essential modulator deficiency. *J Allergy Clin Immunol* 122:1113–1118, e1111
 52. Fuleihan RL (2001) The hyper IgM syndrome. *Curr Allergy Asthma Rep* 1:445–450
 53. Quartier P, Bustamante J, Sanal O et al (2004) Clinical, immunologic and genetic analysis of 29 patients with autosomal recessive hyper-IgM syndrome due to activation-induced cytidine deaminase deficiency. *Clin Immunol* 110:22–29
 54. Levy J, Espanol-Boern T, Thomas C et al (1997) Clinical spectrum of X-linked hyper-IgM syndrome. *J Pediatr* 131:47–54
 55. Blaeser F, Kelly M, Siegrist K et al (2005) Critical function of the CD40 pathway in parvovirus B19 infection revealed by a hypomorphic CD40 ligand mutation. *Clin Immunol* 117:231–237
 56. Seyama K, Kobayashi R, Hasle H et al (1998) Parvovirus B19-induced anemia as the presenting manifestation of X-linked hyper-IgM syndrome. *J Infect Dis* 178:318–324
 57. Erdos M, Garami M, Rakoczi E et al (2008) Neuroendocrine carcinoma associated with X-linked hyper-immunoglobulin M syndrome: report of four cases and review of the literature. *Clin Immunol* 129:455–461
 58. Malhotra RK, Li W (2008) Poorly differentiated gastroenteropancreatic neuroendocrine carcinoma associated with X-linked hyperimmunoglobulin M syndrome. *Arch Pathol Lab Med* 132:847–850
 59. Zirkin HJ, Levy J, Katchko L (1996) Small cell undifferentiated carcinoma of the colon associated with hepatocellular carcinoma in an immunodeficient patient. *Hum Pathol* 27:992–996
 60. Nonoyama S, Hollenbaugh D, Aruffo A, Ledbetter JA, Ochs HD (1993) B cell activation via CD40 is required for specific antibody production by antigen-stimulated human B cells. *J Exp Med* 178:1097–1102
 61. Agematsu K, Nagumo H, Shinozaki K et al (1998) Absence of IgD-CD27(+) memory B cell population in X-linked hyper-IgM syndrome. *J Clin Invest* 102:853–860
 62. Ameratunga R, Lederman HM, Sullivan KE et al (1997) Defective antigen-induced lymphocyte proliferation in the X-linked hyper-IgM syndrome. *J Pediatr* 131:147–150
 63. Inwald DP, Peters MJ, Walshe D, Jones A, Davies EG, Klein NJ (2000) Absence of platelet CD40L identifies patients with X-linked hyper IgM syndrome. *Clin Exp Immunol* 120:499–502
 64. Farrington M, Grosmaire LS, Nonoyama S et al (1994) CD40 ligand expression is defective in a subset of patients with common variable immunodeficiency. *Proc Natl Acad Sci U S A* 91:1099–1103
 65. Conley ME, Notarangelo LD, Etzioni A (1999) Diagnostic criteria for primary immunodeficiencies. Representing PAGID (Pan-American Group for Immunodeficiency) and ESID (European Society for Immunodeficiencies). *Clin Immunol* 93:190–197
 66. Facchetti F, Appiani C, Salvi L, Levy J, Notarangelo LD (1995) Immunohistologic analysis of ineffective CD40-CD40 ligand interaction in lymphoid tissues from patients with X-linked immunodeficiency with hyper-IgM. Abortive germinal center cell reaction and severe depletion of follicular dendritic cells. *J Immunol* 154:6624–6633
 67. Nowak-Wegrzyn A, Crawford TO, Winkelstein JA, Carson KA, Lederman HM (2004) Immunodeficiency and infections in ataxia-telangiectasia. *J Pediatr* 144:505–511
 68. Chou J, Hanna-Wakim R, Tirosh I et al (2012) A novel homozygous mutation in recombination activating gene 2 in 2 relatives with different clinical phenotypes: Omenn syndrome and hyper-IgM syndrome. *J Allergy Clin Immunol* 130:1414–1416
 69. Fuleihan RL (1998) The X-linked hyperimmunoglobulin M syndrome. *Semin Hematol* 35:321–331
 70. Van Hoeyveld E, Zhang PX, De Boeck K, Fuleihan R, Bossuyt X (2007) Hyper-immunoglobulin M syndrome caused by a mutation in the promotor for CD40L. *Immunology* 120:497–501
 71. Revy P, Geissmann F, Debre M, Fischer A, Durandy A (1998) Normal CD40-mediated activation of monocytes and dendritic cells from patients with hyper-IgM syndrome due to a CD40 pathway defect in B cells. *Eur J Immunol* 28:3648–3654
 72. Arpin C, Déchanet J, Van Kooten C et al (1995) Generation of memory B cells and plasma cells in vitro. *Science* 268:720–722
 73. Jain A, Atkinson TP, Lipsky PE, Slater JE, Nelson DL, Strober W (1999) Defects of T-cell effector function and post-thymic maturation in X-linked hyper-IgM syndrome. *J Clin Invest* 103:1151–1158
 74. Subauste CS, Wessendarp M, Sorensen RU, Leiva LE (1999) CD40-CD40 ligand interaction is central to cell-mediated immunity against *Toxoplasma gondii*: patients with hyper IgM syndrome have a defective type 1 immune response that can be restored by soluble CD40 ligand trimer. *J Immunol* 162:6690–6700
 75. Grewal IS, Flavell RA (1996) The role of CD40 ligand in costimulation and T-cell activation. *Immunol Rev* 153:85–106
 76. Mackey MF, Barth RJ Jr, Noelle RJ (1998) The role of CD40/CD154 interactions in the priming, differentiation, and effector function of helper and cytotoxic T cells. *J Leukoc Biol* 63:418–428
 77. Shu U, Kiniwa M, Wu CY et al (1995) Activated T cells induce interleukin-12 production by monocytes via CD40-CD40 ligand interaction. *Eur J Immunol* 25:1125–1128
 78. Urban JF Jr, Fayer R, Chen SJ, Gause WC, Gately MK, Finkelman FD (1996) IL-12 protects immunocompetent and immunodeficient neonatal mice against infection with *Cryptosporidium parvum*. *J Immunol* 156:263–268
 79. Wang WC, Cordoba J, Infante AJ, Conley ME (1994) Successful treatment of neutropenia in the hyper-immunoglobulin M syndrome with granulocyte colony-stimulating factor. *Am J Pediatr Hematol Oncol* 16:160–163
 80. Foy TM, Page DM, Waldschmidt TJ et al (1995) An essential role for gp39, the ligand for CD40, in thymic selection. *J Exp Med* 182:1377–1388
 81. Quezada SA, Jarvinen LZ, Lind EF, Noelle RJ (2004) CD40/CD154 interactions at the interface of tolerance and immunity. *Annu Rev Immunol* 22:307–328

82. Herve M, Isnardi I, Ng YS et al (2007) CD40 ligand and MHC class II expression are essential for human peripheral B cell tolerance. *J Exp Med* 204:1583–1593
83. Meyers G, Ng YS, Bannock JM et al (2011) Activation-induced cytidine deaminase (AID) is required for B-cell tolerance in humans. *Proc Natl Acad Sci U S A* 108:11554–11559
84. Sousa MM, Krokan HE, Slupphaug G (2007) DNA-uracil and human pathology. *Mol Aspects Med* 28:276–306
85. Nilsen H, Stamp G, Andersen S et al (2003) Gene-targeted mice lacking the Ung uracil-DNA glycosylase develop B-cell lymphomas. *Oncogene* 22:5381–5386
86. Thomas C, de Saint Basile G, Le Deist F et al (1995) Brief report: correction of X-linked hyper-IgM syndrome by allogeneic bone marrow transplantation. *N Engl J Med* 333:426–429
87. Al-Dhekri H, Al-Sum Z, Al-Saud B et al (2012) Successful outcome in two patients with CD40 deficiency treated with allogeneic HCST. *Clin Immunol* 143:96–98
88. Kutukculer N, Aksoylar S, Kansoy S, Cetingul N, Notarangelo LD (2003) Outcome of hematopoietic stem cell transplantation in hyper-IgM syndrome caused by CD40 deficiency. *J Pediatr* 143:141–142
89. Mazzolari E, Lanzi G, Forino C et al (2007) First report of successful stem cell transplantation in a child with CD40 deficiency. *Bone Marrow Transplant* 40:279–281
90. Scholl PR, O'Gorman MR, Pachman LM, Haut P, Kletzel M (1998) Correction of neutropenia and hypogammaglobulinemia in X-linked hyper-IgM syndrome by allogeneic bone marrow transplantation. *Bone Marrow Transplant* 22:1215–1218
91. Jacobsohn DA, Emerick KM, Scholl P et al (2004) Nonmyeloablative hematopoietic stem cell transplant for X-linked hyper-immunoglobulin m syndrome with cholangiopathy. *Pediatrics* 113:e122–e127
92. Hadzic N, Pagliuca A, Rela M et al (2000) Correction of the hyper-IgM syndrome after liver and bone marrow transplantation. *N Engl J Med* 342:320–324
93. Gennery AR, Khawaja K, Veys P et al (2004) Treatment of CD40 ligand deficiency by hematopoietic stem cell transplantation: a survey of the European experience, 1993–2002. *Blood* 103:1152–1157
94. Jain A, Kovacs JA, Nelson DL et al (2011) Partial immune reconstitution of X-linked hyper IgM syndrome with recombinant CD40 ligand. *Blood* 118:3811–3817
95. Fan X, Upadhyaya B, Wu L et al (2012) CD40 agonist antibody mediated improvement of chronic *Cryptosporidium* infection in patients with X-linked hyper IgM syndrome. *Clin Immunol* 143:152–161
96. DiSanto JP, Markiewicz S, Gauchat JF, Bonnefoy JY, Fischer A, de Saint Basile G (1994) Brief report: prenatal diagnosis of X-linked hyper-IgM syndrome. *N Engl J Med* 330:969–973
97. Candotti F, Shaw KL, Muul L et al (2012) Gene therapy for adenosine deaminase-deficient severe combined immune deficiency: clinical comparison of retroviral vectors and treatment plans. *Blood* 120:3635–3646
98. Cavazzana-Calvo M, Hacein-Bey S, de Saint Basile G et al (2000) Gene therapy of human severe combined immunodeficiency (SCID)-X1 disease. *Science* 288:669–672
99. Gaspar HB, Bjorkegren E, Parsley K et al (2006) Successful reconstitution of immunity in ADA-SCID by stem cell gene therapy following cessation of PEG-ADA and use of mild preconditioning. *Mol Ther : J Am Soc Gene Ther* 14:505–513
100. Montiel-Equihua CA, Thrasher AJ, Gaspar HB (2012) Gene therapy for severe combined immunodeficiency due to adenosine deaminase deficiency. *Curr Gene Ther* 12:57–65
101. Hacein-Bey-Abina S, Garrigue A, Wang GP et al (2008) Insertional oncogenesis in 4 patients after retrovirus-mediated gene therapy of SCID-X1. *J Clin Invest* 118:3132–3142
102. Howe SJ, Mansour MR, Schwarzwaelder K et al (2008) Insertional mutagenesis combined with acquired somatic mutations causes leukemogenesis following gene therapy of SCID-X1 patients. *J Clin Invest* 118:3143–3150
103. Brown MP, Topham DJ, Sangster MY et al (1998) Thymic lymphoproliferative disease after successful correction of CD40 ligand deficiency by gene transfer in mice. *Nat Med* 4:1253–1260
104. Datta SK, Kalled SL (1997) CD40-CD40 ligand interaction in autoimmune disease. *Arthritis Rheum* 40:1735–1745
105. Garber E, Su L, Ehrenfels B, Karpusas M, Hsu YM (1999) CD154 variants associated with hyper-IgM syndrome can form oligomers and trigger CD40-mediated signals. *J Biol Chem* 274:33545–33550
106. Seyama K, Osborne WR, Ochs HD (1999) CD40 ligand mutants responsible for X-linked hyper-IgM syndrome associate with wild type CD40 ligand. *J Biol Chem* 274:11310–11320
107. Zhu X, Chung I, O'Gorman MR, Scholl PR (2001) Coexpression of normal and mutated CD40 ligand with deletion of a putative RNA lariat branchpoint sequence in X-linked hyper-IgM syndrome. *Clin Immunol* 99:334–339
108. Tahara M, Pergolizzi RG, Kobayashi H et al (2004) Trans-splicing repair of CD40 ligand deficiency results in naturally regulated correction of a mouse model of hyper-IgM X-linked immunodeficiency. *Nat Med* 10:835–841