



## Novel Aspects of Hypogammaglobulinemic States

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Humoral immunodeficiencies are the most common form of immunodeficiency conditions. Immunoglobulin G is the most important immunoglobulin isotype, and therefore low levels will always be associated with clinical symptoms. IgA deficiency, on the other hand, is usually an asymptomatic condition found in about 1 per 700 healthy individuals. It is important to note that serum immunoglobulin levels begin to increase at an early age and thus Ig levels should always be compared to the age of the patient.

Since the main protective function of the immunoglobulins is against bacteria, pyogenic infections in various organs (lung, gastrointestinal, ear and nose) are characteristic of hypogammaglobulinemic states. Still, as will be discussed below, some viral or opportunistic infections are also typical to specific disorders. Several excellent reviews of all forms of hypogammaglobulinemic states have recently been published [1,2]. In the current review we will concentrate on the two main defects in humoral immunity.

### X-linked agammaglobulinemia

X-linked agammaglobulinemia is the prototypic humoral immunodeficiency, with absence of all immunoglobulin isotopes due to abnormal B cell development [3]. XLA was the first human immunodeficiency in which an underlying defect was clearly identified. In 1952 Dr. Bruton reported on an 8 year old boy who suffered from recurrent infections and in whom serum protein electrophoresis revealed a lack of gammaglobulins. Realizing the association between the absence of gammaglobulin and recurrent infections, Bruton instituted gammaglobulin replacement therapy with considerable clinical improvement. Later on it was found that the disease is linked to the X-chromosome. In 1993, using two different approaches – positional cloning [4] and searching for novel protein kinases expressed in B lymphocytes [5] – the gene

affected in XLA was isolated simultaneously and was found to encode a novel cytoplasmic tyrosine kinase, which was designated Bruton's agammaglobulinemia tyrosine kinase.

### Clinical manifestations

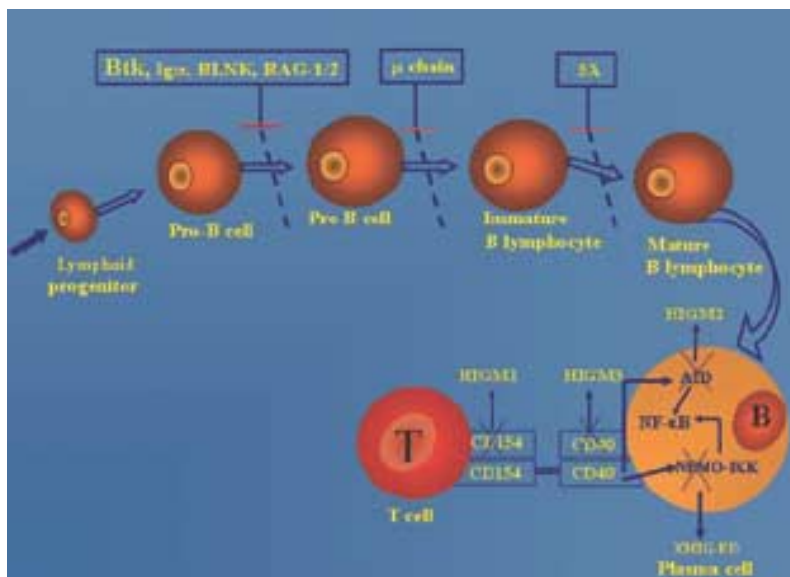
The onset of symptoms, manifested mainly as respiratory and/or gastrointestinal tract infections, is usually within the first year of life after the cessation of maternal IgG transported across the placenta. By age 18 months more than 90% of patients exhibit symptoms [6]. It should be noted that the disease is sometimes diagnosed only in adulthood [7]. Approximately 85% of patients with early-onset recurrent bacterial infections, hypogammaglobulinemia, and markedly reduced numbers of B cells are males with mutations in BTK. The remaining 15% constitute a heterogeneous group of patients with mutations in  $\mu$  chain,  $Ig\alpha$ ,  $\lambda$  and B cell linker (BLNK) [8]. The presenting symptoms at diagnosis are variable, but the most common clinical problems are pneumonia, otitis media and gastroenteritis. Other major infection problems are sinusitis, arthritis, septicemia and meningitis. Infections in XLA are usually caused by the common pyogenic bacteria [3]. *Mycoplasma* causing urinary tract and respiratory tract infections is also common.

A selective anti-polysaccharide antibody deficiency in XLA was recently reported [9]. Resistance to viral infections remains predominately intact except for a susceptibility to enteroviral infections, which can involve the central nervous system and cause chronic meningoencephalitis with neurologic sequelae [3]. Viral infections with disseminated echovirus are the major cause of death in XLA patients. Currently, with prompt replacement therapy and antibiotics, the prognosis for individuals with XLA is much better than before and most patients should have an almost average life span.

The characteristic immunologic findings in XLA reflect the B cell lineage defect with a severely decreased number of B lymphocytes and absence of serum immunoglobulins. In rare cases immunoglobulins or B cells can be found at almost normal range. Normally, T cell number and function is completely intact. Still, a preferential

Ig = immunoglobulin  
XLA = X-linked agammaglobulinemia

BTK = Bruton's agammaglobulinemia tyrosine kinase



**Figure 1.** Human defects (in mice there are several more defects) in B cell development leading to hypogammaglobulinemia, which can also be caused when the interaction with T cells is defective (four types of HIGM).

Th1 profile of T helper cell responses was recently found in patients with XLA [10].

Physical examination shows only rudimentary adenoid and tonsillar tissue and peripheral lymphoid hypoplasia. Histologically, XLA is characterized by the lack of a germinal center in lymphoid tissue and the absence of plasma cell in the lamina propria in the gut. Bone marrow reveals an early block in B cell differentiation. Although there is a normal number of pro-B cells (CD34<sup>+</sup> CD19<sup>+</sup>), there is a marked reduction in the number of cells at the next stage of differentiation, pre-B cells (CD19<sup>+</sup> CD34<sup>+</sup>) [Figure 1] [11]. As there is considerable heterogeneity in the phenotype of XLA, the criteria for definite, probable or possible diagnosis were designated [12].

### Molecular basis of XLA

The BTK gene, which is the primary defect in XLA, is located on the long arm of the X-chromosome at q23.3. It is organized into 19 exons and encodes a 659 amino acid protein. BTK expression is primarily restricted to hematopoietic cells and is found throughout B cell differentiation until the stage of plasma cells. It is expressed also on monocytes, myeloid cells and platelets, but not in T cell lineage. It presents in normal platelets, but its absence in XLA can be used as a simple screening test for the disease [13]. BTK, like other tyrosine kinases, acts as a signal transduction molecule mediating cell surface receptor activation events to downstream pathways. Cross-linking of a number of cell surface receptors, including interleukins 5 and 6 and mainly surface IgM on B cells, results in the recruitment of cytosolic BTK to the plasma membrane and its activation [14].

Despite the large volume of biochemical and signaling data available on BTK activation, a relationship with cellular events and specifically the mechanism of B cell development arrest has yet to be established. Recently, it was found that BTK is essential

for the transcription factor NF- $\kappa$ B, which is essential for reprogramming the expression of genes that control B cell survival and proliferation. The biochemical mechanism by which BTK regulates signaling components that activate NF- $\kappa$ B is still unknown [15].

Identification of the BTK gene has led to research in mutational analysis. Over 500 unique mutations in BTK have been identified. Various types of genetic abnormalities have been found. One-third are missense mutations, but premature stop codon, deletion, and insertions have also been described throughout the gene [16]. Despite the large quantity of mutations and phenotype data available, it has not been possible to make a definite genotype-phenotype correlation. A modest number of mutations with mild phenotypes has been observed, most of which are caused by substitutions. Still, identical genetic changes (in families or sporadic cases) may cause both mild and severe disease. This demonstrates that confounding parameters exist, possibly of both genetic (cytokine gene polymorphism for example) and environmental origin. A knockout mice

(xid) of BTK was generated. In contrast to XLA, the block in B cell maturation is much less severe, with a decrease of only 50% of B cells, and it has the capability to produce various amounts of IgG [17].

From the first description of XLA by Bruton, immunoglobulin replacement is the cornerstone of therapy. The painful and troublesome intramuscular route has now been changed to the intravenous (IVIg) or subcutaneous (SCIg) routes. The goal of therapy is to achieve an IgG serum level of above 600 mg/dl [17]. In most cases 0.4–0.6 g/kg every 3–4 weeks will be needed. While IVIg, which is very safe if given appropriately, is the preferential route in most centers, several recent studies showed equivalent results with SCIg. Despite the excellent clinical response to immunoglobulin replacement therapy in most patients (especially if treatment was started before permanent lung damage occurred), it is still important to monitor patients on a regular basis [18].

Observations in carrier females and murine studies suggest that XLA may be a good candidate for gene therapy. Still, more needs to be known about the function and control of BTK before human trials can begin.

### Hyper-IgM syndrome

Immunodeficiency with hyper-IgM is a rare congenital disorder, characterized by recurrent infections and very low levels of IgA, IgE and IgG, and normal or elevated IgM [19]. Until now four different types have been described, X-linked and autosomal recessive forms. The syndrome was first described in the early 1960s, and was known as dysgammaglobulinemia. Ten years later, in 1974, it was designated immunodeficiency with hyper-IgM. The primary immunologic defect in HIGM has remained elusive for a long time. In the

HIGM = hyper-IgM syndrome

early 1980s an intrinsic B cell inability to undergo immunoglobulin isotype switch was thought to be the primary defect, but it later became clear that a T cell defect must be involved in the pathogenesis. In 1993 Aruffo et al. [20] showed very elegantly that the primary defect in the X-linked form of HIGM (more than 80% of patients with HIGM) is a mutation in the gene encoded for the CD40-ligand (CD154). Several days later, four other groups independently demonstrated the same defect in other patients with X-HIGM. Recently, another three forms of HIGM due to different genetic defects have been described [21] and will be discussed below.

### Clinical manifestations

Although patients with HIGM suffer from pyogenic infections as do patients with hypogammaglobulinemic conditions, the clinical picture is somewhat different. Thanks to the excellent study by Yacob Levy et al. [22], who collected clinical data from 56 patients, the various aspects of the clinical characteristics of the disease are clearly described.

In most patients symptoms will start by the end of the first or during the second year of life. Recurrent upper and lower respiratory tract infections are common. In contrast to other forms of hypogammaglobulinemia, HIGM patients are uniquely susceptible to pneumocystic carinii pneumonia, implying some cellular immune defect in the syndrome. PCP can be present in up to 40% of patients and may be the first manifestation of the disease. Protracted diarrhea with failure to thrive is also common and is due mainly to *Cryptosporidium parvum* infection, which is also involved in sclerosing cholangitis, another serious manifestation. Severe liver disease as a whole, including tumors, is common in HIGM, and its frequency appears to increase with age [23]. Chronic neutropenia occurs in about 50% of patients.

HIGM is characterized by a marked reduction in serum IgG, IgA and IgE with normal to elevated IgM levels and a normal number of circulating B cells. It appears that IgM serum levels increase with age. Levels of specific antibodies of the IgM isotype are normal, but there is little or no production of IgG-specific antibodies. T cell number and T cell subsets are normal in response to various mitogens. In contrast, *in vitro* proliferative response to antigens is often reduced.

Lymphoid hyperplasia distinguishes HIGM from other forms of primary hypogammaglobulinemia and may cause enlargement of tonsils, hepatosplenomegaly and lymphadenopathy. Histologically, lymph nodes of HIGM patients lack a germinal center.

Despite regular use of intravenous gammaglobulin (400–600 mg/kg/3 weeks), the long-term prognosis of HIGM appears to be worse than in other forms of congenital hypogammaglobulinemia. Actuarial survival curves show that less than 30% of patients are alive at 25 years of age. PCP early in life, liver disease and tumors later on, are the major causes of death [22]. Due to the poor prognosis bone marrow transplantation is often performed [24], with recent reports of successful concomitant liver and bone marrow transplants [25].

### Molecular basis

During the late 1980s it became clear that for optimal activation and antibody production by B cells, several signals from T cells must be provided. Cytokines alone (IL-2 and IL-10 for IgG<sub>1</sub> and IgA, IL-4 for IgG<sub>4</sub> and IgE) are not enough to cause isotype switching. A close contact between T and B cells must also occur. This contact is mediated mainly by interaction between CD40, which is constitutively expressed on B cells, and its ligand (CD154), which is expressed only on activated T cells. The molecular basis for signal transduction that follows CD40/CD154 engagement remains to be fully established. The human CD154 protein is 261 amino acids long, with a short intracytoplasmic tail, a transmembrane region, and a long extracellular domain, which shares homology with tumor necrosis factor- $\alpha$ . The gene is organized in five exons and a promoter region. The realization of the importance of the CD154 in B cells isotype switch led to the discovery of mutations in its gene as the cause of the X-linked HIGM syndrome.

In their instrumental work, Aruffo et al. in 1993 [20] showed that B cells from these patients expressed functional CD40, but the T cells did not bind CD40. These patients expressed normal levels of CD154 mRNA, but the mRNA encoded defective CD154 owing to mutations in the TNF- $\alpha$  domain (exon 5). Furthermore, soluble recombinant forms of CD154 containing these mutations were unable to bind CD40 or drive normal B cell proliferation.

A database of CD154 gene mutations has been organized and more than 100 different mutations have been reported [26]. Mutations are scattered throughout the entire length of the gene, although they are more common in exon 5. Nonsense, missense, insertion, deletion and splice-site mutations have been variably reported in families. In general, no strict genotype-phenotype correlation has been identified. While the humoral defect is clearly understood as a consequence of the defective CD40/CD154 interaction, the cellular clinical immune defect (PCP and *Cryptosporidium* infections) was more difficult to explain. The generation of a CD154 knockout mouse helped to solve the problem. Knockout mice have exactly the same immunologic defects as humans, as well as increased susceptibility to PCP. It was found that aside from isotype switching, CD40 binding by CD154 will lead to the expression of B7 on the B cell. B7 will then bind to CD28, which is constitutively expressed on T cells, for an optimal T cell function [27].

### Other forms of HIGM

As discussed above, the first HIGM syndrome to be described (HIGM1) was the X-linked form that results from mutations in the gene encoded CD154. Recently, other genetic defects in patients with HIGM were discovered.

HIGM2 is an autosomal recessive condition in which patients are affected by recurrent bacterial infections, but without opportunistic infections. All patients exhibit markedly diminished IgG and IgA with normal increase in IgM serum level. CD154 gene sequencing is normal and its expression on activated T cell is also normal. In

PCP = pneumocystic carinii pneumonia

IL = interleukin

TNF- $\alpha$  = tumor necrosis factor- $\alpha$

contrast to HIGM1, B cells disclose an intrinsic defect in isotype switching.

Using polymorphic microsatellite markers in consanguineous or multiplex families, a region of the short arm of chromosome 12p13 was found to be associated with HIGM2. A gene coding for a novel molecule, activation-induced cytidine deaminase (AID), was located in this region. All patients with HIGM2 were found to have mutations in this gene [28], which is essential in several crucial steps of B cell terminal differentiation necessary for efficient antibody response. The phenotype of the AID  $-/-$  mice has been shown to be identical to the one observed in HIGM2 patients.

A third HIGM syndrome (HIGM3) has been described in three patients with autosomal recessive forms who failed to express CD40 on the cell surface. Different mutations in the gene encoded CD40 were found [29]. The molecular basis of another form of HIGM syndrome, X-linked hypohydrotic ectodermal dysplasia, was recently delineated [30] as being secondary to missense mutation affecting the zinc-finger domain of the NF- $\kappa$ B essential modulator (NEMO). All patients present with life-threatening infections, including bacterial, opportunistic and microbacterial infections. Low serum IgG with hyper IgM levels and defective antibody response were noted. The B cell abnormality is related to impaired NF- $\kappa$ B signaling following CD40-mediated activation. The B cell defect observed in these patients is similar to the phenotype observed in mice deficient for NF- $\kappa$ B, thus giving evidence for the role of NF- $\kappa$ B in CD40-mediated B cell activation and immunoglobulin class switching.

## References

- Grunbaum E. Agammaglobulinemia caused by defects other than BTK. *Immunol Allergy Clin North Am* 2001;21:45–64.
- Minegishi Y, Rohrer J, Conley ME. Recent progress in diagnosis and treatment of patients with defects in early B-cell development. *Curr Opin Pediatr* 1999;11:528–32.
- Smith CIE, Witte ON. X-linked agammaglobulinemia: a disease of BTK tyrosine kinase. In: Ochs HD, Smith CIE, Puck JM, eds. Primary Immunodeficiency Diseases. Oxford, UK: Oxford University Press, 1999:263–84.
- Vetrie D, Vorechowsky I, Sideras P, et al. The gene involved in x-linked agammaglobulinemia is a member of the Src family of protein-tyrosine kinase. *Nature* 1993;361:226–33.
- Tsukada S, Saffran DC, Rawlings DJ, et al. Deficient expression of a B-cell cytoplasmic tyrosine kinase in human x-linked agammaglobulinemia. *Cell* 1993;72:279–90.
- Gaspar HB, Kinnon C. X-linked agammaglobulinemia. *Immunol Allergy Clin North Am* 2001;21:23–44.
- Stewart DM, Tian L, Nelson DL. A case of x-linked agammaglobulinemia diagnosed in adulthood. *Clin Immunol* 2001;99:94–9.
- Conley ME, Rohrer J, Rapalus L, Boylin EL, Minegishi Y. Defects in early B-cell development: comparing the consequences of abnormalities in pre BCR signaling in the human and the mouse. *Immunol Rev* 2000;178:75–90.
- Wood PMD, Mayne A, Jouce H, Smith CIE, Granoff DM, Kumararatu DS. A mutation in Bruton's tyrosine kinase as a cause of selective antipolysaccharide antibody deficiency. *J Pediatr* 2001;139:148–51.
- Amedei A, Romagnani C, Benagiano M, et al. Preferential Th1 profile of T helper cell response in x-linked (Bruton's) agammaglobulinemia. *Eur J Immunol* 2001;31:1927–34.
- Compana D, Farrant J, Inamdar N, Webster ADB, Janossy G. Phenotypic features and proliferative activity of B-cell progenitors in x-linked agammaglobulinemia. *J Immunol* 1999;145:1675–80.
- Conley ME, Notarangelo LD, Etzioni A. Diagnostic criteria for primary immunodeficiencies. *Clin Immunol* 1999;93:190–8.
- Futani T, Watanabe C, Baba Y, Tsukada S, Ochs HD. Bruton's tyrosine kinase is present in normal platelets and its absence identified patients with x-linked agammaglobulinemia and carrier females. *Br J Haematol* 2001;114:141–9.
- Li T, Rawlings DJ, Park H, et al. Constitutive membrane association potentiates activation of Bruton's tyrosine kinase. *Oncogene* 1997;15:1375–82.
- Khan WN. Regulation of B lymphocyte development and activation by Bruton's tyrosine kinase. *Immunol Res* 2001;23:147–56.
- Vihiran M, Arredondo-Vega F, Casanova JL, et al. Primary immunodeficiency mutation databases. *Adv Genet* 2001;43:103–88.
- Thomas JD, Sideras P, Smith CIE, Vorechowsky I, Chapman V, Paul WE. A missense mutation in the x-linked agammaglobulinemia gene colocalized with the mouse x-linked immunodeficiency gene. *Science* 1993;261:355–8.
- Thampakkul S, Ballou M. Replacement intravenous immune serum globulin therapy in patients with antibody immune deficiency. *Immunol Allergy Clin North Am* 2001;21:165–84.
- Notarangelo LD, Duse M, Ugazio AF. Immunodeficiency with hyper IgM. *Immunodef Rev* 1992;3:101–21.
- Aruffo A, Farrington M, Hollenbough D, et al. The CD40 ligand gp39 is defective in activated T-cells from patients with x-linked hyper IgM syndrome. *Cell* 1993;72:291–300.
- Durandy A, Honjo T. Human genetic defects in class switch recombination (Hyper-IgM syndromes). *Curr Opin Immunol* 2001;13:543–8.
- Levy J, Espanol-Basen T, Thomas C, et al. Clinical spectrum of X-linked hyper IgM syndrome. *J Pediatr* 1997;131:47–54.
- Bonilla FA, Geha RS. CD154 deficiency and related syndromes. *Immunol Allergy Clin North Am* 2001;21:65–89.
- Khawaja K, Gennery AR, Flood TJ, Abinum A, Cant AJ. Bone marrow transplantation for CD40 ligand deficiency: a single centre experience. *Arch Dis Child* 2001;84:509–11.
- Hadzic N, Pagliaca A, Rela M, et al. Correction of the hyper IgM syndrome after liver and bone marrow transplantation. *N Engl J Med* 2001;342:320–4.
- Notarangelo LD, Hayward AR. X-linked immunodeficiency with hyper IgM (XHIM). *Clin Exp Immunol* 2000;120:399–405.
- Jain A, Akinson TP, Lipsky PE, Slotter JE, Nelson DL, Strober W. Defects of T-cell effector function and post thymic maturation in x-linked hyper IgM syndrome. *J Clin Invest* 1999;103:1151–8.
- Revy P, Muto T, Levy Y, Geissmann F, et al. Activation Induced Cytidine Deaminase (AID) deficiency causes the autosomal recessive form of the hyper IgM syndrome (HIGM2). *Cell* 2000;102:565–75.
- Ferrari S, Giliani S, Insalca A, et al. Mutations of CD40 gene cause an autosomal recessive form of immunodeficiency with hyper IgM. *Proc Natl Acad Sci USA* 2001;98:12614–19.
- Jain A, Ma CA, Liu S, Brown M, Cohen J, Strober W. Specific missense mutations in NEMO result in hyper IgM syndrome with hypohydrotic ectodermal dysplasia. *Nat Immunol* 2001;2:223–8.

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