The problems with the IVIG treatment and the potential treatment we could develop to cure patients with X-linked agammaglobulinemia

By Leah Xu

Introduction

X-linked agammaglobulinemia or XLA is one of the most common pediatric primary immunodeficiencies. The name of the condition is descriptive of some of its features. The mutant alleles are carried on the X chromosome with no corresponding allele on the Y chromosome, hence X-linked, while the main symptom of the disease is the absence of immunoglobulin in the serum (agammaglobulinemia). Infants with the disease are usually identified as a result of recurrent infections with pyogenic bacteria and enteroviruses. Although accurate estimates of the incidence and prevalence of XLA are difficult to obtain, it is estimated to affect 1 in 190,000 male births or 1 in 379,000 live births (Winkelstein *et al.*, 2006; Suri, Rawat and Singh, 2016). The first description of immunodeficiency disease was Colonel Ogden Bruton's account, in 1952, of the failure of a male child to produce antibodies. He successfully treated the patient with injection of immunoglobulin as a form of replacement therapy.

Now, 70 years later, the treatment for XLA has remained largely unchanged. There have been no major advancements aside from the switch to intravenous and subcutaneous immunoglobulin therapy (IVIG/SCIG) in the early 1980s (Shillitoe and Gennery, 2017). However, there are still lots of problems with current treatment. Patient need to go to hospital once a week and they require lifelong immunoglobulin replacement therapy for survival (Suri, Rawat and Singh, 2016). The lack of IgA and IgM in the IVIG formulation also leads to increased risk of recurrent sinopulmonary tract infections and bronchiectasis (Shillitoe and Gennery, 2017). This highlights the need for alternative treatment options that circumvent shortcomings of IVIG and improve patient outcomes.

Genetic basis

The defective gene in XLA encodes a protein tyrosine kinase called Bruton's tyrosine kinase (BTK), which is a member of the Tec family of kinases, that transduces signals through the pre-B receptor. In the absence of BTK function, B cell growth and differentiation is partially blocked between the pro-B-cell and pre-B-cell stages, which means the patient has a reduced number of pre-B-cells in the bone marrow (Ochs and Smith, 1996). Furthermore, B-cell maturation is largely arrested at the pre-B cell stage (Murphy *et al.*, 2017, see Figure 1). As a result, there is a failure of B cell development and affected patients have significantly reduced levels (<1 %) of mature B lymphocytes in their peripheral blood circulation (Ochs and Smith, 1996). The lack of B cells in XLA patients will result in the loss of its function, rendering patients unable to produce antibody which would bind pathogens when there is a pathogen invasion.

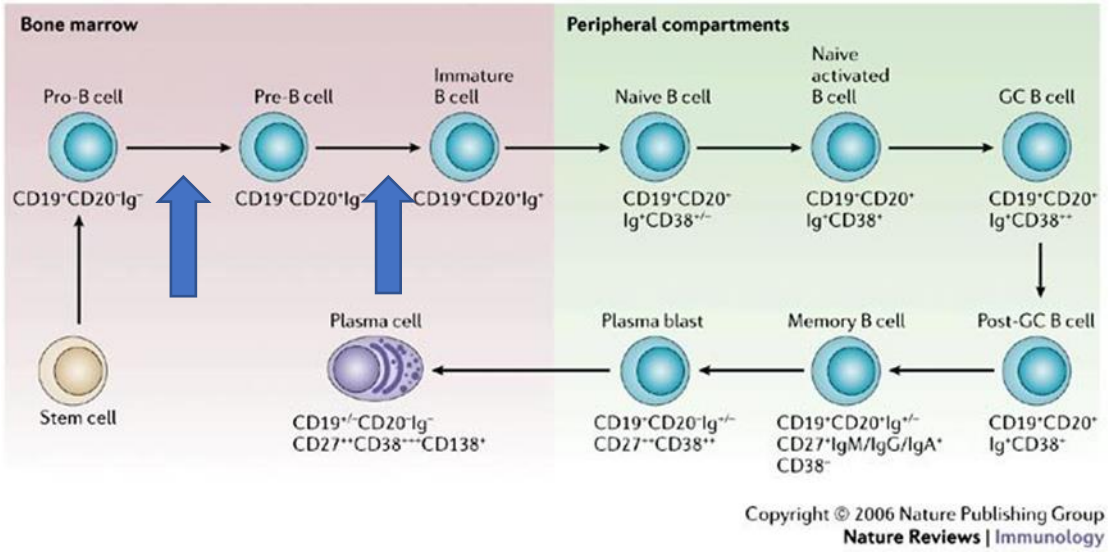

Figure 1: *the mutant BTK gene means there is no signal transduced to pre-B-cell receptor, so that interrupting the transition from pre-B cell to immature B cell. (adapted from (Murphy et al., 2017)). Blue arrows indicate the developmental blockage of B-cells, which is showed in the diagram below.*

Symptoms of XLA

XLA can be either familial or a result of a *de novo* mutation. Diagnosis is therefore either made based on family history or certain signs and symptoms. Generally speaking, the diagnosis of agammaglobulinemia is made at a mean age of 2.5 years among familial cases compared to 3.5 years among non-familial cases (Howard M. Lederman and Jerry A. Winkelstein, 1985). On physical evaluation, there is evidence of growth failure, for example, the absent or atrophied tonsils and lymph nodes are often the most important clinical clue in clinching the diagnosis. Even so, the most common symptoms are still an increased frequency of infections.

Respiratory symptoms

Among all the symptoms, recurrent bacterial infections of the respiratory tract are the most common in XLA. This may manifest in either upper or lower respiratory infections, like otitis media, sinusitis, bronchitis and pneumonia and septicemia (Conley and Howard, 2002). Additional signs of XLA may include complications of the underlying respiratory tract infection and result in postnasal discharge, tympanic membrane perforation, or conjunctivitis (Suri, Rawat and Singh, 2016). Of the 73 XLA patients in Italy, 37 episodes of pneumonia were reported, requiring hospitalization over an average follow-up of 7 years (Plebani *et al.*, 2002). These infections are usually caused by encapsulated pyogenic bacteria (Howard M. Lederman and Jerry A. Winkelstein, 1985).

Gastrointestinal manifestations

Gastrointestinal manifestations are also a frequent complication in XLA patients. An analysis of the United States Immune Deficiency Network (USIDNet) registry reported 35% of XLA patients suffered from gastrointestinal complications ranging from recurrent infections to inflammatory bowel disease (Barmettler *et al.*, 2017). These

symptoms are usually caused by *Giardia lamblia*, which is frequently isolated from stool samples from these patients, and sometimes its eradication may become difficult, resulting in chronic diarrhea and malabsorption. *Campylobacter jejuni* is also a pathogen implicated in causing gastrointestinal manifestations as well as bacteremia and skin lesions (P.J.S.M. Kerstens *et al.*, 1992). The types of pathogens and the age profile of these patients would normally alert doctors about the possibility of an immunosuppressed patient.

Treatment

Current treatment and problems

The current treatment for XLA is immunoglobulin replacement therapy. Although it had some slight changes in the past 70 years, fundamentally the treatment remains unchanged since the first patient described by Colonel Bruton in 1952. Originally, XLA patients injected immunoglobulin through a way called intramuscular injections (IMIG). This was superseded by intravenous immunoglobulin (IVIG) in the early 1980s. Compared with the previous method, IVIG is less painful and allows larger volumes to be administered, which leads to higher IgG injection levels (Garbett, Currie and Cole, 1989). Therefore, there will be a dramatic reduction in invasive infection rates, leading to improved survival outcomes. Statistics about the efficacy of treatment in XLA are lacking. There is, however, a disease similar to XLA, called common variable immune deficiency (CVID), also associated with reduced Ig levels, and patients with both kinds of deficiency depend on IVIG treatment. This treatment, in a large Italian cohort of CVID patients, reduced the prevalence of pneumonia from 49.0% to 20.5% (Quinti *et al.*, 2007). An emerging route of administration is via subcutaneous injections (SCIG), which may prove less of a burden to patients and produce more stable IgG levels, possibly improving clinical outcomes. However, SCIG infusion was limited by the slow infusion rate, although this has improved over the years (Maarschalk-Ellerbroek, Hoepelman and Ellerbroek, 2011). Another issue besides appropriate injection rate for patients is the fact that the immunoglobulin preparation used in the therapy is a blood derived product that is pooled from many donations. As a result, Ig products must undergo a number of initial screening and additional virus inactivation procedures to make sure the risk of infections completely excluded (Rütter, 1994). With improved production protocols, the previous risks of contamination with blood borne viruses have dramatically fallen.

Despite the advances in immunoglobulin therapy, there remain obstacles. Usually, there is a lack of IgM and IgA in the formulation of immunoglobulin replacement. There are five Ig isotypes in our human, each of them has a different function. IgA can be found in the bloodstream, but it mainly acts in the defense of mucosal surfaces while IgM is the class of antibody most efficient at activating complement on microbial surfaces immediately after infection and leading to the clearance of bacteria before they become dangerous. However, most immunoglobulin products continue to be IgA and IgM deplete because there are still some problems with injection of IgM and IgA. IgM is removed because it can rapidly form complexes leading to serious adverse events while most products are IgA deplete as patients can develop anti-IgA antibodies from the IgG they have injected in and will provoke serious anaphylactic

reaction to IgA injected in to patient's blood (Maarschalk-Ellerbroek, Hoepelman and Ellerbroek, 2011). Still, injection of IgM and IgA can benefit patients by reducing infections. Kiani-Alikhan and coworkers' case report of a CVID patient receiving intravenous infusion of high IgA (6 mg/mL) and IgM (6 mg/mL)-containing immunoglobulin demonstrated a marked decrease in her rate of compared with when she was only on another IVIG product (Kiani-Alikhan *et al.*, 2012). Indeed, without sufficient replacement of these two isotypes, patients are much more likely to experience frequent mucosal infections and consequently increases the risk of serious complications such as bronchiectasis (Baumann, Miescher and Vonarburg, 2014). Recent work from Hodkinson et al. shows that patients with a low IgA and IgM are at increased risk of bronchiectasis. This population had a prevalence of 48% compared to 33% in patients with normal IgA and/or IgM (Hodkinson *et al.*, 2017). Clearly though, for the time being, the medical community regards the negative effects of IgM and IgA injection to outweigh the benefits. Recent years have not seen significant developments in this area, making it less likely that current practice would change.

Future treatment strategies

As the world is developing, there is an increasing number of new techniques coming forth in modern medicine. As a result, many complex diseases have been solved. This provides hope for alternative treatment options that could overcome the weakness of IVIG and improve patient outcomes.

Preimplantation genetic diagnosis (PGD)

For those people who have familial cases, instead of having a child with XLA and then cure him with IVIG, it is always better to prevent that child born with a defective BTK gene. As a result, sophisticated ways of genetically screening an early embryo before it is implanted in the uterus have been introduced. This is called preimplantation genetic diagnosis and is based on the technique of *in vitro* fertilization (IVF).

In this technique, the egg is fertilized outside the body. After a few cell divisions, a single cell is removed from each embryo to check its genetic make-up. As a result, only those embryos free of the defective Bruton's tyrosine kinase gene are placed in the mother's uterus to implant and grow. This removes the faulty allele from the gene pool. Amazingly, all the evidence so far suggests that this causes no harm to the embryo. Consequently, people with familial cases can try this method, and this may be the chance to overcome the familial inheritance.

There is limited data available on the use of IVF and PGD in XLA prevention, there are, however, some publications that demonstrate the potential of IVF and PGD. First, in 1990, PDG was introduced by selecting female embryos in order to prevent the birth of male patients affected with X-linked recessive disorders (Chen *et al.*, 2018). 2009 saw the first report on the feasibility of the approach at the prevention of the disease. In this work a 32 years old woman carrying the BTK gene mutation conducted IVF together with PGD for prevention of XLA. Six embryos in total were determined to be normal, although ultimately the woman did not successfully conceive (Xu *et al.*, 2009). Embryo culture techniques have also shown significant improvement. For instance, in

2004, 36.6% of women younger than 35 years of age undergoing IVF had a live birth after being implanted with, on average, 2.5 embryos per cycle. In addition, 32.7% of the women delivered twins and 4.9% delivered triplets (Eskew and Jungheim, 2017). Although there are not too many people using this treatment, the data we already have for the past 30 years demonstrates that this technique is becoming much more mature than it used to be. Overall, preimplantation genetic diagnosis is a feasible scheme in preventing XLA.

Stem Cell Transplantation (SCT)

In addition to those with a familial history of the disease, there are patients whose XLA is caused by a *de novo* mutation of BTK. As a result, their illnesses are unpredictable for the families which means they could not use preimplantation genetic diagnosis to prevent the occurrence of XLA. In this situation, stem cell transplantation is emerging as a promising possibility of lifetime cure for these patients, foregoing the need for long-term immunoglobulin therapy and eliminating the risks brought on by recurrent infections.

As early as 2003, some people began to try this method to treat patients with XLA. Howard et al. reported the first case series of using SCT for six XLA patients. Two of these patients have developed normal sized, nontender cervical lymph nodes 3 to 12 months after transplant but none of the three patients have shown an increase in serum IgM or an increase in the number of peripheral blood B cells (Howard *et al.*, 2003).

Recently, colleagues in Japan have used this experience to successfully transplant an XLA patient using a reduced intensity conditioning regimen (Oncol, 2016). Before the SCT, the level of IgG was normal because of IVIG while that of IgA and IgM were undetectable. The decrease in IgG level at first 100 days was probably because of the discontinuation of IgG replacement. Fortunately, this patient finally shows the normal levels of immunoglobulin within 500 days after the SCT (Figure 2).

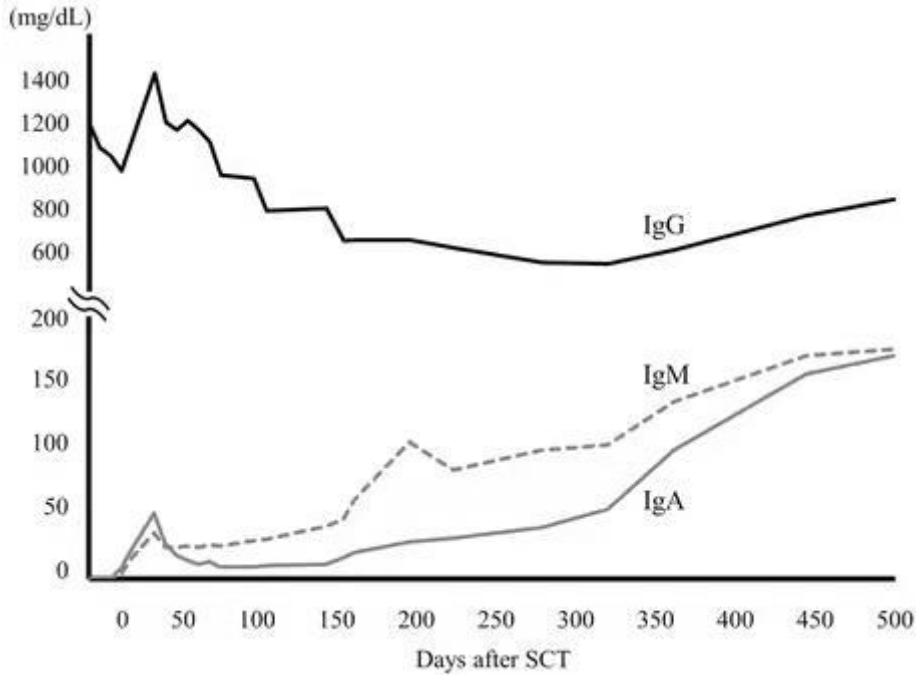

Figure 2: *the variation of blood Ig level with time after SCT. Adapted from* (Oncol, 2016)

Although this patient is only an individual case now, it gives us a right direction and confidence that we can manage a radical cure of the XLA, and that could be what scientists could work on in the future.

Conclusion

In the developing world, the prognosis for XLA has improved dramatically in recent years as the result of early diagnosis, and regular replacement with immunoglobulin. Children with XLA are now surviving into adulthood (Suri, Rawat and Singh, 2016). However, current treatment cannot fix the lost functions of B lymphocytes caused by BTK deficiency. Although the IVIG reduced the consequent infections but many patients finally develop the recurrent infections into serious bronchiectasis and the impact on mortality cannot be overlooked. Nevertheless, scientists are still trying to find new therapies which can solve the current problems.

Whether using preimplantation genetic diagnosis and IVF to prevent the occurrences of XLA among newborns or a potential cure through gene therapy and Stem Cell Transplantation, they all provide feasible future alternatives to current management. Considering the low success rate and immature technology of these new approaches, there is still a large amount of work needed before the application of them in modern medicine.

Bibliography

Barmettler, S. *et al.* (2017) “Gastrointestinal Manifestations in X-linked Agammaglobulinemia,” *Journal of Clinical Immunology*, 37(3), pp. 287–294. doi: 10.1007/s10875-017-0374-x.

Baumann, U., Miescher, S. and Vonarburg, C. (2014) "Immunoglobulin replacement therapy in antibody deficiency syndromes: Are we really doing enough?," *Clinical and Experimental Immunology*, 178(S1), pp. 83–85. doi: 10.1111/cei.12521.
Chen, H. F. *et al.* (2018) "Preimplantation genetic diagnosis and screening: Current status and future challenges," *Journal of the Formosan Medical Association*. Elsevier B.V., pp. 94–100. doi: 10.1016/j.jfma.2017.08.006.
Conley, M. E. and Howard, V. (2002) *clinical findings leading to the diagnosis of X-linked agammaglobulinemia*. Available at: www.mosby.com/jpeds.
Eskew, A. M. and Jungheim, E. S. (2017) *A History of Developments to Improve in vitro Ferti lizati on*. Available at: www.fertility.wustl.edu.
Garbett, N. D., Currie, D. C. and Cole, P. J. (1989) "Comparison of the clinical efficacy and safety of an intramuscular and an intravenous immunoglobulin preparation for replacement therapy in idiopathic adult onset panhypogammaglobulinaemia," *Clinical and experimental immunology*, 76(1), pp. 1–7.
Hodkinson, J. P. *et al.* (2017) "Low IgA and IgM Is Associated with a Higher Prevalence of Bronchiectasis in Primary Antibody Deficiency," *Journal of Clinical Immunology*, pp. 1–3. doi: 10.1007/s10875-017-0381-y.
Howard M. Lederman and Jerry A. Winkelstein (1985) "X-Linked Agammaglobulinemia: An Analysis of 96 Patients."
Howard, V. *et al.* (2003) "Stem cell transplants for patients with X-linked agammaglobulinemia," *Clinical Immunology*, 107(2), pp. 98–102. doi: 10.1016/S1521-6616(03)00045-7.
Kiani-Alikhan, S. *et al.* (2012) "Immunoglobulin replacement therapy: Is there a role for IgA and IgM?," *Journal of Allergy and Clinical Immunology*, pp. 553–554. doi: 10.1016/j.jaci.2012.04.032.
Maarschalk-Ellerbroek, L. J., Hoepelman, I. M. and Ellerbroek, P. M. (2011) "Immunoglobulin treatment in primary antibody deficiency," *International Journal of Antimicrobial Agents*. Elsevier B.V., pp. 396–404. doi: 10.1016/j.ijantimicag.2010.11.027.
Murphy, K. (Kenneth M.) *et al.* (2017) *Janeway's immunobiology*.
Ochs, H. D. and Smith, C. I. E. (1996) "X_Linked_Agammaglobulinemia_A_Clinical_and.1."
Oncol, H. (2016) "Allogeneic stem cell transplantation for X-linked agammaglobulinemia using reduced intensity conditioning as a model of the reconstitution of humoral immunity," 14. doi: 10.1186/s13045-016-0240-y.
P.J.S.M. Kerstens *et al.* (1992) "Erysipelas-Like Skin Lesions Associated with Campylobacter jejuni Septicemia in Patients With Hypogammaglobulinemia."
Plebani, A. *et al.* (2002) "Clinical, immunological, and molecular analysis in a large cohort of patients with X-linked agammaglobulinemia: An Italian Multicenter Study," *Clinical Immunology*, 104(3), pp. 221–230. doi: 10.1006/clim.2002.5241.
Quinti, I. *et al.* (2007) "Long-term follow-up and outcome of a large cohort of patients with common variable immunodeficiency," *Journal of Clinical Immunology*, 27(3), pp. 308–316. doi: 10.1007/s10875-007-9075-1.
Rütter, G. H. (1994) "Requirements for safety and quality of intravenous immunoglobulin G preparations," *Journal of Neurology, Neurosurgery and Psychiatry*, 57(SUPPL), pp. 2–5. doi: 10.1136/jnnp.57.Suppl.2.

Shillitoe, B. and Gennery, A. (2017) "X-Linked Agammaglobulinaemia: Outcomes in the modern era," *Clinical Immunology*. Academic Press Inc., pp. 54–62. doi: 10.1016/j.clim.2017.07.008.

Suri, D., Rawat, A. and Singh, S. (2016) "X-linked Agammaglobulinemia," *Indian Journal of Pediatrics*. Springer India, pp. 331–337. doi: 10.1007/s12098-015-2024-8.

Winkelstein, J. A. *et al.* (2006) "X-linked agammaglobulinemia: Report on a United States registry of 201 patients," *Medicine*, 85(4), pp. 193–202. doi: 10.1097/01.md.0000229482.27398.ad.

Xu, C. *et al.* (2009) "Preimplantation genetic diagnosis for X-linked agammaglobulinemia: a case report," *Fertility and Sterility*, 91(5), pp. 1958.e5-1958.e7. doi: 10.1016/j.fertnstert.2009.01.093.