X-linked Agammaglobulinemia With Normal Immunoglobulin and Near-Normal Vaccine Seroconversion

Kahn Preece, BSc, MBBS, FRACP[a], Graeme Lear, BSc, FRCPCH, FRACP[b]

abstract

We present a 22-month-old boy with X-linked agammaglobulinemia masked by normal immunoglobulin levels and vaccine seroconversion. Diagnosis was made after strong clinical suspicion of immune deficiency led to identification of markedly reduced B-cell numbers and confirmation with identification of a novel Bruton tyrosine kinase gene mutation. He was commenced on replacement immunoglobulin therapy with excellent clinical improvement. This case highlights the variability of phenotypic presentation and apparent disunity between routine immunologic investigations and severe disease in X-linked agammaglobulinemia, necessitating clinical acumen to make the diagnosis.

Presentation of immunodeficiency conditions varies widely. Lists of "red flags" or "warning signs" have been developed and promoted to assist clinicians.[1] Unfortunately, these tools have limitations (inadequate sensitivity, specificity, negative and positive predictive value[2]) and we are left with clinical suspicion to drive further investigation. Disorders of antibody production are generally considered to be caused by defects in B-cell production and/or function, which occasionally are a consequence of defective T-cell help. It is often considered that a hallmark of these conditions is a defect in production of normal amounts of antigen-specific antibodies.[3]

X-linked agammaglobulinemia (XLA) (OMIM # 300755) is a humoral immunodeficiency classically characterized by defective B-cell development with extremely reduced numbers of mature B cells causing a severe hypogammaglobulinemia.[4] This condition was first described in 1952 by Col Ogden Bruton,[5] giving rise to the alternate name of Bruton agammaglobulinemia. Mutation of the *BTK* (Bruton tyrosine kinase) gene was identified in 1993 as the causative defect.[6] Since this time, more than 800 mutations have been identified spanning the length of the BTK gene.[7]

The case was a boy born at 35 weeks' gestation weighing 2320 g after an unremarkable pregnancy with poor antenatal attendance and exposure to maternal smoking. Apart from mild jaundice needing phototherapy, there were no neonatal problems. He was healthy and thrived over the first 6 months of life. At 7 months he was admitted with cough and fever with small ulcers noted on his lips. Investigations showed bilateral lung infiltrates on chest x-rays, profound neutropenia on blood count, and C-reactive protein of 222 mg/L (Normal 0–8 mg/L). He was also found to be iron deficient. Viral and bacterial cultures were negative and he was treated empirically with broad-spectrum antibiotics, making a good recovery. His neutrophil count fluctuated over the next 2 months (Fig 1). Initially, an autoimmune phenomenon was thought to be the cause in view of the normal neonatal blood count.

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Dr Preece assisted with the draft of the initial manuscript and prepared the final manuscript as submitted, including figures and tables; and Dr Lear assisted with the initial draft and reviewed the revised manuscript before submission.

www.pediatrics.org/cgi/doi/10.1542/peds.2014-3907

DOI: 10.1542/peds.2014-3907

Accepted for publication Jul 30, 2015

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PEDIATRICS (ISSN Numbers: Print, 0031-4005; Online, 1098-4275).

FINANCIAL DISCLOSURE: The authors have indicated they have no financial relationships relevant to this article to disclose.

FUNDING: No external funding.

POTENTIAL CONFLICT OF INTEREST: The authors have indicated they have no potential conflicts of interest to disclose.

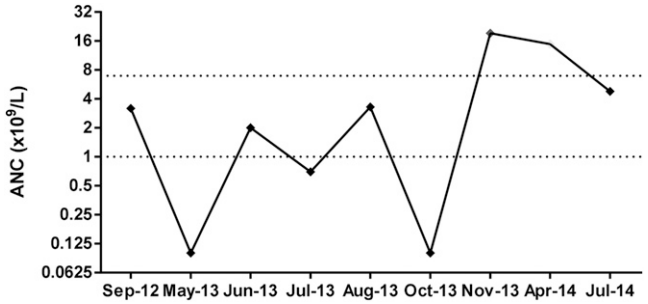

FIGURE 1
Log 2 scale of absolute neutrophil count over time demonstrating protracted low neutrophil count but generally within the normal range (dotted lines).

At 10 months of age, both neutropenia and bilateral lower lobe consolidation continued. Sputum was positive for *Haemophilus influenzae* and his stool grew *Campylobacter*. He was treated again with intravenous antibiotics and neutrophil count had normalized by discharge, 5 days later. Between 12 and 18 months old he had a series of infections, including pharyngitis (growth of *Candida* on throat swab); a 6-cm-diameter cervical lymphadenitis, associated with neutrophilia (19.3×10^9/L); cellulitis of a great toe; left lower lobe pneumonia; periorbital cellulitis with *Streptococcus pneumoniae* sepsis; Coxsackie viral infection; and rotavirus gastroenteritis.

Family history was limited due to significant social stressors and minimal contact with maternal extended family. He is an only child with no known family members previously diagnosed with immune deficiency or suffering severe childhood infections. His mother continues to smoke but is otherwise well.

Examination revealed a happy, thriving, developmentally appropriate boy who measured on the 50th to 75th percentile for both height and weight. He had a frequent, productive cough, but no peripheral signs of chronic lung disease, and normal vesicular breath sounds bilaterally on auscultation. He had generalized mild skin dryness and small cervical and inguinal lymph nodes palpated. Small tonsillar tissue was also identified in his oropharynx.

The recurrent infections and persistent neutropenia had raised the possibility of an immune deficiency. Immunoglobulin levels were first measured at 11 months of age, and were repeatedly found to be normal (eg, immunoglobulin G 7.8 g/L, IgA 0.8 g/L, IgM 0.8 g/L). After routine immunization, protective levels of IgG to *H influenza b*, *Tetanus*, and *Diphtheria* were demonstrated (Table 1) but there was no boost on revaccination. The standard vaccination schedule included 10-valent pneumococcal polysaccharide conjugate vaccine. There are no published age-dependant normal ranges for pooled pneumococcal IgG after vaccination with 10-valent pneumococcal polysaccharide conjugate vaccine, but extrapolation from limited 7-valent pneumococcal vaccine data[8] would suggest his pooled antibody response was low. This was not included during revaccination and boost was not assessed. Further investigation showed marked reduction of B cells (<2% total lymphocytes) on both peripheral blood and bone marrow lymphocyte phenotype assessment.

The patient was referred to a tertiary center for further assessment (Table 1). Genetic testing confirmed a novel 3 base-pair substitution in exon 18 (c.1906_1908GAG>TTT, p.E636F). This mutation caused an amino acid substitution (glutamate to phenylalanine) predicted to be pathogenic by polymorphism phenotyping v2.[9] Maternal carrier status was confirmed, but due to social constraints further heritage analysis and investigation was unable to be done. The combination of profound B-cell deficiency and clinical history consistent with humoral immune deficiency meant a diagnosis of XLA was made. Serum IgM has subsequently dropped to a level that is undetectable. This may represent deteriorating B-cell function or reduced infection and inflammatory drive with the introduction of replacement immunoglobulin therapy.

Intravenous immunoglobulin replacement was started at 460 mg/kg per month and has been well tolerated. Prompt treatment of sinopulmonary infections was advised but there has been a dramatic improvement in respiratory symptoms and requirement for antibiotic prescription. Subcutaneous immunoglobulin replacement was discussed but remains unacceptable to his mother for the time being.

DISCUSSION

Clinical presentation of XLA is generally typical of a primary humoral deficiency. A US registry for XLA was set up in the late 1990s and data compiled and published on 201 patients in 2008.[10] Increased susceptibility to infection was the most common initial presentation in 86%. Infections preceding diagnosis included otitis media, pneumonia, sinusitis, diarrhea, sepsis, and cellulitis, with >50% presenting by 1 year of age and almost all presenting by 5 years.[10] *Pneumocystis jiroveci* pneumonia has been seen in patients with XLA[10,11] and supports a role for opsonizing antibody in the host defense against *P jiroveci* pneumonia.[12]

Neutropenia was present in 15% of the American cohort[10] and is a known association with XLA. The mechanism of neutropenia is poorly understood.

TABLE 1 Results at Diagnosis (Age 22 Months)

	Result	Normal	Units
CBC–Hb	96	105–140	g/L
WCC	15.3	5.0–14.5	$\times 10^9$/L
Neutrophils	4.8	1.0–7.0	$\times 10^9$/L
Lymphocytes	8.3	2.0–8.0	$\times 10^9$/L
IgG	6.3	3.0–10.5	g/L
IgA	0.54	0.1–1.2	g/L
IgM	0.72	0.3–1.5	g/L
Hib IgG	0.84	>0.15[a]	mg/L
Diphtheria toxoid IgG	0.18	>0.01[a]	IU/mL
Tetanus IgG	0.77	>0.1[a]	IU/mL
Pooled pneumococcal IgG	4	—	mg/L
Lymphocyte subsets			
CD3 (T cells)	7849	1500–7500	$\times 10^6$/L
CD4 (T-helper cells)	3773	900–5000	$\times 10^6$/L
CD8 (cytotoxic T cells)	3513	400–2200	$\times 10^6$/L
CD19 (B cells)	56	600–2900	$\times 10^6$/L
CD16+56+ (NK cells)	783	200–1300	$\times 10^6$/L
Bone marrow	Hypercellular with increased granulopoiesis. Very small mature B-cell population (<0.4% of lymphocytes).		
BTK gene analysis	Three base-pair substitution (GAG > TTT) at position 1906. Amino acid substitution glutamate to phenylalanine		

CBC, complete blood count; Hb, hemoglobin; Hib, *Haemophilus influenza* type b; Ig, immunoglobulin; WCC, white cell count; —, no normal values available for pooled pneumococcal IgG after 10-valent pneumococcal vaccine.

[a] Minimum concentration considered protective to infection.

Possible mechanisms include reduced phagocytosis and chemotaxis of monocytes as well as a diminished response to lipopolysaccharide that may suggest a role of BTK in myeloid cell host defense.[13] Neutropenia is also suggested as an associated cause of increased infection susceptibility and generally improves when immunoglobulin replacement is commenced.[13,14]

The critical role of BTK in B-cell development is evident by the universal B-cell deficiency (<2%) in patients with pathogenic mutations. Leaky mutations or those near splice sites can allow for some production of wide-type protein with emergence from the bone marrow of a functional B cell.[15,16] This may account for variability in circulating antibody levels and clinical severity. Multiple studies have now demonstrated a poor genotype-phenotype correlation in this condition.[17–19] Identical mutations or those in close proximity often result in quite different clinical outcome and disease severity, even varied within a single family.[20] Some objective measures have been suggested to predict a more severe disease, such as younger age at clinical diagnosis (<13 months) and level of circulating IgM (<0.1g/L).[17] The presence of low pooled pneumococcal IgG may have provided a clue to the defective humoral immune response. A previous case of isolated selective polysaccharide antibody deficiency was described in 2001 (before routine conjugated pneumococcal vaccination).[21] This patient was diagnosed at age 25 years of age with a relatively mild course.

A limitation in this case is the lack of confirmation that the mutation identified in the BTK gene is pathogenic. The amino acid substitution is predicted pathogenic by using computer modeling but functional analysis would be useful in confirming a dysfunctional gene product. Mutations in coding regions of the BTK gene can be found in healthy individuals and are thought to be variants, even when the mutations were predicted to be deleterious.[22] The relevance of clinical findings and investigations in our patient should not be understated. B-cell deficiency and history consistent with humoral immune deficiency strongly suggests an abnormal and poorly functioning BTK protein.

In summary, XLA is a rare primary immunodeficiency that can result in severe morbidity and mortality if untreated. Early replacement of immunoglobulin, before infection-related end-organ damage, can result in an excellent prognosis.[10] In the absence of a positive family history, diagnosis requires a high index of clinical suspicion and is supported by a low or undetectable B-cell count on flow cytometry. XLA is not excluded by normal immunoglobulin levels or apparent "protective" titers of IgG to vaccination.

ABBREVIATIONS

BTK: Bruton tyrosine kinase
XLA: X-linked agammaglobulinemia

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Pediatrics 2015;136;e1621
DOI: 10.1542/peds.2014-3907 originally published online November 2, 2015;

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