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RELATO DE CASO

Immunodeficiency phenotypes in two siblings with Bloom Syndrome

Fenotipos de imunodeficiência em dois irmãos com Síndrome de Bloom

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Abstract

Bloom's Syndrome is a rare chromosomal instability disorder due to DNA repair defects. The defective gene BLM has been mapped in chromosome 15q and causes significant reduction of DNA-helicase expression in the nucleus of the cell. This enzyme is important in the DNA repair mechanisms. Patients usually have high susceptibility to development of cancer and recurrent bacterial infections.

We have evaluated the immune system of two siblings (13-year-old male and 6-year-old female) with Bloom's Syndrome who had similar susceptibility to recurrent respiratory infections but different underlying immune abnormalities. The oldest sibling has presented persistent low IgM levels and CD4+ numbers. His IgA levels decreased when he was 11 years old. The youngest has presented persistent low IgM and IgA levels and no response to polysaccharide vaccine. Her IgG levels decreased when she was 6 years old.

The different immunologic abnormalities are discussed.

Rev. bras. alerg. imunopatol. 2007; 30(1):32-35 Bloom's syndrome; recurrent infections; immunodeficiency; humoral immunity; cellular immunity.

Resumo

A síndrome de Bloom é um raro distúrbio de instabilidade cromossômica, devido a defeitos de reparo do DNA. O gene responsável BLM foi mapeado no cromossomo 15q e causa redução significante da expressão da enzima DNA-helicase no núcleo celular. Esta enzima é importante para os mecanismos de reparo do DNA. Pacientes geralmente apresentam elevada suscetibilidade para desenvolvimento de câncer e infecções bacterianas recorrentes.

Nós avaliamos o sistema imunológico de dois irmãos (menino de 13 anos e menina de 6 anos) com síndrome de Bloom, os quais tinham similar suscetibilidade para infecções respiratórias recorrentes, mas diferentes anormalidades imunológicas. O menino apresentou níveis persistentemente baixos de IgM e de células CD4+. Os níveis de IgA diminuíram quando ele tinha 11 anos. A menina apresentou níveis persistentemente baixos dos níveis de IgM e IgA, e não respondeu à vacina polissacarídica. Os níveis de IgG diminuíram quando ela tinha 6 anos.

As diferentes anormalidades imunológicas são discutidas.

Rev. bras. alerg. imunopatol. 2007; 30(1):32-35 síndrome de Bloom; infecções recorrentes; imunodeficiência, imunidade humoral; imunidade celular

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Introduction

Bloom's Syndrome (BS) is an autosomal recessive syndrome of chromosomal instability due to DNA repair defects. The BS gene has been mapped to chromosome 15q. Mutations in this gene lead to severe DNA repair abnormalities[1,2] in which spontaneous chromosomal aberrations, sister chromatids exchanges, an elevated number of mitotic chiasms and chromosomal instability have been demonstrated by cytogenetic methods[3]. These DNA abnormalities increase the risk for the development of cancer, including leukemia and lymphoma in younger patients and carcinomas of larynx, lung, esophagus, colon, breast, and cervix in adults[4]. BS patients have delayed growth, fine face and nose, mandibular hypoplasia, anteverted auricles, syndactylia, polydactylia, facial erythematous telangiectasias, sun hypersensitivity, hypercromic spots ("café au lait"), short legs, congenital crooked foot, anular pancreas, cryptochidism and testicle atrophy[3, 4, 5].

Most BS patients present with early onset recurrent infections, especially of the respiratory and gastrointestinal tracts. Sino-pulmonary infections and otitis media, caused by gram-positive or gram-negative bacteria, are common in children. Adults have an elevated risk of developing severe chronic pulmonary disease, such as chronic bronchitis, bronchiectasias and cavitary pulmonary tuberculosis[4, 5].

Different immunologic abnormalities have been described in BS patients. These include defects in cellular immunity and antibody mediated immunity. Phagocytes and the complement system usually are normal. Previous studies have showed that affected homozygote patients presented a weak cellular and humoral response after antigenic challenge[6]. T-cell abnormalities described included a depressed *in vitro* cellular immune response to phytohemagglutinin (PHA)[7] and reduced levels of CD4 cells[8]. The most common defects in antibody mediated immunity described were low levels of one or more immunoglobulins classes, especially IgM[5, 7, 8, 13-15]

We report two siblings with BS with similar susceptibilities to infections but different underlying immune abnormalities that evolved over time.

Case report

The parents of two white siblings, a 13-year-old boy (Patient A) and a 6-year-old girl (Patient B), are first degree cousins. The boy has been followed for 9 years and the girl for 4 years. Both presented recurrent infections beginning at 2 months of age (otitis media, sinusitis and pneumonia), with poor response to treatment with usual antibiotics. Both patients were hospitalized several times (about two per year) due to pneumonia and received intravenous antibiotics. -. Both had growth rate below the third percentile for weight and height, presented "café au lait" spots in the chest and abdomen, facial erythematous telangiectasias, triangularly-shaped face with fine nose (Figures 1 and 2) and delayed bone age.

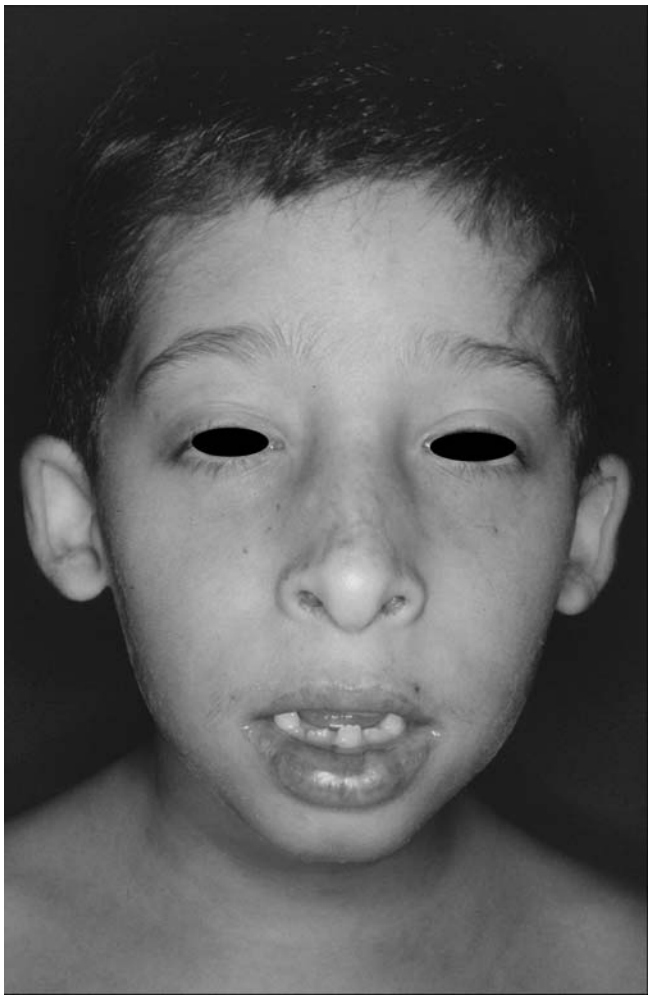

Figure 1 - Patient A. Note the triangularly-shaped face with fine nose and facial erythematous telangiectasia.

Immune evaluations were performed on both patients starting at 4 (Patient A) and 2 (Patient B) years of age due to their recurrent infections (Table I). Patient A presented persistent low levels of IgM (Table II) and CD4+ cells, with a reduced CD4/CD8 rate (Table I). IgA levels were initially normal, but have become bellow normal range to age since he was 11 years old (Table II).

Patient B presented persistent low levels of IgM and IgA (Table II). Reassessed at 5 years of age, she showed inadequate responses to polysaccharide pneumococcal vaccine in all six evaluated serotypes (Table III). IgG levels were initially normal, but have decreased since she was 6 years old (Table II). Other immunologic parameters of Patients A and B were normal (Table 1).

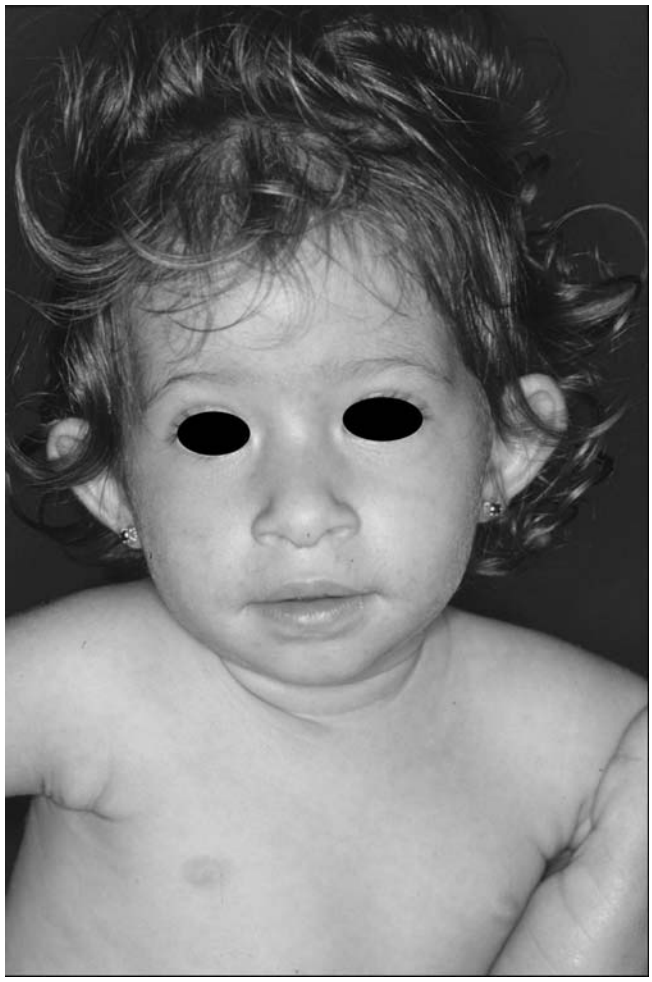

Figure 2 - Patient B. Note the triangularly-shaped face with fine nose.

Table I - Immunologic assessment in patients with BS

	PATIENT A	PATIENT B
Age	**4 yr**	**2 yr**
Sex	**Male**	**Female**
Lymphocytes/mm3	1,500 (1,500-4,500)	5,200 (1,100-3,000)
CD3+/mm3	1,200 (1,100-3,000)	2,200 (1,100-3,000)
CD4+/mm3	304 (400-1,400)	1,540 (400-1,700)
CD8+/mm3	687 (200-800)	773 (200-800)
CD4+/CD8+	0.4 (1.2-4.5)	2.0 (1.2-4.5)
NBT test	85% (>70%)	93% (>70%)
C3 (mg/dl)	130 (74-168)	133 (78-168)
C4 (mg/dl)	15.6 (11-38)	12 (11-38)

Table II - Serum immunoglobulin levels in patients with BS at different ages

PATIENT/AGES	A			B		
Immunoglobulins	4 yr	11 yr	13 yr	2 yr	5 yr	6 yr
IgG (mg/dl)	696 (564-1,318)	764 (739-1,465)	859 (680-1,611)	598 (540-1,116)	572 (564-1,318)	413 (665-1,465)
IgM (mg/dl)	39 (58-176)	39 (65-134)	26 (46-152)	26 43-194)	39 (59-166)	33 (49-218)
IgA (mg/dl)	63 (28-215)	55 (113-248)	65 (113-254)	10 (11-192)	33 (50-191)	32 (47-267)

Table III - Pre and postimmunization levels of antipneumococcal polysaccharide antibodies in patients with BS

PATIENT	A		B	
Serotypes*	Pre	Post	Pre	Post
1 (µg/ml)	1.1	1.5	0.20	0.20
3 (µg/ml)	0.9	1.7	0.47	0.51
5 (µg/ml)	1.3	1.4	0.37	0.32
6B (µg/ml)	1.2	1.9	0.63	0.64
9V (µg/ml)	1.6	1.7	0.14	0.12
14 (µg/ml)	1.9	2.8	0.85	0.90

* An adequate response was defined as a value of postimmunization specific IgG equal or greater than 1.3 µg/ml and/or an increase of at least four-fold in postimmunization levels as compared to baseline (preimmunization) levels, for at least 70% of the analyzed serotypes (15, 17).

The cytogenetic analysis, performed at 4 (Patient A) and 2 (Patient B) years of age, showed a high number of spontaneous chromosome breakage in lymphocytes. Sister chromatids exchanges were ten-fold higher than in normal lymphocytes, which confirmed the diagnosis of BS. Both siblings presented the same changes.

Patient A developed an abdominal Burkitt non-Hodgkin lymphoma when he was 9 years old. He was treated with chemotherapy and has been in complete remission for two years. His low levels of IgM and CD4+ cells were present before the development of the lymphoma and have remained constant after chemotherapy treatment. However, his IgA levels were normal before the development of the lymphoma and decreased since treatment. He has had no recurrent infections for the last 7 years.

Patient B was treated with Amoxicillin for three continuous months (May, June and July) each year between 3 and 5 years of age, with a significant reduction of infections during this period. However, when she was 6 years old the recurrent respiratory infections began again, especially otitis media, sinusitis and pneumonia. Due to the recurrent infections and low levels of IgG, Patient B receives intravenous immunoglobulin replacement (400mg/kg) every four weeks.

Discussion

DNA is constantly submitted to aggressive factors that can compromise its integrity. These cells have repair mechanisms that maintain genomic stability. Healthy repair mechanisms recognize damage and make repairs, maintain cellular checkpoints and apoptosis. Damaged cells are removed by this method. People with DNA repair mechanism defects can present different phenotypes, such as predisposition to cancer, neurological degeneration, growth retardation and immunodeficiency[1]. Lymphocytes of BS patients present many spontaneous chromosomal breakages, with sister chromatids exchanges ten-fold higher than normal[3]. Patients A and B presented clinical and cytogenetic features compatible to BS. However, they did not present neurological degeneration.

BS patients frequently present high susceptibility to respiratory infections, especially otitis media, sinusitis and pneumonia, with onset in the first months of life. The main agents involved are capsulated bacteria[5, 10]. Association between BS and immunodeficiency occurs on a regular basis, but not in all cases[11, 12].

Patient A presented a suggestive history of immunodeficiency, with recurrent respiratory infections, poor response to treatment using the usual antibiotics and several hospitalizations. He presented persistent low levels of IgM, what was observed in other studies[1, 8, 13]. Normal levels of Patient A's IgA were observed but decreased at a later time when compared to the normal levels according to age. It could be that this finding had occurred due to the chemotherapy treatment. Low levels of CD4+ cells observed in this patient have also been reported by other authors. Van Kerckhove *et al*[8] assessed the immunity of four BS patients and observed reduced levels of CD4+ cells in two of them. T- lymphocytes responded normally to phytohemmaglutinin (PHA) and concanavalin A (CON-A). Our patient also presented low levels of CD4+ cells in spite of normal responses to PHA and CON-A. These findings suggest no relationship between T- cell numbers and function. Etzioni *et al*[14] assessed one BS patient with bacterial and fungal recurrent infections and observed normal T-lymphocyte subpopulations. I*n vitro* lymphoproliferative responses to mitogens were reported in spite of a defective regulatory T-cell function for the generation of IgG.

Patient B presented a history suggestive of immunodeficiency, with recurrent respiratory infections and some hospitalizations due to infections. This patient presented persistent low levels of IgM and IgA, like her brother. Similar results were observed by other authors. Kondo *et al*[13] assessed two BS patients over a 10 year period and observed low levels of IgM and mildly reduced levels of IgG and IgA that became normal with age. IgM levels remained low. Van Kerckhove *et al*[8] observed reduction of at least one immunoglobulin class in three of the four patients studied and an absence of *in vitro* pokeweed mitogen (PWM) inducing IgM production in two of three patients. Patient B had borderline levels of IgG which decreased when she was 6 years old. At this time, recurrent respiratory infections had begun again, probably due to the IgG reduction. Interestingly, humoral immunity studies in BS have shown divergent results. Weemaes *et al*[9] assessed five BS patients and showed reduced levels of IgG, IgA and IgM. They observed an increase of only IgA with age. This did not occur in IgG

and IgM. In contrast, Etzioni *et al*[14] observed reduced levels of IgG and elevated levels of IgM.

An important aspect of the immunologic assessment in Patient B was the inadequate anti-pneumococcal antibody production in all six studied serotypes. This patient was immunizated with polysaccharide pneumococcal vaccine when she was five years old. An adequate response to pneumococcal vaccination has been defined as an absolute value of postimmunization specific IgG equal or greater than 1.3 µg/ml and/or an increase of at least four-fold in postimmunization levels as compared to baseline (preimmunization) levels, for at least 70% of the analyzed serotypes[15, 17]. Patient B presents specific antibody deficiencies that can also justify her recurrent respiratory infections. We did not find reports in the literature of specific antibody deficiencies to pneumococcal polysaccharides associated with BS. Probably this immunologic abnormality is related to the number and severity of infections and the need for IgG replacement therapy. Weemaes *et al*[18] assessed the secondary response to diphtheria, tetanus toxoids and poliomyelitis vaccines and did not find abnormalities in BS patients.

Different immunologic phenotypes in siblings were described by Antonio *et al*[7] who assessed two BS siblings with different clinic phenotypes. One of them presented recurrent respiratory and intestinal infections and the other presented neuroblastoma. Both siblings presented low levels of IgG, IgM and IgA and normal numbers of CD3, CD4 and CD8 cells. *In vitro* proliferative response to PHA was reduced only in the second patient. Both patients presented the same DNA abnormalities, as observed in our patients. It is possible that our patients have a different mutation in each of their two BLM genes and that a sister-chromatid exchange had separated these, hence producing a "healed" gene and a gene with two mutations, as reported by Woods[19].

We conclude that BS patients with recurrent infections can present different patterns of immunologic abnormalities. The reason is unclear. They must always be submitted to complete immunologic assessment, anticipating the diagnosis and improving their prognosis.

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