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Case Report

**Severe Axillary Lymphadenitis After BCG Vaccination:
Alert for Primary Immunodeficiencies****Alexandra Santos^{a,b*}, Andrea Dias^a, Ana Cordeiro^a, Carolina Cordinhã^a, Sónia Lemos^b,
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The bacilli Calmette-Guérin (BCG) vaccine is administered to all newborns in countries where tuberculosis is endemic. Immunocompromised hosts, namely patients with human immunodeficiency virus infection or primary immunodeficiencies, are especially prone to serious complications from this vaccine. We report three cases of BCG disease in children with primary immunodeficiencies: one with a partial recessive interferon- γ receptor 1 deficiency, who developed BCG dissemination; and two relatives with ZAP70 deficiency, a severe combined immunodeficiency, both of whom presented with regional and distant BCG disease. All had severe axillary lymphadenitis. These clinical cases underline the importance of considering the diagnosis of immunodeficiency in a child with severe axillary lymphadenitis after BCG vaccination and of disseminated BCG disease in an immunodeficient child in the appropriate clinical setting. Moreover, BCG vaccination should be delayed in every newborn with a family history of primary immunodeficiency until the condition has been ruled out.

KEYWORDS: BCG, immunodeficiency, interferon- γ receptor 1 deficiency, tuberculosis, ZAP70 deficiency**Introduction**

Tuberculosis incidence and mortality are increasing worldwide, particularly in developing countries,¹ where infection

is often acquired in childhood. The human immunodeficiency virus (HIV) pandemic, emerging mycobacterial drug resistance and limitations of the bacilli Calmette-Guérin (BCG) vaccination are contributing factors to this increased incidence and mortality.

The World Health Organization recommends administering BCG vaccine to all infants as soon as possible after birth, in countries with a high incidence of tuberculosis.² Countries with a different epidemiological situation may choose to limit BCG vaccination to defined high-risk groups for the disease and to skin-test negative older children, besides intensifying case detection, supervised early treatment, preventive treatment and infection control measures. In Portugal and other countries where tuberculosis is endemic,

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the BCG vaccine is included in the national childhood immunization program and administered within the first days of life.

Severe adverse effects of BCG vaccination are extremely rare in immunocompetent children.³ In contrast, immunocompromised hosts such as HIV-infected individuals or those suffering a primary immunodeficiency (PID),⁴ are especially prone to complications from this vaccine and tend to be unresponsive to treatment. Therefore, the World Health Organization recommendations have recently been reviewed concerning infants at risk for HIV infection.⁵ Commonly, PID remains undiagnosed at the time of BCG vaccination and has been associated with severe complications from this vaccine.⁶⁻⁸ We report three cases of BCG disease after vaccination in children with PID.

Case Reports

Case 1

A healthy girl, the first child of first cousin parents, was born at full term. She had two healthy maternal half-brothers, aged 11- and 3-years-old. She was given the routine vaccinations in Portugal, including the BCG vaccine, in the first days of life. At the age of 3.5 months, she presented with hypovolemic shock, severe anemia, consumption coagulopathy, hepatosplenomegaly and ulcerated left axillary lymphadenitis. Thoraco-abdominal ultrasound and computed tomography scans revealed heterogeneous splenomegaly. The transfontanelar ultrasound results and bone marrow cellular evaluation were normal. Serological studies for toxoplasma, rubella virus, herpes virus, adenovirus, parvovirus, cytomegalovirus, HIV and *Treponema pallidum* were negative.

She was initially prescribed ceftriaxone (100 mg/kg/day), which was later substituted with teicoplanin (10 mg/kg/day) and fluconazole (12 mg/kg/day). She was also given red blood cells, platelets and plasma transfusions. The possibility of a giant splenic hemangioma (Kasabach-Merritt Syndrome) was put forward, supported by eco-Doppler results. Despite treatment with interferon- α and prednisolone (2 mg/kg/day), splenomegaly persisted and intermittent high temperatures (38–39°C) developed. A few weeks later, she was admitted to the intensive care unit with right pneumothorax, pneumonia with pneumatoceles,

sepsis, heart failure, worsened hepatosplenomegaly, portal hypertension and elevated liver function tests.

During diagnostic investigations, *Mycobacterium bovis* was cultured from the drainage of the left axillary lymphadenitis, bone marrow, liver (Figure 1) and skin. Despite oral treatment with rifampicin (10 mg/kg/day), isoniazid (10 mg/kg/day), pyrazinamide (30 mg/kg/day) and clarithromycin (15 mg/kg/day) at age 5.5 months, she developed generalized BCG cutaneous lesions at the age of 14 months, which were confirmed by biopsy and culture. However, clinical improvement and reduction of the lymphadenitis were observed with anti-bacillar treatment.

Initial immunological assessments encompassing serum immunoglobulins, complement, T cell and monocyte cytokine production profiles and lymphocyte subsets and proliferation tests were normal. Interleukin (IL)-12 receptor expression in CD4⁺ and CD8⁺ T cells was normal. The expression of interferon- γ receptor 1 (IFN- γ R1) was decreased in both CD4⁺ and CD8⁺ T cells (Figure 2). A homozygous I87T mutation in the *IFN- γ R1* gene was identified, clinically presenting as a partial recessive IFN- γ R1 deficiency, as previously described.¹⁹ The child received subcutaneous IFN- γ (50 μ g/m² of body area) three times a week for 10 months from the age of 8 months.

Case 2

A girl with adequate somatometry for her gestational age was born at full term. Her parents were first cousins, as well as her father's parents (Figure 3). This girl was given BCG and anti-hepatitis B virus (HBV) vaccines at birth and anti-diphtheria, tetanus, pertussis, poliomyelitis and

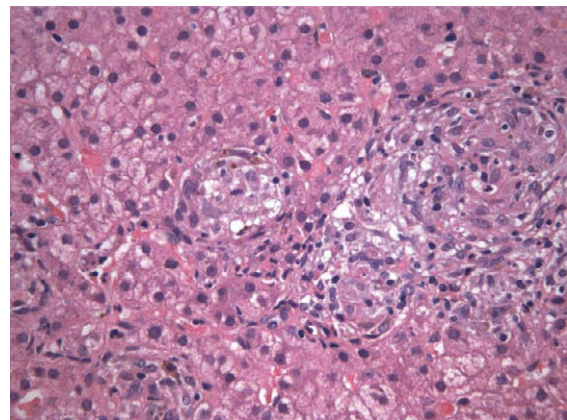


Figure 1. Liver biopsy showing hepatic granuloma (hematoxylin and eosin, original magnification, 150 \times).

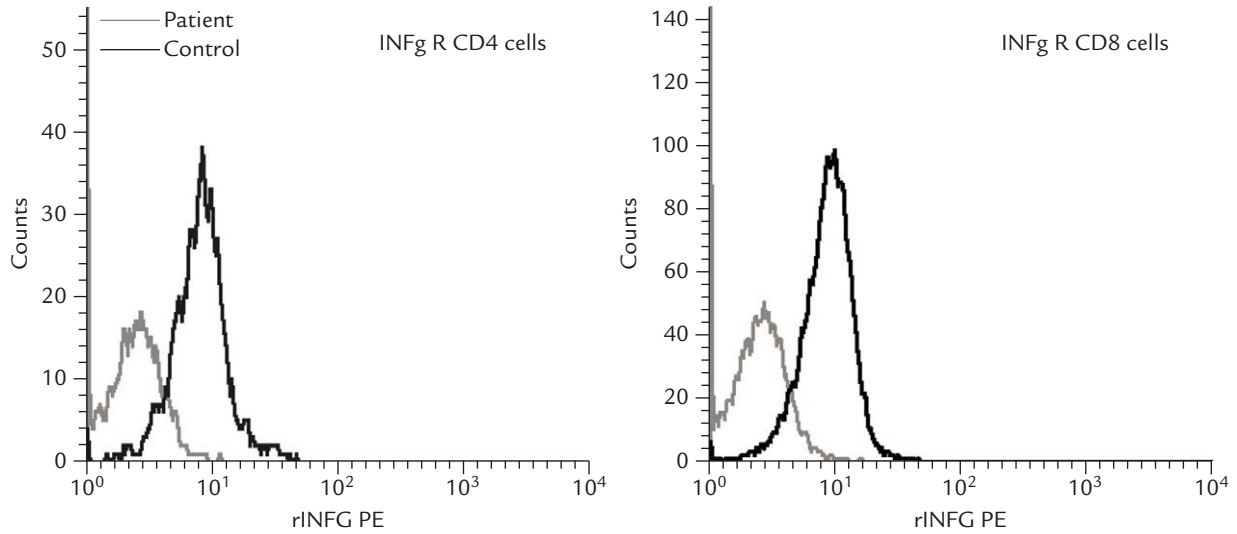


Figure 2. Decreased expression of the IFN- γ receptor on CD4⁺ and CD8⁺ T cells analyzed by flow cytometry and compared with an age-matched healthy control.

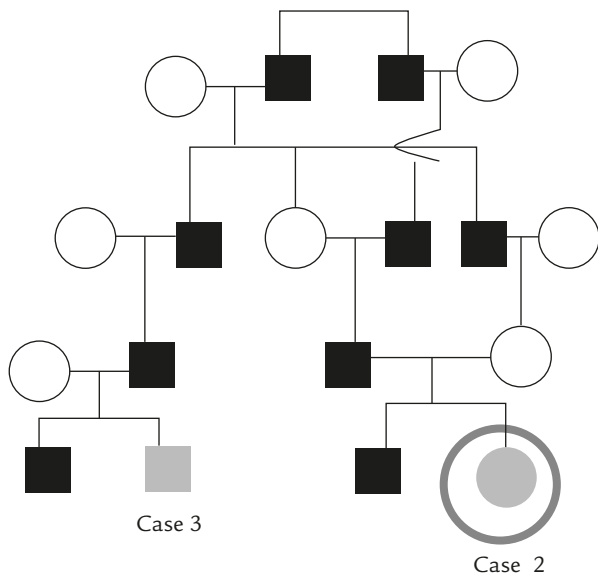


Figure 3. Family trees for cases 2 and 3.

Haemophilus influenza b vaccines along with a second dose of anti-HBV vaccine 2 months later.

At the age of 5 months, she was referred to our department with generalized, cutaneous erythematous papules, which had appeared 3 months before. She also had recurrent wheezing and failure to thrive since the age of 3.5 months. Upon physical examination, she had small left axillary lymphadenopathies.

The histopathology of the biopsied skin lesions showed tuberculoid granuloma (Figure 4). She further developed *Pneumocystis jiroveci* pneumonia and other less severe

bacterial and viral recurrent infections, mainly localized to the respiratory tract. She was subjected to intravenous immunoglobulin therapy (500 mg/kg every 3 weeks) and prophylaxis with trimethoprim/sulphamethoxazole (450 mg/m² for 3 days per week), pending bone marrow transplant, which the parents refused.

Serum immunoglobulins and complement test results were normal. Decreased numbers of CD8⁺ T cells (2%, 42/ μ L) and CD4⁺:CD8⁺ cell ratios (0.028) were detected. T cell cytokine production (IL-2 and tumor necrosis factor- α) after stimulation with phytohemagglutinin was absent. The lymphocyte transformation test was decreased for different mitogens. The expression of ZAP70 was normal in B, NK and T cells. The ZAP70 and Syk (spleen tyrosine kinase) western blot analysis carried out to evaluate the presence and phosphorylation of these two proteins revealed an increase in Syk and an absence of phosphorylation of ZAP70. Molecular analysis showed a R170C mutation in the ZAP70 gene corresponding to an arginine at position 170, which is important in the interaction with the phosphorylated tyrosine in the ITAM (Immunoreceptor tyrosine-based activation motif) motifs of the CD3 complex (Taylor et al, unpublished data).

Case 3

A boy, the second son of a non-consanguineous young couple, was born at full term, with adequate somatometry for the gestational age. When he was 6 months old, he

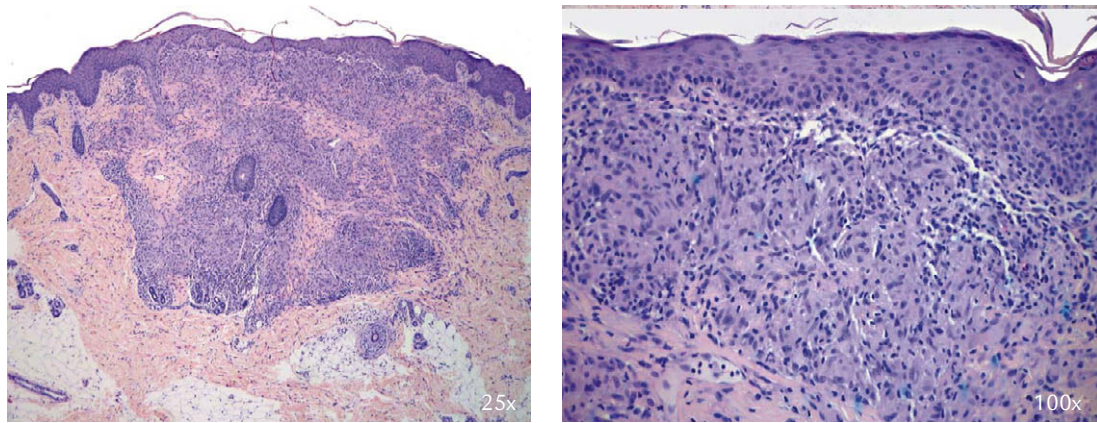


Figure 4. Cutaneous tuberculoma (hematoxylin and eosin, original magnification, 25× and 100×).



Figure 5. Left axillary lymphadenitis in case 3.

presented with acute nasopharyngitis for 5 days, and 1 week later he presented with irritability, respiratory distress syndrome, skin pallor, peripheral and central cyanosis and dry cough without fever or anorexia. He had received only the BCG and HBV vaccinations at the maternity hospital. A large left axillary lymphadenitis was evident (Figure 5). He started failing to thrive at the age of 2 months. He had a cousin with a suspected immunodeficiency later diagnosed as a ZAP70 deficiency (case 2) and followed up in our PID clinic.

At the time of admission, the leukocyte count was 13,620/ μ L (60% lymphocytes), the platelet count was 554,000/ μ L, hemoglobin was at 11.2 g/dL and the C-reactive protein level was 0.2 mg/dL. Urine and blood cultures were negative. The chest radiography showed a bilateral interstitial pneumonia (Figure 6). He was hospitalized and initially treated with intravenous amoxicillin (90 mg/kg/day) and clavulanic acid, as well as oxygenotherapy. *Pneumocystis jiroveci* was detected in the nasopharynx

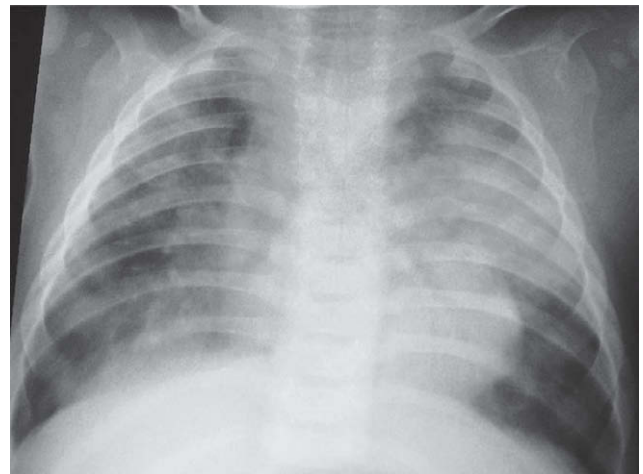


Figure 6. Bilateral pneumonia (*Pneumocystis jiroveci*).

aspirate by indirect immunofluorescence. Consequently, he was put on ceftriaxone (100 mg/kg/day) and trimethoprim/sulphamethoxazole (120 mg/kg/day) and an immunodeficiency was suspected.

The immunological evaluation revealed increased levels of serum IgA (2.55 g/L) and IgM (1.77 g/L), and a decreased number of CD56⁺ (138/ μ L, 3%) and CD8⁺ (46/ μ L, 1%) cells. Serological evaluation for HIV-1 and HIV-2 was negative. The expression, genetic analysis and functional assessment of ZAP70 were similar to his cousin in case 2 (Taylor et al, unpublished data).

Mycobacterium bovis was isolated in the lymphadenitis exudate and nasopharynx aspirate. Besides anti-bacillar treatment with isoniazid, rifampin and etambutol, he was submitted to intravenous immunoglobulin therapy and antimicrobial prophylaxis with trimethoprim/sulphamethoxazole. Since bone marrow transplantation was performed at the age of 14 months, he has thrived and developed well, despite minor infections.

Discussion

We report three cases of BCG disease after vaccination in children with PID: one with a partial recessive IFN- γ R1 deficiency, who developed BCG dissemination, and two with ZAP70 deficiency, a severe combined immunodeficiency, who presented with regional and distant BCG disease. All had severe axillary lymphadenitis, which should be a sign for PID in BCG-vaccinated children.

The etiologies of infections developing in the immunodeficient child are an important clue to the diagnosis of underlying immune defects in patients with PID.^{9,10} Few PIDs confer susceptibility to mycobacteria.¹⁰ PIDs with high susceptibility to mycobacterial infections include IL-12/IFN- γ axis defects, chronic granulomatous disease, severe combined immunodeficiencies (SCID), idiopathic CD4 lymphopenia and defects of nuclear factor- κ B essential modulator (NEMO)-dependent NF- κ B activation. The latter two show increased susceptibility to tuberculosis and non-tuberculosis mycobacteria but not to BCG. The particular immune defects explain the susceptibility to mycobacterial infections and complications of BCG vaccination, however one has to bear in mind that both tuberculosis and BCG disease may occur.^{4,11}

The first reported case was an infant with a defect in the IL-12/IFN- γ axis, an IFN- γ R1 deficiency.^{12,13} The IFN- γ -IL-12/23 circuit is crucial in the immune response to intracellular microorganisms such as *Salmonella*, *Listeria*, mycobacteria¹⁴ and certain viruses. Specific genetic

mutations resulting in the impairment of this functional axis have been identified and include deficiency of IFN- γ R1, the receptor which binds onto macrophages; deficiency in the signaling chain of the IFN- γ R2; deficiency of the IL-12 R subunit β_1 , which is expressed in T and NK cells; a deficiency in the p40 subunit of IL-12; and, finally, STAT-1 deficiency, a critical molecule in the transduction of signals from the IFN- γ and IFN- α/β receptors.

A correlation between the IFN- γ R1 genotype, cellular response to IFN- γ and the clinical phenotype has been established.¹⁵ The autosomal dominant form is the most common and the least serious. Among the autosomal recessive forms, a complete loss of the protein expression or a partial defect can occur. These rare patients tend to develop severe mycobacterial diseases during infancy or childhood, as they fail to form well-circumscribed tuberculoid granuloma. When BCG vaccine is administered, disseminated infection typically develops.¹⁶ In complete defects, fatal infection from BCG vaccination has been reported.^{17,18}

The major difficulty in these cases is that the routine immunological test results are normal and specific investigation of IL-12/IFN- γ axis is needed. In the reported case, that fact may have delayed the diagnosis. In the end, a homozygous I87T mutation in the IFN- γ R1 gene was detected, which has already been described in non-related Portuguese children.¹⁹

The second and third patients are cases of ZAP70 deficiency, a rare autosomal recessive SCID.²⁰ ZAP70 is an intracellular tyrosine kinase recruited to the CD3/T cell receptor and required for T cell activation following T cell receptor engagement. Mutations in the gene on chromosome 2q12 result in defective expression and/or function of ZAP70 and consequently, a decreased number of CD8⁺ cells, a normal number of CD4⁺ cells and inability of peripheral lymphocytes to respond to activation signals. Circulating B cells are normal, serum immunoglobulins may be normal or decreased and specific antibody responses are variable. Both our patients exhibited no ZAP70 phosphorylation, which means that despite the presence of ZAP70 protein in the immune cells it was not activated after encountering an antigen. Patients with ZAP70 deficiency usually present early in life with typical clinical features of SCID: severe respiratory infections, including those from opportunistic pathogens, chronic diarrhea, failure to thrive,

Table. Guidelines for classification, diagnosis and management of BCG disease in immunocompromised children⁴

Classification of BCG disease	<p>Local BCG disease</p> <ul style="list-style-type: none"> ● Abscess on the BCG injection site with diameter greater or equal to 10 mm, and/or ● Severe BCG scar ulceration <p>Regional BCG disease</p> <ul style="list-style-type: none"> ● Involvement of vaccination site, and ● Enlargement, suppuration and/or fistula on any regional lymph nodes (e.g. ipsilateral axillary, supraclavicular, cervical and upper arm glands) or other regional lesions <p>Distant BCG disease</p> <ul style="list-style-type: none"> ● BCG confirmed from one site beyond a local or regional ipsilateral process [e.g. pulmonary secretions (gastric or tracheal aspirate), cerebrospinal fluid, urine, osteitis, distant skin lesions] <p>Disseminated BCG disease</p> <ul style="list-style-type: none"> ● BCG confirmed from more than 1 distant site and/or from at least 1 blood or bone marrow culture
Diagnosis of BCG disease	<p>All children</p> <ul style="list-style-type: none"> ● Detailed clinical history including documentation of local and regional BCG lesions ● Fine needle aspirate for mycobacterial culture ● HIV testing <p>Immunocompromised children</p> <ul style="list-style-type: none"> ● Chest radiography (antero-posterior and lateral) ● Gastric washings for mycobacterial culture (minimum of 2) ● Mycobacterial blood culture (if febrile) ● CD4⁺ T lymphocyte count and viral load (if HIV+ and not performed in previous 2 months) ● Full blood and differential count ● Baseline liver function tests (for monitoring of toxicity) <p>Additional tests for immunocompromised children with suspected distant or disseminated BCG disease</p> <ul style="list-style-type: none"> ● bone marrow aspirate/biopsy for mycobacterial culture ● mycobacterial blood culture (even if afebrile) ● abdominal ultrasound (for intra-abdominal lymphadenopathy) ● radiography (if osteitis is suspected) ● other systemic investigations as clinically indicated
Management of BCG disease in immunocompromised children	<p>Local or regional BCG disease</p> <ul style="list-style-type: none"> ● Medical treatment: <ul style="list-style-type: none"> – isoniazid 15–20 mg/kg/day – rifampicin 20 mg/kg/day – pyrazinamide 20–25 mg/kg/day (2 months until tuberculosis is excluded) – ethambutol 20–25 mg/kg/day – ofloxacin 15 mg/kg/day or ciprofloxacin 30 mg/kg/day ● Therapeutic aspiration if node fluctuant ● Excision biopsy if no improvement or deterioration of adenitis after 6 weeks of antituberculosis therapy ● If HIVC+ on HAART, ensure that HAART is antituberculosis-drug compatible ● Monitor for drug toxicity ● Report as vaccine-related adverse event to Expanded Programme on Immunization <p>Distant or disseminated BCG disease</p> <ul style="list-style-type: none"> ● Medical treatment as above ● Consider expedited initiation of HAART ● Monitor for drug toxicity ● Report as vaccine-related adverse event to Expanded Programme on Immunization

persistent candidiasis and oral ulcers. Unlike patients with other forms of SCID, these patients tend to have palpable lymph nodes and a normal thymic shadow.

Severe BCG disease has previously been described in SCID infants,^{8,21} including cases of cutaneous BCG dissemination,²² but not in children with ZAP70 deficiency. These cases illustrate the importance of taking a detailed clinical history before routine vaccination, as it would have been important to avoid BCG vaccination in this child with a family history of PID, before appropriate immunological evaluation had been performed.

After Talbot et al designed a BCG disease classification system,⁶ Hesselning et al revised this system regarding the pediatric age group in order to more accurately reflect all relevant disease categories in HIV-infected and uninfected children.⁴ More recently, Bernatowska et al also proposed some diagnostic criteria for disseminated BCG infection in PID patients.⁷ Guidelines for the diagnosis and treatment of BCG disease in immunocompromised patients as recommended by Hesselning et al⁴ and summarized in the Table are helpful for early recognition.

The practice of compulsory administration of the BCG vaccine early in life, as performed in many countries where tuberculosis is endemic, puts immunodeficient children at high risk of developing BCG disease, as common PIDs have not been diagnosed at the time of vaccination. BCG vaccination for all neonates should be reconsidered in some countries²³ due to the changing epidemiology of tuberculosis and the poor protection that BCG vaccine confers. In any country, BCG vaccination should be delayed when a PID is suspected and/or a newborn has a family history of PID, until the same condition has been ruled out.

In summary, BCG dissemination in an immunodeficient child may be the presenting clinical picture of a PID, and may not be associated with a local or regional disease. Severe axillary lymphadenitis (or any other form of BCG disease) after BCG vaccination should alert physicians for an immunodeficiency, including PID. The diagnosis of disseminated BCG disease should be considered in a child with a known PID in the appropriate clinical setting. An accurate diagnosis of BCG dissemination demands a search for specific immune defects to allow for an early life-saving treatment. Moreover, every adverse reaction to the BCG vaccine should be reported in the medical literature and to the relevant authorities. A new tuberculosis

vaccine, more immunogenic, effective and safer than the old BCG vaccine, is urgently needed.²⁴

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