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CASE REPORT

Disseminated Bacille Calmette-Guérin Disease as the Initial Presentation of X-linked Severe Combined Immunodeficiency - A Case Report

Li-Hsin Huang[1], Shyh-Dar Shyur[1], Jyh-Der Weng[1], Shin-Chi[2], Chin-Yuan Tzen[3] and Fu-Yuan Huang[2]

SUMMARY Bacille Calmette-Guérin (BCG) vaccination is used to prevent severe *M. tuberculosis* infection. It has been used in many countries for a long time. However, complications do occur, including localized abscesses, regional lymphadenitis and disseminated disease. The latter is often associated with underlying immunodeficiency. We report an 8-month-old male infant presenting with cough and fever who had had a generalized pigmented skin rash for one month. Skin biopsy revealed mycobacterial infection, but his response to treatment was poor and he had a persistent mild fever. Immunological studies revealed an IgG of 49 mg/dl, IgA 4 mg/dl, IgM 28 mg/dl, IgE < 1 mg/dl, CD_3 1.1%, CD_4 0.6%, CD_8 0.6%, CD_{19} 93.9%, CD_{57} 1.1%, activated T cells 0.9%, and CH_{50} < 6.3%. These findings are compatible with the diagnosis of $T^-B^+NK^-$ severe combined immunodeficiency. Sequence analysis was performed and showed the presence of missense mutation in IL2Rγ gene. An X-linked recessive inheritance pattern was proved by sequence analysis of his mother and grandmother. In order to identify the strain of the microorganism, we reviewed pathology of the skin biopsy which consisted of diffuse histiocytic infiltrate with poorly formed granulomas and no necrosis and used polymerase chain reaction (PCR) with the stain-positive clinical specimen and verify the organism found in the child's biopsy as *M. bovis* BCG strain. The diagnosis of disseminated BCG disease must be considered in any infant with cutaneous mycobacterial lesions, especially with atypical histologic findings. Such a patient's immunologic status should be evaluated and further family study is suggested. A high index of suspicion is needed to make a timely diagnosis, as early intervention with intensive treatment and bone marrow transplantation may be life-saving.

Bacille Calmette-Guérin (BCG) vaccination is practiced in many countries. It prevents severe infection with *M. tuberculosis,* particularly in children and is considered to have an excellent safety profile.[1] However, complications do occur, ranging from local lymphadenitis to disseminated disease.[2] Most patients with disseminated disease have underlying immunodeficiency.[3] SCID is a phenotypic term for a wide variety of congenital and hereditary immunologic defects characterized by early onset of infections, defects in both B- and T-cell systems, lymphoid

From the Sections of [1]Allergy and Immunology, [2]Infectious Diseases, Department of Pediatrics, [3]Department of Pathology, Mackay Memorial Hospital, Taipei, Taiwan.
Correspondence: Shyh-Dar Shyur
E-mail: abc1016@ms2.mmh.org.tw

aplasia and thymic dysplasia, which should be one of the most important considerations. We report a case of disseminated BCG disease in an infant with X-linked severe combined immunodeficiency, which was both confirmed by sequence analysis.

CASE REPORT

An 8-month-old male infant was brought to the outpatient department of Mackay Memorial Hospital, Taipei, with a chief complaint of productive cough for months and a fever off and on for 2 weeks. The child had been immunized in the left deltoid muscle with Moreau BCG vaccine at 3 days of age. He had been relatively well until 6 months of age when he began to have frequent upper respiratory tract infections and acute bronchiolitis. Pigmented papules were noted beginning at age 7 months, initially on buttocks and then progressing to involve the entire trunk one week later. He was admitted to another hospital for evaluation of the skin lesions. A skin biopsy revealed granulomatous inflammation with mycobacteria on smear. Isoniazid 10 mg/kg/day, pyrazinamide 30 mg/kg/day, and rifampin 10 mg/kg/day were prescribed. However, the skin lesions persisted and he continued to have cough with intermittent fever. He was brought to our hospital for further evaluation and treatment.

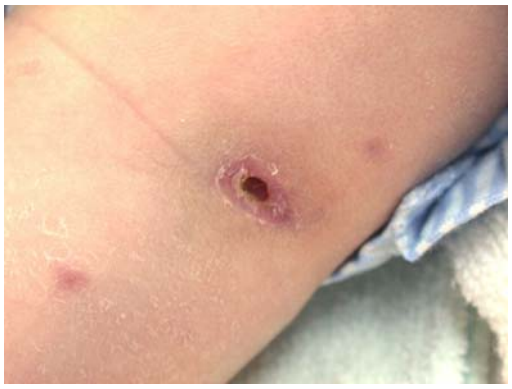

Fig. 1 Unhealed BCG scar with discharge in the left deltoid area

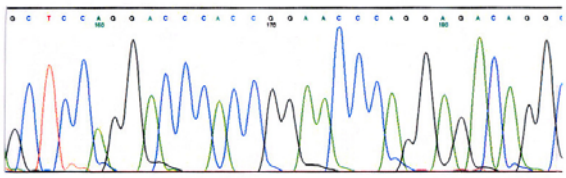

Fig. 2 A missense mutation at codon 136 (CGG to CCG) in the exon 2 of IL2Rγ gene.

Upon admission, his general appearance was fair. His body temperature was 38.4°C, pulse rate 120/minute, respiratory rate 30/minute, and blood pressure 93/66 mmHg. His length, weight, and head circumference were all less than the 3rd percentile, consistent with failure to thrive. There was moderate oral thrush and no tonsils could be seen. Patchy erythematous rashes with subcutaneous nodules were noted on the trunk and all four extremities. There was an unhealed ulcer on the left deltoid muscle (Fig. 1). No lymph nodes were palpable in any area.

Laboratory studies at the time of admission revealed a hemoglobin of 9.0 gm/dl, hematocrit 29.1%, WBC count 1020/mm^3 (3% bands, 12% segments, and 73% lymphocytes), and platelet count 222,000/mm^3. The examination of human immunodeficiency virus antibody of the patient and his mother revealed negative. Chest X-ray revealed perihilar and peribronchial infiltrates and absence of a thymic shadow. Immunologic studies showed an IgG of 49 mg/dl, IgA 4 mg/dl, IgM 28 mg/dl, IgE < 1 mg/dl, CD_3 1.1%, CD_4 0.6%, CD_8 0.6%, CD_{19} 93.9%, CD_{57} 1.1%, activated T cells 0.9%, and CH_{50} < 6.3%. These findings are compatible with the diagnosis of $T^-B^+NK^-$ severe combined immunodeficiency. On further questioning about his family history, we learned that all his maternal grandmothers' brothers died before 1 year of age with unknown infections. This indicated an X-linked recessive inheritance pattern, with his mother and grandmother being asymptomatic carriers. Sequence analysis shows a missense mutation at codon 136 (CGG to CCG) in the exon 2 of the IL IL2Rγ, resulting in a mutant of R136P (Fig. 2).[4] Carrier detection was performed by mutation analysis. His pedigree is shown in Fig. 3. He was given intravenous immunoglobulin at a dose of 400 mg/kg/day. However, the second night of the admission, he suddenly developed massive hematemesis and melena. Despite of fluid resuscitation and antibiotics treatment, he developed *Pseudomonas aeruginosa* sepsis and expired 6 days later.

The patient's family refused an autopsy. However, we were able to obtain the skin biopsy specimens from the first hospital. The histologic lesions consisted of diffuse histiocytic infiltrate with poorly formed granulomas and no necrosis. A Kinyoun carbol fuchsin stain (acid-fast stain) showed plump histiocytes engorged with numerous intracellular and extracellular acid-fast bacilli (Fig. 4). The findings were compatible with atypical mycobacterial infection. We did a polymerase chain reaction (PCR) on material from the stain-positive specimen using the following primer sets: 1. spacer region-specific primers, spacer region 33 specific (5'-ACA CCG ACA TGA CGG CGG-3') and spacer region 34 specific (5'-CGA CGG TGT GGG CGA GG-3'); 2. IS*6110*, *M. tuberculosis* complex-specific primers, TB284 (5'-GGA CAA CGC CGA ATT GCG-3') and TB850 (5'-TAG GCG TCG GTG ACA AAG GCC AC-3'); 3. *Mycobacterium* genus-specific (65-kDa antigen gene) primers, TB11 (5'-ACC AAC GAT GGT GTG TCC A-3') and TB12 (5'-CTT GTC GAA CCG CAT ACC CT-3'); 4. mycobacterial interspersed repetitive units (MIRU), the senX3-regX3 intergenic region, C5 (5'-GCG CGA GAG CCC GAA CTG C-3') and C3 (5'-GCG CAG CAG AAA CGT CAG C-3').[5-7] The negative controls contained the PCR mixture without DNA template. The positive controls contained the extracted mycobacterial DNA from BCG vaccine (Tokyo 172). The PCR products were analyzed by electrophoresis on a 2.0% agarose gel and were visualized with ethidium bromide. The specimen showed positive reaction with the above 4 primers. For further differentiation between BCG and other virulent strains of the *M. tuberculosis* complex, the PCR products of the MIRU region were then cloned and sequenced with ABI PRISM model 377 apparatus

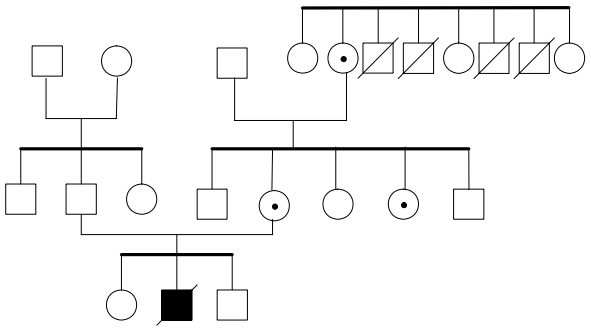

Fig. 3 The patient's family pedigree showing X-linked recessive inheritance pattern. Squares represent males and circles represent females; open square box with line across, died before one year of age with unknown infection; solid square with line across, index patient; open circles with dots at center, asymptomatic carriers.

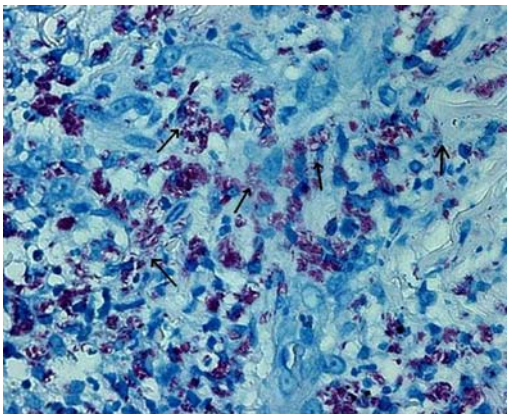

Fig. 4 Plump histiocytes engorged with numerous intracellular and extracellular acid-fast bacilli. (Kinyoun carbol fuchsin stain; 1,000x).

CTCACAATCAACACACTGGTCATCAGCGCCACTCTCCCCCGCAAGCGGGTGGTGCC
CCCACCTCATCGCTACGCTCTGCATCGTCGTCGGCGCAGGTCATCAGCGCCACTCTC
CCCCGCAAGCGGGTGGTGCCCCCACCTCATCGCTACGCTCTGCATCGTCGTCGGCG
CAGGTCATCAGCGCCACTCTCCCCCGCAAGCGGGTGGTGCCCCCACCTCATCGCTA
CGCTCTGCATCGTCGTCGGCGCAGGTCATCGGCTCANCTCTTCCTCTCGTTGTGACC
TGTTTGACCGCAGTTCGGGCTCTCGCGCA

Fig. 5 Sequence analyses of the PCR products of C3/C5 primers in alphabetical format. Nucleotides marked and underlined comprise the 3 sets of 77-bp MIRU, indicating the sample is BCG.

(Perkin Elmer Applied Biosystems, Foster City, CA, USA). Sequence analysis showed 99.36% homology with a BCG positive control (Figs. 5,6). The final diagnosis was X-linked SCID with disseminated BCG disease.

DISCUSSION

BCG vaccines have been given to four billion people and have been used routinely since the 1960s in almost all countries, including Taiwan.[8] The vaccine was originally derived from multiple passages of wild-type *M. bovis* to diminish the virulence.[9] It has proved remarkably safe. Localized abscesses and ulcers or regional suppurative lymphadenitis occur in infants at a rate of only 0.4 per thousand vaccines.[10] Disseminated disease is much rarer, occurring in 1 per 3.4 per million infants vaccinated.[3] Widespread dissemination is associated with a high mortality and usually develops only in the presence of underlying immunodeficiency.[11,12] Casanova *et al.*[13] analyzed a series of 121 children with disseminated BCG disease from 1951 to 1994 and showed that 50% of cases occurred in children with identifiable immunodeficiency syndromes. The other 50% were considered idiopathic, but an undiagnosed genetic immune defect was strongly suspected.

SCID is a phenotypic term for a wide variety of congenital and hereditary immunologic defects characterized by early onset of infections, defects in both B- and T-cell systems, lymphoid aplasia and thymic dysplasia. Inheritance can be X-linked, autosomal recessive, or sporadic. The most common form is X-linked $T^-B^+NK^-$SCID caused by mutations in the X-linked gene IL2Rγ, which encodes the common gamma chain of the leukocyte receptors for interleukin-2 and multiple other cytokines.[4] The immunologic deficiency in SCID results in increased susceptibility to intracellular pathogens, such as mycobacterial, viral, bacterial, fungal and protozoal infections. In the absence of specific immunologic treatment, such patients usually die within the first year of life due to overwhelming infection.[14] Infants with lymphocyte count persistently less than 2,800/mm^3 should be investigated for SCID.[15] A greatly improved prognosis could be achieved in infants with SCID if it is diagnosed and treated before they develop overwhelming infection. If the diagnosis is not made immediately, patients may be given live-attenuated vaccine, as in our case, which causes the disease it was intended to prevent. BCG vaccination is the only live-attenuated vaccine in Taiwan which is given within the first month of life. Therefore, disseminated BCG disease should be considered in patients presenting with compatible symptoms if the diagnosis of SCID is made subsequently.

The most commonly reported symptoms in disseminated BCG disease are fever, lymphadenopathy, weight loss, failure to thrive and hepatosplenomegaly. Widespread cutaneous lesions occur occasionally. A retrospective review of all reported BCG cases between 1980 and 1995 found only 28 cases with a definitive diagnosis of disseminated disease, 6 of which included disseminated cutaneous lesions. Of these 6 patients, 4 had SCID, one had cell-mediated immunodeficiency and only 1 had no immune defect. In the rest 22 cases, only one patient had SCID.[16] This suggests that the clinical manifes-

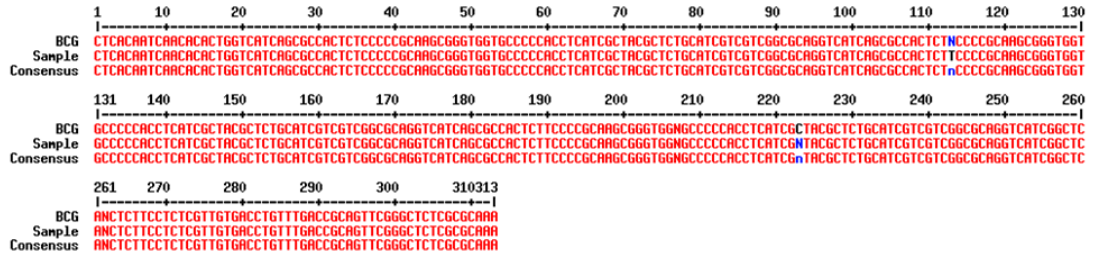

Fig. 6 Alignment of the sample and the positive control (BCG Tokyo172) with TB284/885 primers showed 99.36% homology.

tations of disseminated BCG disease differ by the immunologic status. Generalized pigmented papules may be the only initial manifestation in patients with SCID, a finding that is easily ignored. Cutaneous infections in these patients have an unusually persistent or progressive character. Failure to respond to treatment should suggest the presence of underlying immunodeficiency.[17,18]

Cutaneous histologic examination often confirms the presence of disseminated infection,[19] and may give a clue as to the patient's immunologic status. In the immunocompetent patient, there are well-circumscribed, well-differentiated granulomas, with epithelioid and multinucleated giant cells containing very few acid-fast bacilli, surrounded by lymphocytes and fibrosis and occasionally with central caseous necrosis. In the immunocompromised patient, the lesions usually consist of ill-defined and poorly differentiated granulomas, with few if any giant cells and lymphocytes but widespread histiocytes loaded with acid-fast bacilli.[20]

Mycobacterial isolates are frequently identified in clinical microbiology laboratories only to the level of *M. tuberculosis* complex, which includes *M. tuberculosis, M. bovis, M. africanum,* and *M. microti.* The combination of biochemical and growth features may strongly suggest that an isolate is BCG, but none of these features are definitive. PCR only requires a small specimen from a clinical isolate. It can be used even with heat-killed cultures and in smear-positive specimens on slides.[21] It was the material we used, since the family refused an autopsy. The PCR using the primers C3 and C5 and subsequent sequencing allowed us to confirm the identity of the organism as BCG. Further alignment confirmed the source of infection was from the BCG vaccine (Tokyo 172) the patient received at 3-day-old. Only three sets of 77-bp MIRU were detected in our specimen but a unique 53-bp MIRU in addition to the common variable copies of 77-bp MIRU was present in all virulent *M. tuberculosis* complex strains. This method has a sensitivity of 100% and specificity of 100% as reported by Magdalena *et al*.[5] and has been employed in our previously reported case.[22]

The most commonly used antimycobacterial regimens for disseminated infection are isoniazid, rifampin, ethambutol and streptomycin. However, response is usually poor in immunocompromised patients, who have a reported mortality of 83%.[16] Heyderman *et al*.[23] and Ikinciogullari *et al*.[24] have reported good success with early bone marrow transplantation and aggressive treatment with clofazimine, claruthromycin, ciprofloxacin in addition to the classical agents. Prenatal diagnosis and female carrier detection is useful for preventing the disaster of the same disease in subsequent deliveries. The carrier detection can be accomplished by analysis of an unbalanced pattern of X-chromosome inactivation or direct mutation analysis, by which we do the carrier detection to the family. The prenatal diagnosis can be made by fetal blood sampling at 20 weeks gestation through enumeration of lymphocyte subsets and functional studies, and it can also be accomplished as early as the tenth week of gestation through the pattern of X-chromosome inactivation or direct mutation analysis.[25] Evaluation of the mutation analysis should be performed in the following male siblings of the family and live attenuated vaccines should be avoided before the immunologic status is confirmed to prevent overwhelming infection. In conclusion, the diagnosis of disseminated BCG disease must be considered in any infant with cutaneous mycobacterial lesions, especially those with atypical histologic findings and PCR could help us in precise diagnosis. A high index of suspicion for immunodeficiency is needed in such cases, as early intervention with intensive treatment and bone marrow transplantation may be life-saving.

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