the upstream rpoBF1 but with a DNA uptake sequence (in lower case) that was added at the 5′ end to permit DNA transformation (*7*). The transformant strain NM05-08 was resistant to rifampin (MIC >32 mg/L), and the sequence of the *rpoB* gene confirmed the His→Tyr mutation. When compared to the parental isolate (LNP21362), strain NM05-08 showed reduced virulence. Indeed, bacterial loads were similar to those observed for the resistant isolate LNP22330 (Figure). These results strongly suggest a direct negative impact of *rpoB* mutations on meningococcal virulence. Mutations in the *rpoB* gene have been reported to confer pleiotropic phenotypes (*8*).

The data reported here show that rifampin-resistant isolates were not clonal but belonged to different genetic lineages. The results of virulence assays in mice suggest that mutations in *rpoB* in resistant isolates may have a major biological cost for *N. meningitidis*, which can be defined as lower bacterial fitness in terms of survival in the bloodstream. This biological cost could explain the lack of clonal expansion of meningococcal isolates that acquired resistance to rifampin.

Muhamed-Kheir Taha,*
Maria Leticia Zarantonelli,*
Corinne Ruckly,* Dario Giorgini,*
and Jean-Michel Alonso*
*Institut Pasteur, Paris, France

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Address for correspondence: Muhamed-Kheir Taha, Directeur Adjoint du Centre, National de Reference des Meningocoques, Unite des Neisseria, Institut Pasteur, 28 Rue du Dr Roux, Paris 75724 CEDEX 15, France; email: mktaha@pasteur.fr

Vaccination-related *Mycobacterium bovis* BCG Infection

To the Editor: The high prevalence of tuberculosis (TB) underlines the important role of BCG (bacillus Calmette-Guérin) immunization. The vaccine, however, is not free from complications, which could be local or disseminated. Disseminated BCG infection as a result of TB vaccination is a rare complication with an incidence of 0.06 to 1.56 cases per million vaccinations; it occurs exclusively in patients with immune deficits. However, in these cases, the prognosis is unfavorable; up to 70% of patients die, despite intensive antituberculous treatment (*1–4*).

A 4-month-old-girl exhibited enlargement of left axillary lymph nodes during a 1.5-month period. She was the second child of healthy parents, with no family history of genetic disorders or TB. She was vaccinated according to the regimen compulsory in Poland: the first dose of BCG and anti–hepatitis B virus (HBV) vaccination on the first day of life, followed by vaccination against diphtheria, tetanus, pertussis, poliomyelitis, and the second dose of anti-HBV after 6 weeks. BCG vaccination was performed intradermally in the upper part of left arm by administration of 0.1 mL Brazilian Moreau strain (Biomed, Lublin, Poland).

On hospital admission, the patient was in reasonably good condition but pale, with grossly enlarged, adjacent left axillary lymph nodes and hepatosplenomegaly. Laboratory tests showed anemia, thrombocytopenia, elevated transaminase activity, a high C-reactive protein level, and high level of immunoglobulin M (IgM) class anti-cytomegalovirus (CMV) reactive antibodies.

Based on clinical manifestations and biochemical and serologic signs, CMV infection was suspected. The patient was administered a 14-day regimen of ganciclovir (10 mg/kg/day); results of liver function tests and blood count normalized, and hepatosplenomegaly decreased. However, the lymph nodes continued to enlarge, and diagnostic excision and bone marrow aspiration were performed to exclude a neoplastic process. A histopathologic image of the excised lymph nodes showed caseating granulomas, and tuberculous lymphadenitis was suggested (Figure).

At that time, a diagnosis of disseminated BCG infection as a complication of TB vaccination in a presumed immunocompromised patient

was proposed. This idea was based on suggestive lymph node pathology, which showed caseating granulomas, a history of TB vaccination, and the exclusion of other pathologic changes. Flow cytometry measurements showed abnormally low expression of the α chain of the interferon (IFN)-γ receptor on peripheral blood lymphocytes. Only 20% lymphocytes expressed CD 119 (IFN-γ receptor outer subunit R1).

Three-drug anti-tuberculous therapy (with rifampin, isoniazid, and streptomycin) was introduced despite chest and bone radiographs that were negative for infection, no abnormalities found on funduscopy, and negative results of Ziehl-Neelsen staining of lymph node tissue. Despite this therapy, the child's condition worsened; she exhibited a high temperature, hemolysis, and progressive neutropenia, thrombocytopenia, cholestasis, and renal failure. Uncontrolled sepsis developed, and she died.

At postmortem examination, the diagnosis of disseminated BGC infection was made on the basis of multiple TB-like granulomas in the lungs, lymph nodes, meninges, liver, spleen, and kidneys. However, direct microbiologic confirmation of BCG infection was lacking because cultures were negative and Ziehl-Neelsen and periodic acid–Schiff staining did not show acid-fast bacilli, other bacteria, or fungi in these specimens.

This case represents a rare complication of antituberculous vaccination, that is a progressive, disseminated BCG infection in a patient with deficiency of IFN-γ receptor. Concomitant CMV infection was diagnosed by positive IgM antibody response. Transient response to the ganciclovir treatment made the final diagnosis of BCG infection more difficult and probably postponed implementation of the anti-TB therapy. Until now ≈100 cases have been reported in the literature, most of them in infants and young children. These patients also had clear predisposition to other severe infections with intracellular microorganisms such as atypical mycobacteria, *Salmonella* spp., *Listeria monocytogenes*, and *Leishmania* spp. (*1–5*).

The INF-γ receptor is present on many cell types; however, its deficiency on macrophages may be responsible for the inhibition of phagocytosis and intracellular killing and the observed deficit of an antimycobacterial immunity. Among children with a clinical syndrome of IFN-γ–receptor deficiency, a clear genetic defect was identified in ≈20%. In our patient, the diagnosis was made by detection by flow cytometry of abnormally low expression of the α chain of the IFN-γ receptor on peripheral blood lymphocytes. This method appears to have high diagnostic value, given the fact that genetic methods are not always available and are expensive and often insensitive.

The prognosis in patients with BCG infection secondary to IFN-γ–receptor deficiency is unfavorable. A few cases of successful treatment with allogenic bone marrow transplantation have been reported with long-term improvement of general condition and stable receipt of the graft as shown by molecular analysis of peripheral leukocytes (*4*,*6–8*). However, as specific and efficient therapy for this condition has not been as yet proposed, supportive measures with early diagnosis and institution of anti-TB and antimicrobial drug treatment appear to be important in managing this rare immune deficiency. The level of IFN-γ–receptor expression in populations known to be susceptible to TB, and its potential role in this phenomenon, appears to be a promising area of study.

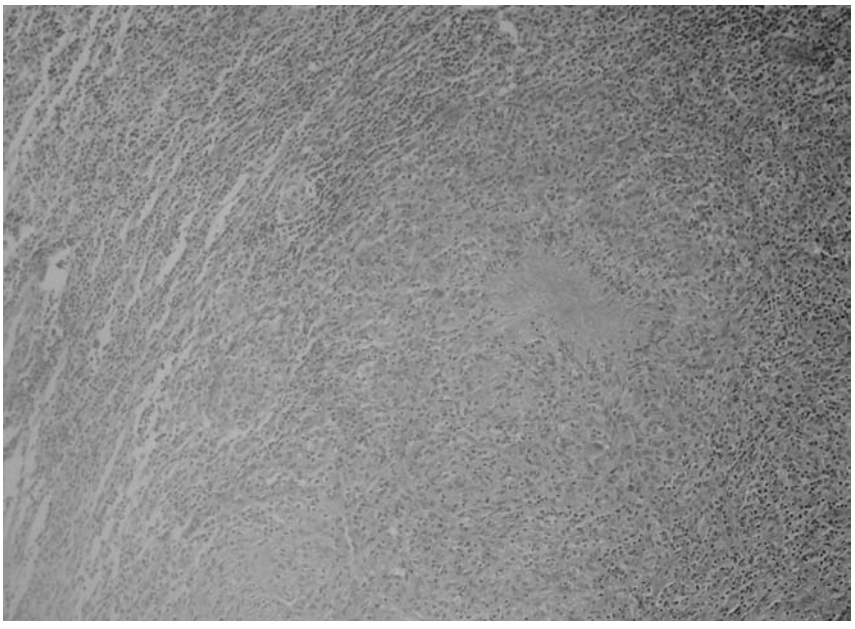

Figure. Digitally processed hematoxylin-eosin staining of the excised lymph nodes, showing caseating, tuberculosislike granulomas (original magnification ×100).

Anna Liberek,* Maria Korzon,* Ewa Bernatowska,† Magdalena Kurenko-Deptuch,† and Marlena Rytlewska*

*Medical University, Gdansk, Poland; and †Children's Memorial Health Institute, Warsaw, Poland

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Address for correspondence: Anna Liberek, Department of Paediatrics, Children's Gastroenterology and Oncology, Medical University, Ul, Nowe Ogrody 1-680-803, Gdansk, Poland; email: tlib@amg.gda.pl

Human Bocavirus in Children

To the Editor: Respiratory tract infection is a major cause of illness in children. Despite the availability of sensitive diagnostic methods, detecting infectious agents is difficult in a substantial proportion of respiratory samples from children with respiratory tract disease (*1*). This fact suggests the existence of currently unknown respiratory pathogens.

A new virus has been recently identified in respiratory samples from children with lower respiratory tract disease in Sweden (*2*). Analysis of the full-length genome sequence showed that this virus is closely related to bovine parvovirus and canine minute virus and is a member of the genus *Bocavirus*, subfamily *Parvovirinae*, family *Parvoviridae*. This virus has been provisionally named human Bocavirus (HBoV) (*2*). HBoV in respiratory samples from Australian children was also recently reported (*3*). Involvement of this new virus in respiratory tract diseases merits further investigation. We have therefore retrospectively tested for HBoV nasopharyngeal samples collected from children <5 years of age hospitalized with respiratory tract disease.

Samples were collected from 262 children from November 1, 2003, to January 31, 2004. The samples were tested for respiratory viruses by using direct immunofluorescence assays with monoclonal antibodies to respiratory syncytial virus; influenza virus types A and B; parainfluenza virus types 1, 2, and 3; and adenovirus. Samples were also placed on MRC5 cell monolayers for virus isolation and tested for human metapneumovirus by reverse transcription–polymerase chain reaction (RT-PCR). Nucleic acids were extracted from samples, stored at –80°C, and tested for HBoV DNA by PCR with primers specific for the predicted NP1 gene as previously described (*2*). The expected product size was 354 bp. In each experiment, a negative control was included, and positive samples were confirmed by analyzing a second sample. Amplification specificity was verified by sequencing.

Nine (3.4%) samples were positive. Comparison of PCR product sequences of these 9 isolates (GenBank accession nos. AM109958–AM109966) showed minor differences that occurred at 1 to 4 nucleotide positions, and a high level of sequence identity (99%–100%) was observed with the NP1 sequences of the previously identified ST1 and ST2 isolates (*2*). This finding indicates that HBoV is a highly conserved virus.

HBoV was the only virus identified in 6 children and was associated with respiratory syncytial virus in 3 other children. An infection with other respiratory viruses was detected among 153 (60.5%) of the 253 HBoV-negative children. The viruses identified were respiratory syncytial virus in 114 (43.5%) samples, human metapneumovirus in 27 (10.3%) samples, influenza A virus in 14 (5.4%) samples, rhinovirus in 4 (1.5%) samples, adenovirus in 2 (0.8%) samples, and parainfluenza virus type 3 in 1 (0.4%) sample. Respiratory syncytial virus was associated with human metapneumovirus in 9 (3.4%) samples.

Clinical characteristics of the HBoV-infected children are shown in the Table. Children infected with only HBoV had mild-to-moderate fevers. Leukocyte counts and C-reactive protein levels were normal or moderately elevated. Chest radiographs obtained for 7 children showed abnormalities such as hyperinflation and interstitial infiltrates. Bronchiolitis was the major diagnosis. Dyspnea, respiratory distress, and cough were the most common respiratory symptoms observed. Four (44%) HBoV-infected children were born preterm, which suggests that these children have an increased susceptibility to HBoV-associated diseases. All children