Mycobacterial disease in patients with chronic granulomatous disease: A retrospective analysis of 71 cases

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Background: Chronic granulomatous disease (CGD) is a rare primary immunodeficiency caused by inborn errors of the phagocyte nicotinamide adenine dinucleotide phosphate oxidase complex. From the first year of life onward, most affected patients display multiple, severe, and recurrent infections caused by bacteria and fungi. Mycobacterial infections have also been reported in some patients.

Objective: Our objective was to assess the effect of mycobacterial disease in patients with CGD.

Methods: We analyzed retrospectively the clinical features of mycobacterial disease in 71 patients with CGD. Tuberculosis and BCG disease were diagnosed on the basis of microbiological, pathological, and/or clinical criteria.

Results: Thirty-one (44%) patients had tuberculosis, and 53 (75%) presented with adverse effects of BCG vaccination; 13 (18%) had both tuberculosis and BCG infections. None of these patients displayed clinical disease caused by environmental mycobacteria, *Mycobacterium leprae*, or *Mycobacterium ulcerans*. Most patients (76%) also had other pyogenic and fungal infections, but 24% presented solely with mycobacterial disease. Most patients presented a single localized episode of mycobacterial disease (37%), but recurrence (18%), disseminated disease (27%), and even death (18%) were also observed. One common feature in these patients was an early age at presentation for BCG disease. Mycobacterial disease was the first clinical manifestation of CGD in 60% of these patients.

Conclusion: Mycobacterial disease is relatively common in patients with CGD living in countries in which tuberculosis is endemic, BCG vaccine is mandatory, or both. Adverse reactions to BCG and severe forms of tuberculosis should lead to a suspicion of CGD. BCG vaccine is contraindicated in patients with CGD. (J Allergy Clin Immunol 2016;138:241-8.)

Key words: *Mycobacteria, BCG, chronic granulomatous disease, tuberculosis, primary immunodeficiency*

Chronic granulomatous disease (CGD) is a primary immunodeficiency (PID) characterized by the production of reactive oxygen species in small amounts, if at all, by phagocytes because of a deficiency of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase.[1-3] The phagocyte NADPH oxidase is an enzymatic complex composed of a membrane-bound core, the heterodimeric flavocytochrome, consisting of gp91^{phox} (encoded by *CYBB*) and p22^{phox} *(CYBA)*, and the cytosolic subunits p47^{phox} *(NCF1)*, p67^{phox} *(NCF2)*, and p40^{phox} *(NCF4)*. Mutations in any of the 5 genes (*CYBB*, *CYBA*, *NCF1*, *NCF2*, and *NCF4*) encoding the membrane-bound or cytosolic components of the phagocyte NADPH oxidase are responsible for CGD.[4-7]

Affected patients experience severe and recurrent infections caused by a diverse but relatively specific set of bacteria and fungi and from uncontrolled inflammation that can lead to granuloma

Abbreviations used
AR: Autosomal recessive
CGD: Chronic granulomatous disease
EM: Environmental mycobacteria
MSMD: Mendelian susceptibility to mycobacterial disease
NADPH: Nicotinamide adenine dinucleotide phosphate
PID: Primary immunodeficiency
XR: X-linked recessive

formation.[3,8] The main infections observed in patients with CGD are pneumonia, skin lesions, liver abscesses, and osteomyelitis. The most commonly isolated pathogens are *Aspergillus*, *Burkholderia*, *Nocardia*, and *Staphylococcus* species,[9] but gram-negative extracellular bacteria, such as *Serratia* species,[1] and other fungi, such as *Scedosporium* species,[10] are also frequently identified. Mycobacterial infections are not negligible among the pathogens causing infectious diseases in patients with CGD, especially in countries in which BCG vaccine is routinely administered, tuberculosis is endemic, or both.[11]

In a review of the literature, Deffert et al[12] reported a total of 297 cases of mycobacterial infections in patients with CGD.[13-24] BCG disease has been reported in 220 (74%) patients with CGD.[11] Similarly, tuberculosis has been reported in 59 (20%) patients.[11] Disease caused by environmental mycobacteria (EM) or unidentified species was reported in 18 (6%) patients.[12,25-33] However, the clinical features of mycobacterial disease remain poorly described. We examine the clinical manifestations of mycobacterial disease in 71 patients with CGD from 20 countries on 4 continents.

METHODS

Subjects and kindreds

Patients with CGD were recruited retrospectively for this study through extensive collaboration with clinicians in Latin America, Africa, Europe, and Asia, particularly in regions and countries in which tuberculosis is endemic and BCG vaccination is routine.[10] These patients were referred to the laboratory of human infectious diseases because of mycobacterial infections, and therefore they might not be representative of the entire CGD population. Informed consent forms were signed by the parents, as requested and approved by the institutional review boards of the various institutions involved. Data were collected from 2007 to 2013 and sent to Dr Bustamante.

A detailed questionnaire was completed by the physicians, including demographic data (age, sex, and country), biological tests for CGD diagnosis, mutations (where available), and infectious diseases. CGD was diagnosed on clinical grounds and confirmed with at least 1 of 3 laboratory tests,[34] the nitroblue tetrazolium reduction assay, dihydrorhodamine 123 oxidation, and/or superoxide production (cytochrome c reduction) assay, on whole-blood samples and/or EBV-transformed lymphoblastoid cell lines. Our analysis focused exclusively on mycobacterial infectious disease in patients with CGD and did not take into account other clinical signs. This constitutes one of the limitations of this retrospective study. Mycobacterial infections were diagnosed on the basis of clinical and radiologic findings, staining for acid-fast bacilli, supportive histology, serology (ELISA), and molecular (PCR) findings and microbiological culture results, when available.

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Adverse reactions to BCG vaccination were classified as follows: localized BCG-itis was defined as a local abscess or severe ulcer at the site of injection, and regional BCG-itis was defined as the involvement of regional ganglia in the axillary, supraclavicular, and cervical areas, including enlargement, suppuration, and/or fistula formation. Disseminated BCG-osis was defined as the presence of BCG at more than 1 remote site, a positive blood or bone marrow culture, or both.[13,35,36]

Tuberculosis infection was diagnosed according to the criteria proposed by Graham et al.[37] Basically, confirmed tuberculosis infection was defined as the presence of at least 1 sign or symptom suggestive of tuberculosis, with microbiological confirmation in the form of a positive culture for *Mycobacterium tuberculosis*. Patients with at least 1 sign or symptom suggestive of tuberculosis but negative cultures were classified as having probable or possible infection.[37] Patients were considered to have a probable infection if they had 1 sign or symptom suggestive of tuberculosis, a chest radiograph consistent with intrathoracic tuberculosis, and at least 1 of the following: (1) positive clinical response to antituberculosis treatment, (2) documented exposure to tuberculosis or close contact with a patient with tuberculosis, or (3) immunologic evidence of *M tuberculosis* in the form of a positive tuberculin skin test result or tuberculosis IFN-γ release assay. Possible tuberculosis was defined as a sign or symptom suggestive of tuberculosis infection and a positive clinical response to antituberculosis treatment but with a chest radiograph that was not consistent with pulmonary tuberculosis or documented exposure to tuberculosis, close contact with a patient with tuberculosis, or immunologic evidence of *M tuberculosis* in the tuberculin skin test or IFN-γ release assay.

TABLE I. Distribution of genetic and clinical phenotypes of patients with CGD

Sex	
Male	n = 58
Female	n = 13
Age at CGD diagnosis (mo), mean (range)	48.76 (2-180)
Genetic findings	
CYBB	n = 41
CYBA	n = 4
NCF1	n = 8
NCF2	n = 6
No reported	n = 12
Patients receiving BCG vaccine	n = 70
BCG-associated manifestations	n = 53
Localized	n = 33
Disseminated	n = 20
Age at BCG diagnosis (mo), mean (range)	9.22 (0-84)
Tuberculosis-associated manifestations	n = 31
Localized	n = 23
Disseminated	n = 8
Age at tuberculosis diagnosis (mo), mean (range)	56.7 (15-108)
Follow-up	
Alive	n = 58
Deceased	n = 13

RESULTS

Demographics

We enrolled and studied 71 children from 62 families in 20 countries on 4 continents (Table I and see Table E1 in this article's Online Repository at www.jacionline.org). All these children had mycobacterial disease in the context of CGD. Thirty-one (44%) children were from Latin America (Argentina, n = 7; Brazil, n = 4; Chile, n = 1, Colombia, n = 1; El Salvador, n = 2; and Mexico, n = 16), 6 were from China (8%), 4 were from France (6%), 3 were from Eastern Europe (4%; Slovakia, n = 2; Ukraine, n = 1), 20 were from the Middle East or Asia (28%; Iran, n = 1; Lebanon, n = 1; Pakistan, n = 1; Saudi Arabia, n = 1; Syria, n = 1; Turkey, n = 15), and 7 (10%) were from Africa (Algeria, n = 1; Congo, n = 1; Morocco, n = 2; and Tunisia, n = 3). Fifty-four (76%) of the patients referred to us displayed a wide spectrum of infectious diseases, including mycobacterial infections, and had been given a diagnosis of CGD.

Mean age at CGD diagnosis was 48.76 ± 43.63 months (range, 2-180 months; median, 34.5 months). CGD was diagnosed before the age of 5 years in 70% of the patients. Of the 71 patients, 58 (82%) were male, and 13 (18%) were female, reflecting the high proportion of X-linked CGD (Table I and see Table E1). Consanguinity was identified in 22 (35%) of 62 kindreds. CGD was autosomal recessive (AR) in 22% of these patients and X-linked recessive (XR) in 77% of cases, and there was 1 *de novo* XR mutation. In 7 consanguineous kindreds CGD was XR (kindreds 7, 23, 47, 52, 53, 59, and 61; Fig 1 and Table I).

Diagnosis of CGD

CGD was diagnosed in 71 patients (62 index cases and 9 siblings) from 62 kindreds. The biochemical diagnosis of CGD was based on nitroblue tetrazolium tests on granulocytes in 26 (37%) patients, dihydrorhodamine 123 in granulocytes in 19 (27%) patients, and cytochrome c reduction in EBV-transformed lymphoblastoid cell lines in 2 (3%) patients (see Table E1). A combination of 2 or more of these assays had been carried out for 13 (18%) of the 71 patients. In 11 (15%) cases CGD was suspected on clinical grounds and diagnosed directly by using genetic investigations to search for a specific mutation previously identified in another family member. Overall, 85% of the patients were identified as having CGD by their referring clinicians, with the remaining 15% being initially and, with hindsight, interestingly referred for Mendelian susceptibility to mycobacterial disease (MSMD) because of the occurrence of infections caused by mycobacteria.[38]

For 12 (17%) of the 71 patients, no genetic material was available for molecular diagnosis, and CGD was diagnosed on the basis of a clinical and biochemical phenotype of the disease. The sequencing of phagocyte NADPH component genes revealed 44 different mutations (see Table E1). Mutations in *CYBB*, which encodes gp91^{phox}, were found in 41 (57.8%) patients (see Table E1). Other mutations were found in the autosomal genes *NCF1* (n = 8 [11%]), *NCF2* (n = 6 [8%]), and *CYBA* (n = 4 [5.6%]). No mutations were found in *NCF4*. There were 22 (31%) patients carrying missense mutations and 12 (17%) patients carrying nonsense mutations; 12 (17%) patients had a deletion, 12 (17%) patients had an intronic splice-site mutation, and 1 patient had a compound heterozygous mutation, including a missense mutation and a deletion (see Table E1). In total, 34 of these mutations had already been identified in other patients with CGD.[5,6,39]

BCG disease

In total, 70 (98%) of the 71 children were vaccinated with BCG at birth or shortly thereafter. The vaccine strain was reported in 54 patients from Denmark (n = 19), Japan (n = 2), Moreau (n = 4), Pasteur (n = 12), and Russia (n = 17); the strain was not reported for 16 patients. The vaccine was injected into the deltoid region in all cases. Mean age at vaccination was 1.69 months (SD, ±5.05; range, 0-30 months; 77% were vaccinated by the age of 1 month).

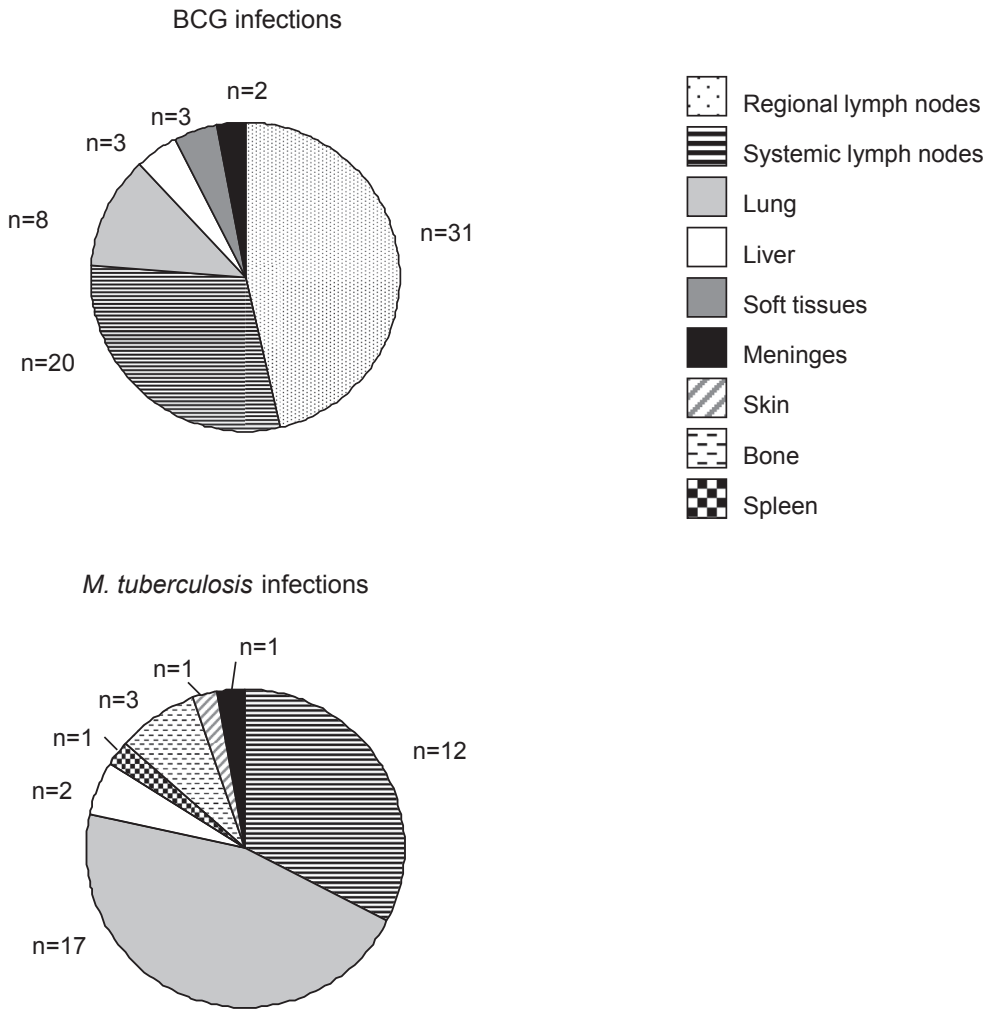

FIG 1. Mycobacterial infection sites in patients with CGD. Numbers of patients with BCG and *M tuberculosis* infections are shown.

Complications caused by BCG vaccination were found in 75% (n = 53) of the patients with mycobacterial diseases. Fifty-three patients had BCG disease (localized, n = 33; disseminated, n = 20), resulting in an attack rate of 76% in this case series; 17 (24%) patients had no adverse reaction. Mean age at diagnosis of an adverse reaction to BCG was 9.22 months (SD, ±14.8 month; range, 0-84 months; median, 3 months; 80% of adverse reactions were identified by age 12 months).

Clinical suspicion of an adverse reaction to BCG is usually straightforward because a local abscess or severe ulcer at the site of injection or involvement of the regional ipsilateral ganglia with suppuration, fistula formation, or both is observed shortly after vaccination. Accordingly, BCG-itis was diagnosed on purely clinical grounds in 17 (24%) patients. Positive cultures were obtained for 9 (13%) patients, and diagnosis was based on a combination of laboratory, histologic, and clinical criteria in 23 (32%) patients (Fig 1 and Table I). The adverse reaction to BCG was the first sign of the disease in 39 (55%) of the 53 children with such a reaction.

A local or regional reaction (BCG-itis) was reported in 33 (63%) of the 53 patients with an adverse reaction, and a disseminated reaction (BCG-osis) was observed in the other 20 (37%) patients. BCG was the infectious agent most frequently identified as responsible for the first clinical signs, as in patients with MSMD in countries in which BCG vaccination is mandatory. Most of the 70 patients presented only a single adverse reaction to BCG (42 [59%] patients). A single relapse was reported in 8 (12%) patients (Fig 1 and Tables II and III). Regional BCG-itis was observed in 33 (46%) patients, all of whom displayed local or regional involvement at the site of vaccine injection, with development of ipsilateral regional lymph node enlargement after BCG vaccination (Fig 1).

No treatment for BCG-itis was given to 13 patients (20% of cases), 1 patient underwent axillary lymph node excision, and antituberculosis antibiotics were given to 35 (49%) of the children with adverse reactions to BCG. However, the therapeutic regimen for mycobacterial infectious diseases (BCG and tuberculosis) differed between countries and medical centers in terms of the drugs used and the duration of treatment. Therefore it was not possible to evaluate and compare the effect of the various treatments in this cohort.

Disseminated BCG disease (BCG-osis) occurred in 12 (28%) patients. BCG infection was diagnosed at various anatomic sites. Most episodes (n = 8 [40%]) involved the lymph nodes and lung

TABLE II. Number of episodes of mycobacterial infections in patients with CGD

Site of disease	No. of patients with 1 episode	No. of patients with >1 episode
BCG infection sites		
Regional lymph nodes	26	5
Systemic lymph nodes	6	14
Lung	0	8
Liver	0	3
Soft tissues	0	3
Meninges	0	2
Mtb infection sites		
Lymph nodes	5	7
Lung	5	12
Liver	0	2
Spleen	0	1
Bone	1	2
Skin	0	1
Meninges	1	0

Mtb, *Mycobacterium tuberculosis*.

TABLE III. Patients displaying BCG and *M tuberculosis* infections

Site of tuberculosis infection	No. of patients	Site of BCG infection	No. of patients
Lymph nodes	4	Regional lymph nodes	8
Lung	10	Systemic lymph nodes	5
Liver	3	Lung	0
Spleen	1	Liver	0
Bone	0	Soft tissues	0
Skin	0	CNS	0
Meninges	0	Bone	0

CNS, Central nervous system.

tissue; 3 (16%) patients presented with BCG dissemination to the lymph nodes and liver. Soft-tissue BCG infection was observed in 4 (20%) patients, 2 of whom also presented with meningeal involvement. The other patients (n = 5) presented with multiple systemic lymph node involvement (Fig 1 and Tables II and III). Only 1 of the patients did not receive antibiotic treatment for the disease. Three patients died from BCG-osis (11.III.5, 24.II.3, and 65.II.6).

Tuberculosis

Thirty-one (44%) children with CGD had tuberculosis based on the criteria proposed by Graham et al.[37] Thirteen (18%) had both an adverse reaction to BCG (a reaction in 8 patients and disseminated BCG in 5 patients) and tuberculosis (Fig 1 and Table III). The diagnosis of tuberculosis was confirmed by means of culture in 6 (19.3%) patients, whereas probable tuberculosis was diagnosed in 15 (48.4%) patients and possible tuberculosis was diagnosed in 10 (32.3%) patients. Mean age at tuberculosis diagnosis was 56.7 months (SD, ±26.5 months; range, 15-108 months). Tuberculosis was intrathoracic in 23 (74%) patients and disseminated in the other 8 (26%) patients. The most common site for tuberculosis was the lungs (n = 19 [61%]), followed by the peripheral lymph nodes (n = 8 [26%]), bones (n = 1), and meninges (n = 1); 2 patients had disseminated intrathoracic and bone tuberculosis (19.II.3 and 50.II.3), and 2 children had miliary tuberculosis (54.II.7 and 61.II.1, Fig 1). The only patient not vaccinated with BCG (38.II.2) who had pulmonary tuberculosis (Fig 1) had no other mycobacterial diseases. Clinical signs of inflammation were not reported during the period of mycobacterial infectious disease, and the patients did not receive steroid treatment. All patients were treated with multiple-drug therapy. In most patients long-term remission was achieved with a combination of 3 or 4 antibiotics (rifampicin, ethambutol, isoniazid, and/or streptomycin). However, relapses occurred in 5 (16%) patients. None of the patients in this cohort displayed EM disease, as suspected or documented by culture or clinical response to specific therapy.

Other infections

Fifty-four (76%) patients had other bacterial and fungal infections caused by common microorganisms affecting children with CGD. The organisms most frequently isolated were *Staphylococcus*, *Aspergillus*, and *Salmonella* species, followed by *Candida*, *Klebsiella*, *Serratia*, *Pseudomonas*, *Acinetobacter*, *Nocardia*, *Streptococcus*, *Burkholderia*, *Enterobacter*, *Giardia*, *Leishmania*, *Citrobacter*, *Actinomyces*, and *Entamoeba* species. Twenty-three (32%) patients had intracellular bacterial infections other than mycobacterial infections, and 11 (15%) of these patients had *Salmonella* species infections. Pneumonia was the most common clinical sign of bacterial or fungal infection, followed by lymphadenitis, skin, liver, and perianal abscesses. Mean age at first pyogenic infection was 24.97 months (SD, 37.9 months; range, 0-180 months).

Clinical outcome

Fifty-eight (82%) patients are still alive, and their most recent follow-up visit occurred at a mean age of 7.11 years (SD, ±4.67 years; range, 1-20 years). Seven patients with XR-CGD underwent hematopoietic stem cell transplantation; none displayed signs of mycobacterial infections after immune reconstitution. Thirteen (18%) of the 71 patients died during the observation period: 2 patients with XR-CGD, 7 patients with the AR form of the disorder, and 4 with an unidentified genetic cause. Surprisingly, more patients died with the AR form of the disorder (54%) than with the XR form (15%). Four (31.3%) patients (3 male and 1 female) died without a genetic diagnosis.

Infections caused by *Aspergillus* species were the most common cause of death, accounting for more than one third of all deaths. Five patients with CGD, including 3 with AR-CGD (54.II.7, 57.II.1, and 66.II.1) and 2 with an unidentified genetic cause (21.II.4 and 47.II.1, Table I), died of sepsis caused by *Aspergillus* species in the major organs. One patient died of Hodgkin lymphoma (41.V.1). Three patients died from septicemia without isolation of the causal microorganism (4.II.5, 39.II.1, and 58.II.1). Four deaths were attributed to disseminated mycobacterial infection: 3 cases of BCG-osis (11.III.5, 24.II.3, and 65.II.6) and 1 case of miliary tuberculosis (21.II.4). Patient 11.II.5 was vaccinated with BCG after the age of 1 month. At 6 months of age, he was admitted to the hospital for disseminated disease affecting the liver, spleen, lungs, and lymph nodes and multiple cutaneous abscesses. He was treated with isoniazid, pyrazinamide, and rifampicin but died soon after admission. Necropsy showed multiple granulomas with bacilli. Patient 24.II.3 was vaccinated with BCG at birth. Three months

later, she had axillary adenitis with fistulization. Despite surgical excision, the infectious disease continued to spread, eventually reaching the central nervous system. The patient was treated with 4 antimycobacterial antibiotics but died at the age of 13 months. Finally, patient 65.II.6 was vaccinated with BCG at birth. Three months after vaccination, an enlarged lymph node was observed in the right armpit. At the age of 2 years, the lymph node ruptured and suppurated. The patient was hospitalized for generalized disease affecting the lymph nodes and lungs, with negative cultures. He received isoniazid, pyrazinamide, rifampicin, and ethambutol. He remained asymptomatic until the age of 2 years, when he was hospitalized for a relapse of mycobacterial disease; he died at age 5 years. These 4 patients (11.III.5, 24.II.3, 65.II.6, and 21.II.4) also had other infections of various severities during their lives.

DISCUSSION

We describe here a retrospectively collected international series of 71 patients with CGD and mycobacterial diseases. We reviewed only the clinical manifestations caused by mycobacterial diseases. Fifty-three patients had BCG disease, 33 of whom had a localized or regional form, with the others displaying disseminated disease; 31 patients had tuberculosis. None of the patients had a diagnosis of inflammatory disease, and none were treated with steroids (or any other immunosuppressant) before or during mycobacterial infections. Fifty-four patients also displayed susceptibility to other bacterial or fungal infections, and the microorganisms isolated were among the pathogens known to be associated with CGD.[8] The clinical manifestations of mycobacterial disease and the microbes implicated (BCG and *M tuberculosis*) in patients with CGD reflected an impairment or abolition of NADPH oxidase activity in macrophages. Patients with MSMD caused by particular *CYBB* mutations (or patients with X-linked recessive type 2 MSMD)[40,41] specifically disrupting the respiratory burst in monocyte-derived macrophages (respecting granulocytes and monocyte-derived dendritic cells) provided a cellular explanation for the susceptibility to mycobacteria in patients with CGD.[41,42] It is difficult to exclude the possibility of susceptibility to *Mycobacterium ulcerans*, the causal agent of Buruli ulcer, or *Mycobacterium leprae*, the causal agent of leprosy, in patients with CGD because of the low probability of patients from this series (and of previously described patients with CGD) to have these microbes. Nevertheless, the lack of EM disease in our series is consistent with the well-documented observation that NADPH is not critical for the control of these diverse and ubiquitous pathogens.[43]

Adverse reactions to BCG in the general population are rare, depending on the vaccine strain used in different countries.[11] CGD is a rare PID affecting about 1 in every 250,000 live births. Adverse reactions to BCG present as a very early event, when children are only a few months old, in many cases before they have been given a diagnosis of CGD and often before they have an infection caused by pyogenic bacteria or fungi. BCG-related disease might be the first sign of CGD, reflecting early exposure caused by mandatory vaccination at birth. In the most recent report on CGD, Roos et al[5,6] described 1150 patients with XR and AR deficiencies. Adverse reactions to BCG are probably not a rare sign of CGD, as shown by the experience of countries in which BCG vaccination is routinely carried out at birth or in which tuberculosis is endemic.[14,15,44,45] The proportion of patients with CGD with mycobacterial infections can be up to 25%. For instance, before BCG vaccination at birth was proscribed in France, 22% of French patients with CGD had a reaction to BCG.[29,45] In a Moroccan series, 2 (16.7%) of 12 vaccinated patients presented with clinical BCG disease,[19] and in a Mexican series, 16 (32%) of 50 patients with CGD vaccinated with BCG presented with clinical BCG disease (Blancas Galicia, personal communication).

Another key finding of this study is the pertinent proportion of patients having *M tuberculosis* infection. Genetic factors make a major contribution to tuberculosis, as shown by the occurrence of tuberculosis in children displaying PIDs.[46] CGD is a PID frequently associated with *M tuberculosis* infection, providing support for the notion that tuberculosis might be both an infectious and a genetic disease, at least in children, and that NADPH activity plays an important role in mycobacterial immunity. In countries in which tuberculosis is endemic, patients with CGD have been shown to be highly vulnerable to tuberculosis. In Argentina, Hong Kong, and Iran, up to 11%,[24] 54.5%,[15] and 31.7%[44] of patients with CGD, respectively, were found to have tuberculosis. Moreover, severe tuberculosis has been reported in children with IL-12 receptor β1 deficiency, providing further evidence to support the hypothesis that childhood tuberculosis is in some cases also a Mendelian disease.[46-48] In any event children with severe forms of tuberculosis should be tested for PIDs.[46]

The oxidative burst is an important component of human immunity to mycobacteria, as suggested by studies in animal models[49-51] and of patients with X-linked recessive type 2 MSMD, who carry macrophage-tropic germline mutations of *CYBB*.[41,42] Moreover, increasing numbers of patients with CGD have been identified as susceptible to mycobacterial disease. However, this association has been overlooked in countries in which exposure to mycobacteria is low. Susceptibility to mycobacterial infections is not the best known feature of CGD, and therefore CGD is often diagnosed long after the initial adverse reaction to BCG. Greater awareness of this aspect of the disease might accelerate diagnosis.

In conclusion, CGD should be considered in children with BCG-itis and BCG-osis and in selected patients with severe tuberculosis, even in the absence of the cardinal features of CGD, which can occur later in life. Likewise, BCG vaccination should be formally contraindicated in patients with a diagnosis or suspicion of CGD and their newborn siblings, as already advised for children with other PIDs affecting T cells, phagocytes, or IFN-γ immunity.[52]

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Clinical implications: The susceptibility of patients with CGD to mycobacteria highlights the importance of this pathway in human immunity to mycobacteria, providing new insight into the IFN-γ–dependent mechanisms involved in protective immunity against mycobacteria within phagocytes.

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TABLE E1. Familial, genetic, geographic, and clinical phenotypes of patients with CGD

Patient no.	Kindred	Code	Country	Sex	Test	Gene	Mutation	Follow-up	BCG	*M tuberculosis*	*Salmonella* species	Other	Reference
1	1	II.2	Argentina	Male	NBT/DHR	*CYBB*	c.45+3_5delAAG	Alive	L	Confirmed	Yes	*Staphylococcus aureus* *Aspergillus* species	
2	2	II.2	Argentina	Male	NBT/DHR	*CYBB*	p.Cys329Arg	Alive	NR	Confirmed	–	*Nocardia* species	Barese et al[24]
3	3	II.4	Argentina	Male	DHR	*CYBB*	c.617delC	Alive	D	–	–	*Aspergillus* species	
4	4	II.5	Argentina	Male	NBT	*CYBB*	p.Gly359Ala	Deceased	L	–	–	*Nocardia* species	
5	5	II.1	Argentina	Male	NBT/DHR	*CYBB*	p.Trp361Gly	Alive	L	–	–	*Staphylococcus aureus*	
6	6	II.1	Argentina	Male	DHR	*CYBB*	p.Arg130X	Alive	L	–	–	*Acinetobacter* species *Klebsiella* species	
7	7	II.1	Argentina	Male	DHR	*CYBA*	p.Ser118Arg	Alive	L	–	–	*Nocardia* species *Aspergillus* species *Candida* species	
8	8	II.2	Brazil	Male	NBT/DHR	*CYBB*	p.Cys185Arg	Alive	L	–	–	UE	de Oliveira-Junior et al[39]
9	9	II.5	Brazil	Male	NBT/DHR	*CYBB*	c.1166G>A	Alive	L	–	–	*Staphylococcus aureus*	de Oliveira-Junior et al[39]
10	10	III.4	Brazil	Male	NBT/DHR	*CYBB*	p.His101Arg	Alive	L	–	–	UE	de Oliveira-Junior et al[39]
11	10	III.5	Brazil	Male	NBT/DHR	*CYBB*	p.His101Arg	Deceased	D	–	–	–	de Oliveira-Junior et al[39]
12	11	II.1	Chile	Male	DHR	*CYBA*	c.114delT c.268C>G	Alive	D	Probable	–	*Staphylococcus aureus*	de Oliveira-Junior et al[39]
13	12	II.2	Colombia	Male	NBT	*CYBB*	p.Met1Val	Alive	D	Probable	Yes	–	
14	13	II.1	El Salvador	Male	NBT	E ?	E ?	Alive	NR	Confirmed	–	*S epidermidis*	
15	13	II.2	El Salvador	Male	NBT	E ?	E ?	Alive	NR	Confirmed	Yes	*S epidermidis* *Giardia lamblia* *Ascaris lumbricoides*	
16	14	III.1	Mexico	Male	NBT	*CYBB*	p.Pro339His	Alive	L	–	–	*Staphylococcus* species *Acinetobacter* species *Pseudomonas* species	de Oliveira-Junior et al[39]
17	15	II.2	Mexico	Male	NBT	*CYBB*	p.Trp483Arg	Alive	L	–	–	UE	de Oliveira-Junior et al[39]
18	15	II.3	Mexico	Male	NBT	*CYBB*	p.Trp483Arg	Alive	L	–	–	UE	de Oliveira-Junior et al[39]
19	16	II.3	Mexico	Male	NBT	*CYBB*	p.Trp206X	Alive	L	Possible	–	*Staphylococcus aureus*	de Oliveira-Junior et al[39]
20	17	II.1	Mexico	Male	NBT	E ?	E ?	Alive	L	–	–	*Serratia* species *Pseudomonas* species *Streptococcus viridans*	
21	18	II.4	Mexico	Male	NBT	E ?	E ?	Deceased	NR	Confirmed	–	*Aspergillus* species	de Oliveira-Junior et al[39]
22	19	II.1	Mexico	Male	NBT/DHR	*NCF1*	p.Tyr26HisfsX26	Alive	D	–	Yes	–	de Oliveira-Junior et al[39]
23	20	II.1	Mexico	Male	NBT	E ?	E ?	Deceased	D	–	–	*Citrobacter freundii* *Burkholderia cepacia* *Candida krusei*	de Oliveira-Junior et al[39]
24	21	II.3	Mexico	Female	NBT	E ?	E ?	Deceased	NR	Probable	–	*Serratia* species *Staphylococcus aureus*	de Oliveira-Junior et al[39]
25	22	II.5	Mexico	Female	NBT	E ?	E ?	Alive	L	–	–	*Serratia marcescens* *Staphylococcus aureus*	de Oliveira-Junior et al[39]
26	23	II.1	Mexico	Male	NBT	*CYBB*	p.Arg226X	Alive	L	–	–	*Klebsiella oxytoca*	de Oliveira-Junior et al[39]
27	23	II.2	Mexico	Male	NBT	*CYBB*	p.Arg226X	Alive	NR	Probable	–	*Pseudomonas* species *Burkholderia cepacia* *Giardia lamblia* *Entamoeba histolytica*	de Oliveira-Junior et al[39]
28	24	II.1	Mexico	Male	NBT	*CYBB*	p.Gln93X	Alive	L	–	–	UE	de Oliveira-Junior et al[39]

(Continued)

TABLE E1. *(Continued)*

Patient no.	Kindred	Code	Country	Sex	Test	Gene	Mutation	Follow-up	BCG	*M tuberculosis*	*Salmonella* species	Other	Reference
29	25	II.1	Mexico	Male	NBT	*CYBB*	p.Trp28X	Alive	NR	Possible	Yes	–	
30	26	II.3	Mexico	Male	NBT	*E ?*	E ?	Alive	NR	Probable	–	*Burkholderia cepacia* UE	de Oliveira-Junior et al[39]
31	27	II.1	Mexico	Female	NBT	*NCF1*	p.Tyr26HisfsX26	Alive	L	–	–	*Klebsiella pneumoniae* UE	
32	28	II.3	China	Male	NE	*CYBB*	p.Cys445Arg	Alive	D	Probable	–	UE	He et al[21]
33	29	II.2	China	Male	NE	*CYBB*	p.Gly322Glu	Alive	D	Probable	–	*Aspergillus* species	He et al[21]
34	30	II.1	China	Male	NE	*CYBB*	p.Gly412Arg	Alive	L	Probable	–	UE	He et al[21]
35	31	II.1	China	Male	NE	*CYBB*	c.252G>T	Alive	D	–	–	UE	He et al[21]
36	32	II.1	China	Male	NBT	*CYBB*	p.Tyr440X	Alive	L	Probable	–	UE	He et al[21]
37	33	II.1	China	Male	DHR	*CYBB*	c.252G>T	Alive	L	Probable	–	–	He et al[21]
38	34	II.2	France	Male	DHR	*CYBB*	p.Ala55Asp	Alive	NV	Confirmed	–	UE	
39	35	II.1	France	Male	DHR	*NCF1*	p.Tyr26HisfsX26	Deceased	L	–	–	UE	Lugo Reyes et al[20]
40	36	V.3	France	Female	NBT	*NCF1*	p.Tyr26HisfsX26	Alive	L	–	–	UE	Lugo Reyes et al[20]
41	36	V.1	France	Female	Cytochrome c	*NCF1*	p.Tyr26HisfsX26	Deceased	NR	Confirmed	–	–	Lugo Reyes et al[20]
42	37	II.1	Ukraine	Female	NBT + cytochrome c	E ?	E ?	Alive	L	Probable	Yes	*Klebsiella* species	
43	38	II.1	Slovakia	Male	NBT/cytochrome c	*CYBB*	p.Arg84Lys	Alive	L	–	Yes	*Pseudomonas* species	
44	39	II.1	Slovakia	Male	NBT	*CYBB*	p.Arg84Lys	Alive	L	–	–	*Staphylococcus aureus* *Pseudomonas aeruginosa* *Candida* species	
45	40	II.2	Iran	Male	NBT	*CYBA*	g.exon3_6del	Alive	D	–	–	*Candida* species	
46	41	III.2	Lebanon	Male	DHR	*NCF2*	p.Val123_Trp167del	Alive	D	–	Yes	UE	Gentsch et al[23]
47	42	II.1	Pakistan	Male	DHR	E ?	E ?	Deceased	D	–	–	*Staphylococcus* species *Serratia* species *Aspergillus* species	
48	43	II.1	Saudi Arabia	Male	NBT/DHR	*NCF2*	p.Arg77X	Alive	D	–	–	*Serratia* species UE	
49	44	II.2	Syria	Female	DHR	*CYBA*	p.Thr74X78	Alive	L	–	–	–	
50	45	II.3	Turkey	Male	NBT/DHR	*CYBB*	c.483+978G>T	Alive	D	Probable	–	–	Bustamante et al[13]
51	46	II.3	Turkey	Male	DHR	*CYBB*	p.Ala524Val	Alive	L	Probable	Yes	*Klebsiella pneumoniae* *Pseudomonas aeruginosa* *Enterobacter* species *Aspergillus fumigatus* *Candida glabrata*	
52	47	II.1	Turkey	Male	DHR	*CYBB*	p.Pro383Leu	Alive	NR	Possible	–	–	
53	47	II.2	Turkey	Male	DHR	*CYBB*	p.Pro383Leu	Alive	NR	Possible	–	–	
54	48	II.7	Turkey	Female	DHR	*NCF1*	p.Tyr26HisfsX26	Deceased	NR	Confirmed	–	*Aspergillus fumigatus*	
55	49	II.3	Turkey	Male	DHR	*CYBB*	p.Arg91X	Alive	D	–	–	*Staphylococcus* species	
56	49	II.2	Turkey	Male	DHR	*CYBB*	p.Arg91X	Alive	L	Probable	–	–	
57	50	II.1	Turkey	Female	Cytochrome c	*NCF2*	p.Asn42Ser	Deceased	NR	Possible	–	*Aspergillus fumigatus*	
58	51	II.1	Turkey	Male	DHR	*NCF2*	c.175-1G>A	Deceased	L	–	–	*Actinomyces* species *Enterobacter* species	
59	52	II.1	Turkey	Male	Cytochrome c	*CYBB*	c.674+2T>G	Alive	D	–	–	UE	
60	53	II.1	Turkey	Male	DHR	*CYBB*	p.Arg130X	Alive	L	–	–	*Cryptosporidium parvum*	
61	54	II.4	Turkey	Female	DHR	*NCF1*	p.Tyr26HisfsX26	Alive	NR	Possible	–	–	
62	54	II.9	Turkey	Female	DHR	*NCF1*	p.Tyr26HisfsX26	Alive	NR	Possible	–	–	

(Continued)

TABLE E1. *(Continued)*

Patient no.	Kindred	Code	Country	Sex	Test	Gene	Mutation	Follow-up	BCG	*M tuberculosis*	*Salmonella* species	Other	Reference
63	55	II.2	Turkey	Male	DHR	E ?	E ?	Alive	NR	Possible	–	–	
64	56	II.6	Turkey	Female	DHR	E ?	E ?	Alive	L	–	–	–	
65	57	II.6	Tunisia	Male	NBT	*NCF2*	c.257+2T>C	Deceased	D	–	Yes	*Streptococcus* species *Acinetobacter baumannii* *Leishmania* species *Candida tropicalis*	El Kares et al[22]
66	57	II.1	Tunisia	Female	NBT	*NCF2*	c.257+2T>C	Deceased	NR	Probable	–	*Aspergillus* species	El Kares et al[22]
67	58	II.1	Tunisia	Male	DHR	*CYBB*	p.Trp337X	Alive	D	–	Yes	–	
68	59	II.1	Algeria	Male	DHR	*CYBB*	c.809delG	Alive	D	–	–	*Aspergillus* species *Candida albicans*	
69	60	II.1	Congo	Male	DHR	*CYBB*	c.1151+4A>T	Alive	D	–	–	*Klebsiella pneumoniae*	
70	61	II.1	Morocco	Male	NBT/DHR	*CYBB*	c.897+1G>T	Alive	L	–	–	–	Baba et al[19]
71	62	II.3	Morocco	Male	NBT/DHR	*CYBB*	c.674+5G>A	Alive	L	–	–	*Serratia* species	Baba et al[19]

D, Disseminated; *DHR*, dihydrorhodamine 123; *E ?*, genetic status could not be evaluated; *L*, local; *NBT*, nitroblue tetrazolium; *NE*, not evaluated; *NR*, BCG vaccinated with no adverse reaction; *NV*, non-BCG vaccinated; *UE*, unknown cause.