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Perspective

A novel view on the pathogenesis of complications after intravesical BCG for bladder cancer

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ABSTRACT

Intravesical bacillus Calmette-Guérin (BCG) is widely used for high-risk, non-muscle-invasive bladder cancer. This report describes four cases that illustrate the spectrum of BCG-induced complications, varying from granulomatous prostatitis to sepsis. There is considerable debate regarding whether inflammation or infection is the predominant mechanism in the pathogenesis of BCG disease. In two patients with a systemic illness, the symptoms first resolved after adding prednisone, indicating a principal role for inflammation in systemic disease. In vitro testing of T-cell responses and a mycobacterial growth inhibition assay were performed for these patients with systemic disease. The patient with mild symptoms showed more effective in vitro growth reduction of BCG, while the patient with sepsis and organ involvement had high T-cell responses but ineffective killing. While these findings are preliminary, it is believed that immunological assays, as described in this report, may provide a better insight into the pathogenesis of BCG disease in individual patients, justifying further research.

Introduction

Intravesical bacillus Calmette–Guérin (BCG) instillation following transurethral resection (TUR) is considered the gold standard in the treatment of high-risk, non-muscle-invasive bladder cancer. Compared with the use of TUR alone, additional intravesical BCG significantly reduces disease recurrence and the risk of progression to invasive disease (Shelley et al., 2010). However, BCG-related complications are common and may necessitate treatment discontinuation. Side effects limited to the genitourinary tract include chemical cystitis (35%), bacterial cystitis (23.3%), macroscopic haematuria (22.6%), and symptomatic granulomatous prostatitis (0.9%). Systemic complications are persistent high-grade fever as an isolated manifestation (2.9%), pneumonitis with or without hepatitis (0.7%), and sepsis (0.4%) (Brausi et al., 2014; Lamm et al., 1992). The International Bladder Cancer Group recommends deferring BCG instillation in the case of traumatic catheterization, concurrent urinary tract infection, and TUR in the past 2 weeks, since these risk factors may facilitate haematogenous dissemination (Witjes et al., 2008). In a randomized controlled trial comparing one-third dose to full-dose BCG for 1 or 3 years, the incidence of side effects did not differ between the groups (Brausi et al., 2014).

It is unclear whether inflammation or infection underlies these BCG-related complications, and their relative importance may vary between patients. Since a diagnostic tool to make this distinction is

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currently not available, treatment with tuberculostatic drugs is frequently initiated and anti-inflammatory drugs are added in the case of systemic disease, with the risk of side effects.

This report describes four cases illustrating the spectrum of BCG disease. In two patients with a systemic illness, immunological assays including a novel mycobacterial growth inhibition assay (MGIA) were performed, with the aim of studying the individual pathogenesis.

Case reports

The clinical characteristics of the patients are described in detail in Table 1. In short, patient 1 was a 66-year-old man complaining of anejaculation, dysuria, and frequency at 1 month after the sixth BCG instillation. A pelvic computed tomography (CT) scan showed prostate abnormalities (Figure 1A), with chronic granulomatous inflammation in a biopsy (Figure 2). The patient was treated with isoniazid and rifampicin for 6 months, resulting in the resolution of symptoms.

Patient 2, a 70-year-old man, presented with weight loss, malaise, and abdominal pain at 2 months after the 18th BCG instillation. A CT scan revealed a leaking aneurysm of the abdominal aorta, which was repaired via an endovascular approach. Blood cultures were negative (no mycobacterial culture was performed). After 10 months, a follow-up CT scan showed progression of the aneurysm with leakage (Figure 1B) and

Table 1
Overview of clinical characteristics, diagnostic and therapeutic approaches, treatment, and outcomes for four patients with BCG disease.

Patient number Age (years)/ sex	Symptoms	Underlying condition(s)	Type of BCG complication	Number of TURs	Time between last TUR and first BCG dose (days)	Number of instillations	Time between last BCG dose and onset of symptoms (days)
1 66/M	Anejaculation Dysuria Frequency	COPD Partial nephrectomy (renal cell carcinoma)	Granulomatous prostatitis and seminal vesiculitis	1	47	6	30
2 70/M	Weight loss Malaise Abdominal pain	Hypertension EVAR	Mycotic aneurysm	2	21	18	60
3 67/M	Fever (39.2 °C) Weight loss Malaise Fever after second BCG	No relevant history BCG vaccination	Systemic reaction without organ involvement	2	23	1	4
4 77/M	Fever (39.5 °C) Cough Dyspnoea	COPD Gout Mitral valve insufficiency	Pneumonitis and hepatitis	3	43	Since last TUR: 14 Total: 59 Traumatic instillation	1

Patient number Age (years)/ sex	Laboratory results	Radiology	Microbiology	Histology	Antibiotics	Steroids	Outcome
1 66/M	ESR 25 PSA 13.3 AST 34 ALT 49 ALP 66 GGT 43	Pelvic CT scan: Inhomogeneous enlarged prostate with hyperdensity of right seminal vesicle	Urine culture negative for common bacteria and BCG	Chronic granulomatous inflammation in prostate	INH 300 mg/ day RIF 600 mg/ day (6 months)	None	Resolution of BCG disease Cystoprostatectomy due to tumour progression
2 70/M	ESR 33 AST 52 ALT 31 ALP 234 GGT 154	CT scan: Progression of aneurysm with type 1 endoleak Para-aortic lymphadenopathy Splenomegaly	PCR on resected (para)aortic tissue positive for *M. bovis*	Chronic granulomatous inflammation in aortic wall and lymph nodes	None	None	Death due to aortic rupture 1 week after aortic reconstruction, lymph node resection, and splenectomy
3 67/M	ESR 103 AST 32 ALT 54 ALP 75 GGT 39	CT scan (day 20 after onset of symptoms): No relevant findings	Urine and blood cultures negative for common bacteria and BCG	ND	Ciprofloxacin 500 mg twice a day (2 weeks)	Prednisone 15 mg/day with tapering schedule (2 months)	Resolution of BCG disease TUR + intravesical KLH due to recurrence, 2 years recurrence-free
4 77/M	CRP 48 AST 67 ALT 58 ALP 153 GGT 132	CT scan: Miliary lung pattern Hepatomegaly Perihilar lymphadenopathy	Urine and blood cultures negative for common bacteria and BCG	ND	INH 300 mg/ day RIF 600 mg/ day ETH 1600 mg/ day (1 month, due to hepatotoxicity)	Prednisone 20 mg/day with tapering schedule (6 weeks)	Resolution of BCG disease Intravesical KLH → recurrence and death after 2 years

ALP (in U/l), alkaline phosphatase; ALT (in U/l), alanine aminotransferase; AST (in U/l), aspartate aminotransferase; BCG, bacillus Calmette–Guérin; COPD, chronic obstructive pulmonary disease; CRP (in mg/l), C-reactive protein; CT, computed tomography; ESR (in mm), erythrocyte sedimentation rate; ETH, ethambutol; EVAR, endovascular aneurysm repair; GGT (in U/l), gamma-glutamyltransferase; INH, isoniazid; KLH, keyhole limpet hemocyanin; M, male; *M. bovis*, *Mycobacterium bovis*; ND, not done; PSA (in μg/l), prostate-specific antigen; RIF, rifampicin; TUR, transurethral resection.

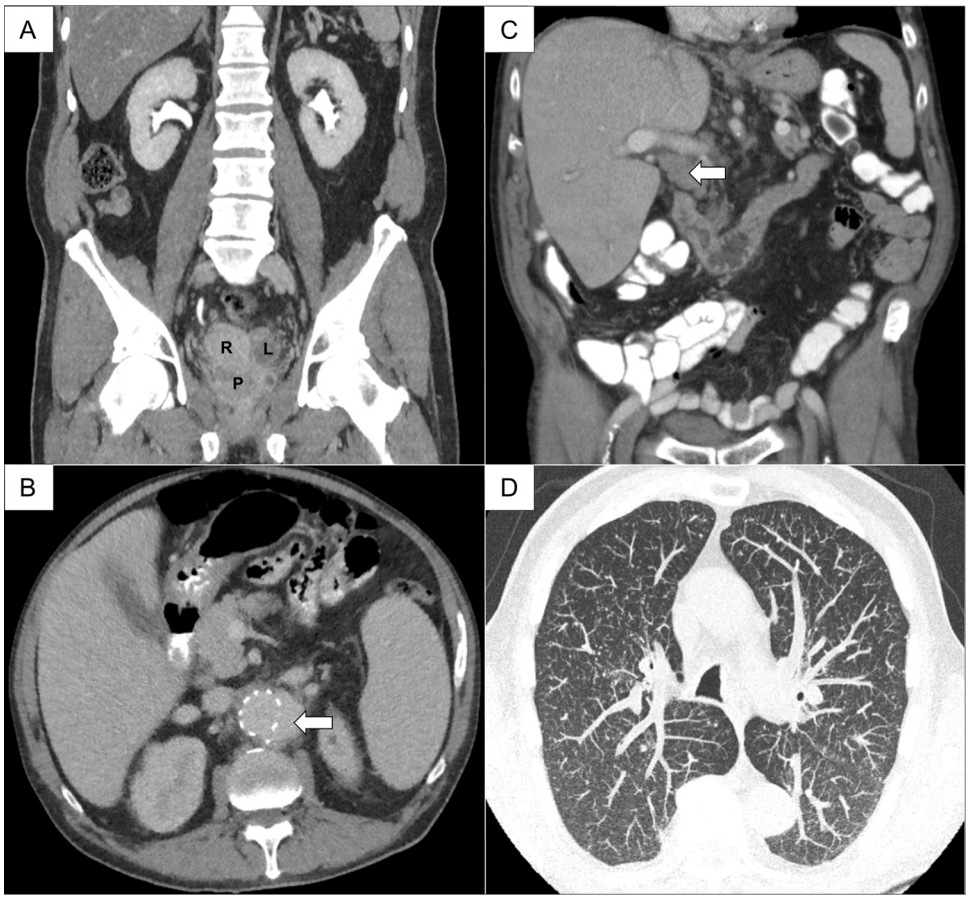

Figure 1. (A) Pelvic CT scan (coronal plane) of patient 1, displaying an inhomogeneous, enlarged prostate (P) and asymmetry of the seminal vesicles with hyperdensity of the right seminal vesicle (R) compared to the left seminal vesicle (L). (B) Transverse abdominal CT scan of patient 2 showing leakage of contrast fluid (arrow) outside the endovascular aortic stent. (C) Abdominal CT scan (coronal plane) of patient 4 showing hepatomegaly (craniocaudal diameter 22 cm) and perihilar lymphadenopathy (white arrow). (D) High-resolution thoracic CT scan (maximum intensity projection) of patient 4 demonstrating a bilateral miliary pattern with multiple sub-millimetre pulmonary nodules.

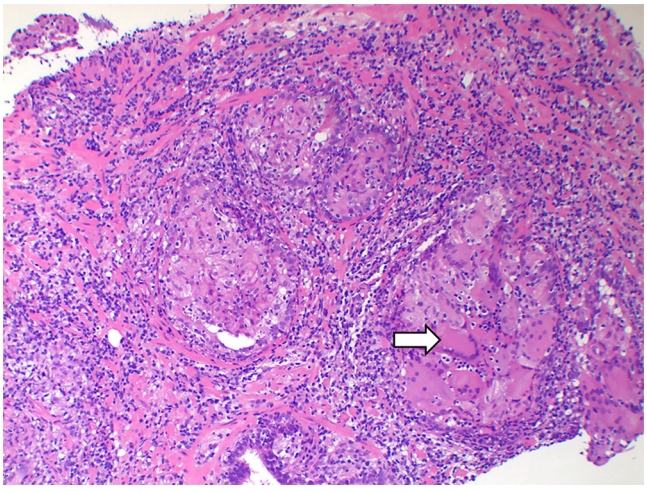

Figure 2. Prostate biopsy of patient 1 showing prostatic glands surrounded by a fibromuscular stroma and granulomatous inflammation with multiple giant cells (arrow).

splenomegaly. The patient underwent an aortic repair with resection of the infected tissue, para-aortic lymph nodes, and the spleen. Histology showed chronic granulomatous inflammation, and PCR on the resected tissue was positive for *Mycobacterium bovis*. However, before antibiotic treatment could be initiated the patient died due to aortic rupture.

Patient 3, a 67-year-old man who had been vaccinated with BCG in the past, developed a persistent high-grade fever, weight loss, and malaise at 4 days after his first BCG instillation. Pulmonary involvement was ruled out and liver enzymes were within normal limits. The fever persisted despite ciprofloxacin and only disappeared after prednisone was started. The high fever recurred after the second BCG instillation and BCG treatment was discontinued. After a renewed TUR and intravesical keyhole limpet hemocyanin (KLH) instillations for local tumour recurrence, the patient has been recurrence-free for 2 years.

Patient 4, a 77-year-old-man, presented with a high-grade fever, cough, and dyspnoea at 1 day after traumatic BCG instillation following many uneventful previous instillations. A CT scan revealed a miliary lung pattern, while elevated liver enzymes and hepatomegaly indicated hepatic involvement (Figure 1C, D). Treatment with isoniazid, rifampicin, and ethambutol was initiated, but his symptoms first resolved after prednisone was added 2 weeks later. The antibiotics were discontinued after 1 month due to serious hepatotoxicity and were not reintroduced given the favourable clinical course. KLH instillations were given for recurrence and a nephroureterectomy was performed after tumour progression. The patient died 2 years later from metastasized disease.

Immunological assays

Immunological assays were performed for patients 3 and 4 who both had early onset systemic complications. The study protocol (P07.048) allowing exploratory research of immune responses in

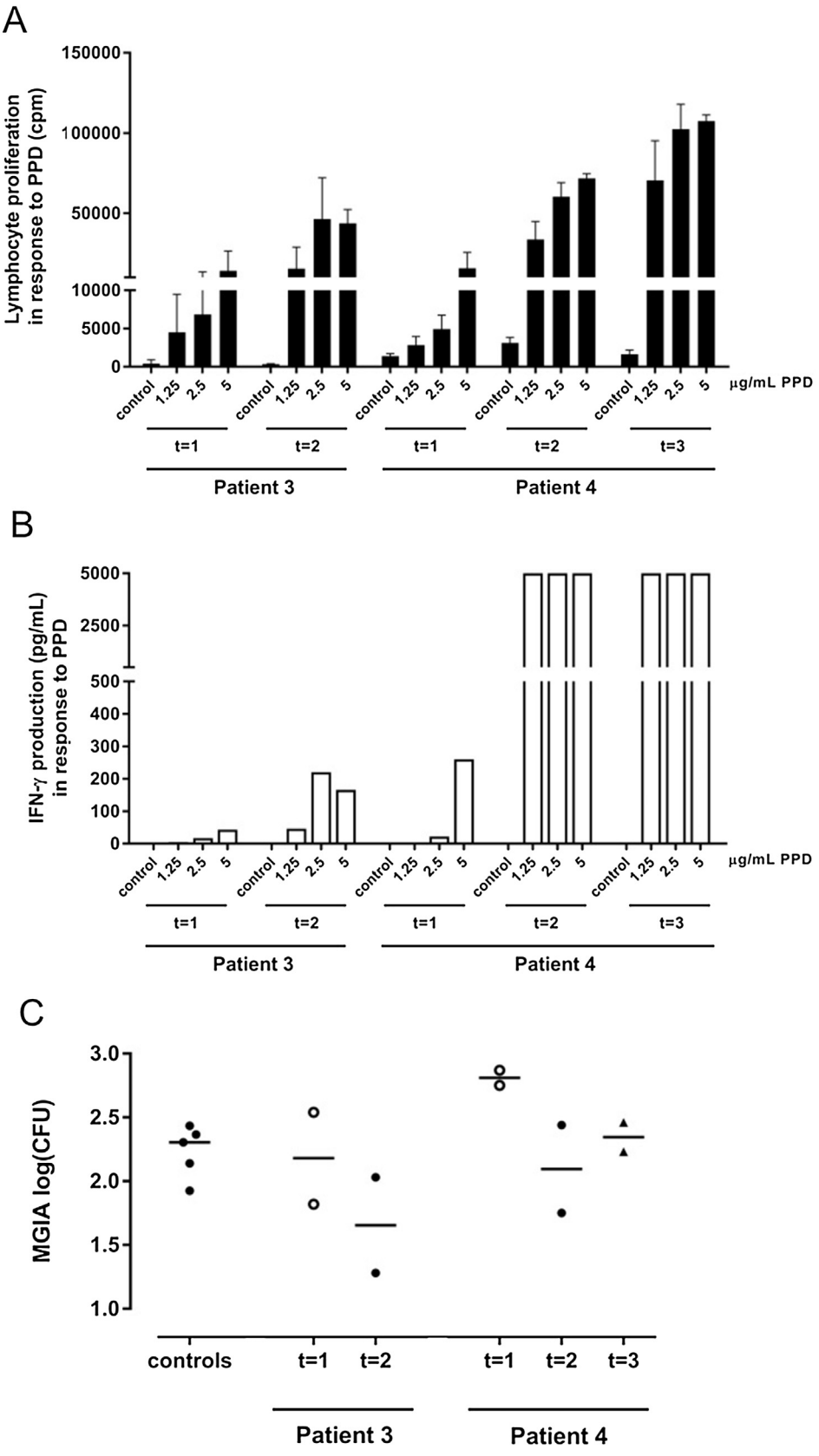

Figure 3. (A) Proliferation of T-lymphocytes in response to *Mycobacterium tuberculosis* purified protein derivative (PPD) or control. Lymphocyte proliferation is measured as the count per minute (cpm; ^{3}H-thymidine incorporation) for three different antigen (PPD) concentrations (µg/ml). Bars are medians with the standard deviation of three measurements. For patient 3, t = 1 is day 25 after BCG instillation (before initiating prednisone) and t = 2 is day 109 (following the cessation of prednisone). For patient 4, t = 1 is day 57 after BCG instillation after tapering of prednisone (dose 2.5 mg every other day), t = 2 is day 138 (no prednisone), and t = 3 is day 216 (no prednisone). (B) Levels of IFN-γ production by peripheral blood mononuclear cells in response to PPD or control medium; for time points, see the caption for Figure 3A. (C) Mycobacterial growth inhibition assay (MGIA): the capacity to control BCG growth is represented as log(CFU), as calculated from the time to positivity of the culture. Patients 3 and 4 are compared to five

mycobacterial disease was approved by the Institutional Review Board of Leiden University Medical Centre, and written informed consent was obtained from the patients.

The lymphocyte stimulation assay was performed as described previously (Arend et al., 2000). In short, peripheral blood mononuclear cells (PBMC) were incubated with *Mycobacterium tuberculosis* purified protein derivative (PPD) (Statens Serum Institut, Copenhagen, Denmark) at 37 °C in a humidified CO_2 incubator. After 6 days, the supernatants were harvested for analysis by interferon-gamma (IFN-γ) ELISA (U-CyTech, Utrecht, The Netherlands), and ^{3}H-thymidine was added for the last 18 h to assess proliferation.

For the MGIA, PBMC (1×10^6) were incubated with 100 colony-forming units (CFU) of BCG on a rotator at 37 °C in a humidified CO_2 incubator. After 4 days, cultures were transferred to Mycobacteria Growth Indicator Tubes (MGIT; Becton and Dickinson, Franklin Lakes, NJ, USA) and placed in a BACTEC incubator until growth was detected (Tanner et al., 2016). The mechanism of the control of BCG outgrowth has been published recently (Joosten et al., 2018).

Test results for patient 3, who had a high-grade fever without organ involvement, showed a moderate mycobacterial antigen-specific T-cell response, but enhanced in vitro growth inhibition of BCG compared to healthy controls. In contrast, the test results for patient 4, who presented with sepsis and organ involvement, were characterized by a higher mycobacterial antigen T-cell response associated with a lack of in vitro growth inhibition of BCG (Figure 3).

Discussion

The broad range of possible complications after intravesical BCG instillation, illustrated by these four patient cases, has been described previously (Perez-Jacoiste Asin et al., 2014). They vary from focal disease limited to the genitourinary tract, e.g., granulomatous prostatitis (patient 1), cystitis, or epididymo-orchitis, to focal disease outside the genitourinary tract, e.g., arthritis, mycotic aneurysm (patient 2), or uveitis, to systemic reaction without organ involvement (patient 3), and finally to full-blown sepsis with pneumonitis and/or hepatitis (patient 4).

In view of the discussion regarding the relative contribution of inflammation and infection to the pathogenesis of BCG-related complications, the finding that acid-fast staining, mycobacterial culture, and PCR often remain negative (as found in three of the four patients presented here), favours the inflammation hypothesis (Perez-Jacoiste Asin et al., 2014). Interestingly, both of the patients with systemic BCG disease did not respond to antibiotic therapy and symptoms only resolved after adding prednisone. A good outcome in patients with systemic disease treated exclusively with corticosteroids has been reported previously (LeMense and Strange, 1994). However, increased mortality was seen after treatment with prednisone alone in a mouse model of intraperitoneal BCG infection (Koukol et al., 1995), indicating that antibiotic therapy should not be withheld if infection is the predominant pathogenic mechanism. A randomized controlled trial comparing intravesical BCG with and without isoniazid prophylaxis around the time of instillations showed no difference in the incidence of focal or systemic complications, further supporting inflammation as an important pathogenic mechanism (Vegt et al., 1997).

Intravesical BCG results in marked leukocyturia, the influx of immune cells into the bladder wall, and the release of cytokines in the urine, which is more pronounced in the case of previous BCG exposure, reflecting recall immune activation (Redelman-Sidi et al., 2014). On the other hand, ofloxacin given after intravesical BCG significantly reduced the incidence of complications compared to placebo (Colombel et al., 2006). Together with the microbiological identification of *M. bovis* in some cases, predominantly with focal disease, this argues in favour of infection.

In agreement with previous large case series studies (Gonzalez et al., 2003; Perez-Jacoiste Asin et al., 2014), the interval between the last instillation and onset of symptoms was shorter for patients 3 and 4 with systemic complications than for patients 1 and 2 with focal disease. In the case series by Gonzalez et al. (2003), a late presentation with mostly focal disease was microbiologically confirmed in two-thirds of the patients, compared to only one-third of the patients with early systemic disease. This suggests that acute inflammatory mechanisms play a relatively larger role in the development of systemic reactions, whereas focal disease is mainly caused by infection-induced chronic inflammation.

Immune tests showed that mycobacterial antigen-specific T-cell responses were highest in patient 4, who presented with sepsis and organ involvement. Intriguingly, the blood cells of this patient had limited capacity for in vitro growth inhibition of BCG, while patient 3, who had fever in the absence of organ involvement, showed enhanced in vitro BCG growth inhibition. While no conclusions can be drawn from only two cases, these immunological assays might provide a clue about the pathogenesis of BCG complications in individual patients. High mycobacterial antigen-specific T-cell responses would point to systemic inflammation as the predominant mechanism, in accordance with the beneficial effect of steroids. In patients with low in vitro growth inhibition of BCG, infection might be more important, explaining why these patients benefit more from tuberculostatic drugs, although the duration of such treatment is debatable, as illustrated by patient 4.

A preliminary model for the mechanism of action of BCG in bladder cancer has been proposed (Redelman-Sidi et al., 2014), consisting of internalization of live BCG by malignant urothelial cells followed by MHC-II up-regulation in infected bladder cancer cells and the presentation of a BCG- and/or tumour-specific antigen to T-cells. This leads to T-cell-mediated cytotoxicity specific to malignant urothelial cells. Since the patient with enhanced in vitro growth inhibition of BCG is now recurrence-free while the patient with limited in vitro growth inhibition died due to tumour progression, one could speculate that differences between individuals in the handling of live BCG by immune cells and perhaps also malignant urothelial cells might be related to this observation. This warrants further research.

In conclusion, the complications of intravesical BCG are manifold and the contribution of inflammation and infection varies between patients. Immunological assays have potential to provide insight into the pathogenesis of BCG disease. Future studies should also explore immunological mechanisms involved in tumour control following BCG instillations.

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healthy non-BCG vaccinated or *M. tuberculosis*-exposed, PPD-negative controls (for time points, see the caption for Figure 3A). Effective in vitro growth inhibition of BCG corresponds with low log(CFU). MGIA were always performed in duplicate. For controls, each dot represents the mean of duplicate values. For patients 3 and 4, duplicates are shown as separate dots. The lines represent the median value.

Conflict of interest

RP has no conflicts of interest, including specific financial interests, relevant to the submitted work. His financial activities outside the submitted work include being on the advisory board of Janssen Biologics BV and Amgen Europe BV, and providing guest lectures for AstraZeneca Netherlands. TO has no conflicts of interest, including specific financial interests, relevant to the submitted work. His financial activities outside the submitted work include being a member of the senior management team of the TB Vaccine Initiative (TBVI; www.tbvi.eu), receiving many external research grants (governmental, European Commission), reimbursement for travel costs for attending meetings, and holding two EU patents. All other authors report no conflicts of interest.

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