Disseminated bacille Calmette–Guérin infection in two patients with CD8+ T-cell lymphopenia

To the Editors:

Intravesical bacille Calmette–Guérin (BCG) therapy is an effective treatment for high-risk superficial bladder cancer. A nonspecific immune response is expected after instillation of BCG, an alive but attenuated strain of *Mycobacterium bovis*. Disseminated BCG disease is a rare but well known complication of this therapy. It occurs in approximately 1% of cases [1]. Severe complications could occur with hepatitis and pneumonitis but bone marrow involvement is exceptional. There are limited data on factors associated with the risk of BCG infection. The role of CD4+ T-cells in controlling mycobacteria infection is well established and the susceptibility of HIV-infected individuals for tuberculosis is an argument for this [2]. Nevertheless, CD8+ T-cells play a significant role in this control, in particular secreting interferon (IFN)-γ and killing infected cells given their cytotoxic potential [3]. Furthermore, experimental studies have shown that CD8+ T-cells play an important role in the early control of mycobacteria infection in older mice [4], suggesting that this may be occurring in elderly people. The present study describes two disseminated BCG infections, including bone marrow involvement, in elderly patients. Severe CD8+ T-cell depletion was found in the two patients, which illustrates the importance of CD8+ T-cells in the early control of mycobacterial infection in humans.

The first patient was a 74-yr-old male, admitted in our department for fever and chills. He had concomitant weight loss of 6 kg and intense fatigue over the last 15 days. 3 months earlier he had undergone a trans-urethral resection for superficial bladder cancer. Treatment was completed by weekly instillation of BCG. A few days after the second instillation, the patient noted gradual onset of fever. 3 weeks after the onset of fever, he was referred to our hospital. Clinical examination gave no conclusive results but the biological results showed elevated C-reactive protein (CRP) at 139 mg·L^{-1} (normal levels 0–5 mg·L^{-1}), with leukopenia (2,150 white blood cells (WBC) per mm^3) and thrombopenia (80,000 platelets·mm^{-3}). Lymphopenia was noted (800 per mm^3), with a very low CD8+ T-cell rate of 12% (normal levels 30–40%), *i.e.* 96 per mm^3 in absolute count (normally 500–900). The CD4+ T-cell count was normal. The haemoglobin level was normal. The liver enzyme levels were elevated: aspartate aminotransferase (AST) 179 IU·L^{-1}; alanine aminotransferase (ALT) 311 IU·L^{-1}; alkaline phosphatase (AP) 280 IU·L^{-1}; and γ-glutamyl transferase (GGT) 640 IU·L^{-1}. Neither viral (A, B and C) nor autoimmune hepatitis was found. The chest radiograph revealed a diffuse interstitial pneumonitis. Abdominal echography showed a homogenous hepatomegaly. A bone marrow biopsy was performed but not a liver biopsy, because of the low platelet count (80,000 per mm^3). Bone marrow biopsy showed noncaseating granulomas, composed of both CD8+ and CD4+ T-cells (fig. 1). The culture failed to find any mycobacteria, including *M. bovis*, in blood, bone marrow, sputum or urine. Isoniazid, rifampin and ethambutol were prescribed with a progressive resolution of symptoms. Fever dropped after 1 week

of treatment but intense fatigue persisted after 6 months of the three-drug regimen; hence, treatment was continued for a further 6 months with only rifampin and isoniazid. The patient's state of health completely returned to normal in 12 months, and blood count normalisation was observed except for CD8+ T-cells, which remained persistently low (<100 per mm^3) 1 yr after the end of the treatment.

The second patient was an 81-yr-old male who underwent trans-urethral resection for *in situ* bladder carcinoma 4 months before his admission in our department. BCG therapy was administered weekly after resection, but after the fourth instillation he consulted his general practitioner with fever, weight loss and asthenia. The clinical examination showed crackles in both lung fields, and the chest radiograph confirmed diffuse interstitial infiltrates. A blood workup was prescribed. CRP was elevated at 47 mg·L^{-1}, as were liver enzymes: AST 140 IU·L^{-1}; ALT 195 IU·L^{-1}; AP 643 IU·L^{-1}; and GGT 589 IU·L^{-1}. The WBC count was 3,200 per mm^3, and severe CD8+ T-cell lymphopenia was detected (the CD8+ T-cell rate was 11%, *i.e.* 77 per mm^3 in absolute count). A bone marrow biopsy was performed and showed noncaseating granulomas, with CD8+ and CD4+ T-cells inside (fig. 2). After 2 months, the bone marrow culture was still negative for mycobacteria, as were blood and sputum culture. The same antituberculous therapy was given (isoniazid, rifampin and ethambutol), with rapid progress in the patient's general health, allowing us to stop the treatment completely after 6 months of the three-drug regimen, whereas a low rate of CD8+ T-cells persisted 1 yr after the end of the treatment.

Disseminated BCG infection was diagnosed in these two patients based on suggestive clinical data and histological examination of the bone marrow, which showed the presence of noncaseating granulomas. The absence of *M. bovis* identification did not exclude the diagnosis, since the culture is positive in less than one case out of two in the largest series published [5]. The patients' favourable progression with antituberculosis treatment confirmed the diagnosis retrospectively. In these two patients, generalised symptoms (fever, chills and weight loss), as well as liver and lung involvement, are suggestive of the early form of the disease, as described by GONZALEZ *et al.* [5]. This early-presentation disease is marked by disseminated involvement of the organs remote from the bladder. However, in this large series only two cases of bone marrow involvement are reported.

One sign, however, retained our attention: in both of these patients, we detected severe CD8+ T-cell depletion that persisted for several months after clinical recovery. We believe that this CD8+ T-cell lymphopenia preceded the infection and promoted it. Unfortunately, we were not able to demonstrate this lymphopenia on previous blood samples for either patient. To confirm our hypothesis that CD8+ T-cell depletion predated and promoted BCG infection, we performed CD8+ T-cell
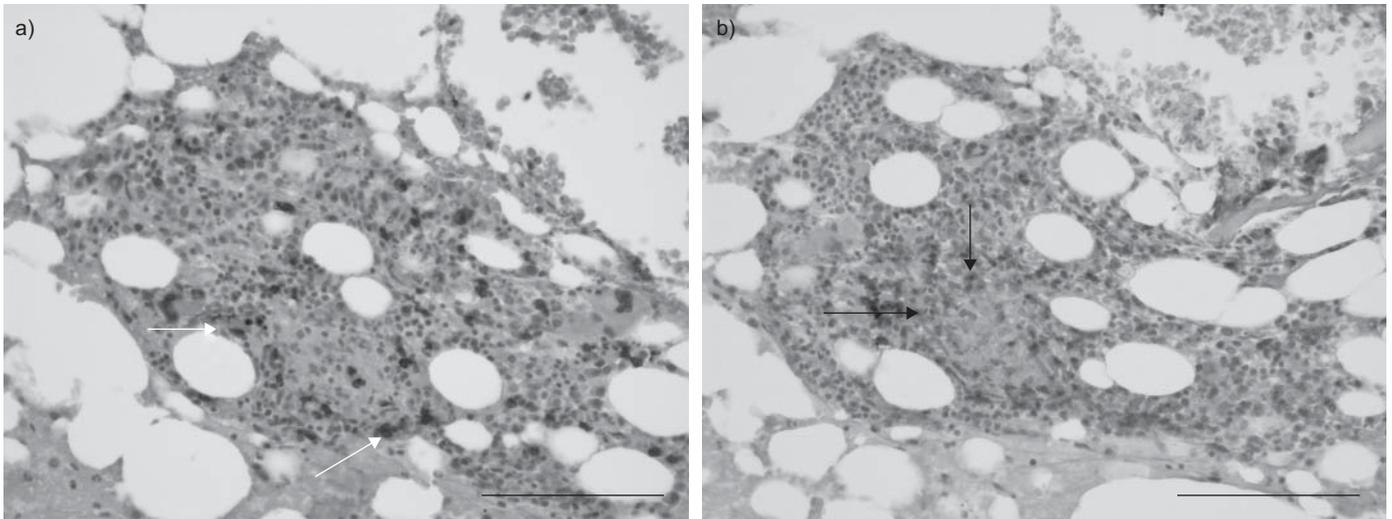
FIGURE 1. Bone marrow granuloma of case 1. a) Microscopic view of CD8+ T-cells (white arrows) and b) microscopic view of CD4+ T-cells (black arrows). Scale bars=0.1 mm.

counts up to 1 yr after the end of antituberculous treatment. For each patient, we noted persistence of low numbers of CD8+ T-cells (<100 per mm³) until 1 yr after the end of treatment. This persistence of low CD8+ T-cell count, a long time after recovery, was considered as indicative that the CD8+ T-cell lymphopenia could have predated the infectious episode.

The role of CD4+ T-cells is well known in the defence against mycobacteria, notably in granuloma formation and IFN-γ production [6, 7]. HIV-infected patients have a great susceptibility for mycobacterial infections and this fact demonstrates the dominant role of CD4+ T-cells in controlling mycobacterial infection [2]. The role played by CD8+ T-cells is more controversial, but many studies have shown their influence on mycobacterial control, notably based on IFN-γ production and its cytotoxic activity [3, 8, 9]. These cells have also been found in granulomas, as confirmed by our study (figs 1 and 2),

but they are not essential to granuloma formation [10]. Finally, CD8+ T-cells can be actively recruited in BCG-induced granulomas when activated by another pathogenic agent, such as during acute concomitant viral infection, thus forming CD8+ T-cell-dominated granulomas [7].

In addition, TURNER et al. [4] showed that older mice presented particularly pronounced early resistance to mycobacteria compared with younger mice. This early resistance was associated with a higher number of CD8+ T-cells and IFN-γ in the lungs of older mice that showed only slight pulmonary involvement. Another mouse experiment has indeed shown that memory CD8+ T-cells are stimulated nonspecifically in the first days of infection by *M. tuberculosis* in older mice [11]. This nonspecific stimulation of memory CD8+ T-cells and the resulting IFN-γ production may play a particularly important role in the early control of *M. tuberculosis* infection. Other data
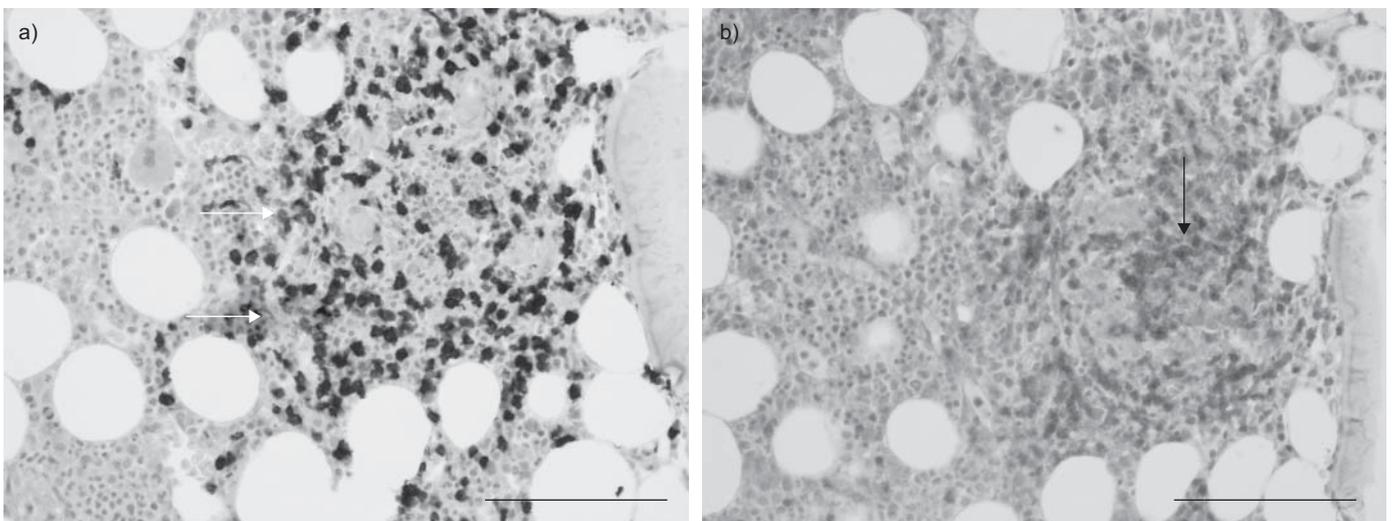

FIGURE 2. Bone-marrow granuloma of case 2. a) Microscopic view of CD8+ T-cells (white arrows) and b) microscopic view of CD4+ T-cells (black arrow). Scale bars=0.1 mm.

from mouse models seem to confirm this hypothesis: mice with a drastically reduced number of peripheral CD8+ T-cells (genetically deficient in β2 microglobulin) succumbed prematurely to tuberculosis [12].

However, there are considerable differences between mice and humans in CD8+ T-cell biology and the mouse model of tuberculosis probably does not completely reflect the immunological responses to mycobacterial infections in humans. Thus, CHEN et al. [13] have recently developed a macaque tuberculosis model, more similar to humans, to examine the contribution of CD8+ T-cells in protection against mycobacterial infection. In this study, BCG-vaccinated macaques were depleted of CD8+ T-cells and then infected with M. tuberculosis. These macaques exhibited more extensive infection, with systemic dissemination of tuberculosis. Furthermore, CARRANZA et al. [14] have shown that CD8+ T-cells of patients recently exposed to M. tuberculosis are capable of contributing ex vivo to the control of M. tuberculosis in autologous infected macrophages. Such findings strongly suggest that CD8+ T-cells are of critical importance for containment of mycobacterial dissemination in humans, notably through memory CD8+ T-cells.

Consequently, we believe that our two patients developed this BCG infection because of a deficit in CD8+ T-cells. It should be noted that these two patients had an early presentation of the disease, demonstrating the insufficiency of their early defence mechanisms, mediated by CD8+ T-cells, as we have described. We suggest performing a CD8+ T-cell count in elderly patients aged >70 yrs who need to have BCG instillations, and contraindicating these instillations if a CD8+ T-cell deficit is found. Finally, this case study exhibits a crucial role for CD8+ T-cells in antituberculosis immunity. Stimulation of this mode of immunity could provide new perspectives in the development of new tuberculosis vaccines or immunotherapeutics.

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