Case Report

Bacteria-associated haemophagocytic syndrome and septic pulmonary embolism caused by *Burkholderia cepacia* complex in a woman with chronic granulomatous disease

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Chronic granulomatous disease (CGD) is a primary immunodeficiency characterized by recurrent infections with certain types of bacteria and fungi. Presented herein is the case of a 29 year old woman with CGD who suffered from bacteria-associated haemophagocytic syndrome and a septic pulmonary embolism following a uterine infection and sepsis, caused by *Burkholderia cepacia* complex.

Introduction

Infections caused by *Burkholderia cepacia* complex (BCC) contribute to morbidity and mortality in patients who suffer from chronic granulomatous disease (CGD). The most common infection in CGD is pneumonia, followed by suppurative adenitis, subcutaneous abscess, liver abscess, osteomyelitis and sepsis (Winkelstein *et al.*, 2000). We report a rare case of bacteria-associated haemophagocytic syndrome (BAHS) and septic pulmonary embolism caused by BCC in a woman with CGD.

Case report

A 29 year old woman at 6 weeks gestation was admitted to our hospital with a high-grade fever, lower abdominal pain and abnormal genital bleeding. In her childhood, she had been diagnosed with CGD caused by a defect in p22-*phox*, one of the four subunits of the superoxide-generating phagocyte NADPH oxidase. The patient had a past history of several hospitalizations due to recurrent bacterial infections such as pneumonia, lymphadenitis, subcutaneous abscess and blepharitis, and oral trimethoprim-sulfamethoxazole had been administered prophylactically. Her vital signs were as follows: body temperature, 39.5 °C; blood pressure, 87/66 mmHg; and heart rate, 117 beats

min⁻¹ (Fig. 1). No significant swelling of superficial lymph nodes or hepatosplenomegaly was observed on a physical examination, and a chest radiograph was normal. Unfortunately, her pregnancy ended in spontaneous abortion at 7 weeks gestation.

Initial laboratory studies showed the following: leukocytosis with a count of 13 300 white blood cells (WBC) µl⁻¹ (neutrophils 92.2 %, lymphocytes 5.7 %, monocytes 2.0 %, atypical lymphocytes 0 %); 10.6 g haemoglobin dl⁻¹; and 295 000 platelets µl⁻¹. Elevated serum ferritin (190.2 ng ml⁻¹, normal range 8–74 ng ml⁻¹) and C-reactive protein (CRP) (133 mg l⁻¹, normal range <3 mg l⁻¹) were noted. Slightly increased levels of fibrinogen (4.3 g l⁻¹, normal range 1.5–4.0 g l⁻¹) and fibrinogen degradation products (FDP) (8.9 µg ml⁻¹, normal range <4 µg ml⁻¹) were also observed. The clinical manifestation was not improved after 3 days of cefazolin treatment. Both blood and vaginal secretion culture specimens, obtained on the 3rd hospital day, yielded Gram-negative rods. These micro-organisms were subsequently identified as BCC, which is resistant to many antibiotics, but susceptible to meropenem, levofloxacin, minocycline and trimethoprim-sulfamethoxazole. On the 4th hospital day, laboratory studies showed leucopenia with a count of 3680 WBC µl⁻¹ and liver dysfunction with 151 U aspartate aminotransferase (AST) l⁻¹ (normal range 10–31 U l⁻¹) and 101 U alanine aminotransferase (ALT) l⁻¹ (normal range 6–26 U l⁻¹). Elevation of lactate dehydrogenase (LDH) (472 U l⁻¹, normal range 115–245 U l⁻¹), ferritin (820.5 ng ml⁻¹), FDP (36.3 µg ml⁻¹), D-dimer (20.4 µg ml⁻¹, normal range <1 µg ml⁻¹) and sIL-2R (2400 U ml⁻¹, normal range 220–530 U ml⁻¹) were noted. Her serum cytokine levels of tumour necrosis factor alpha (TNF-α), interleukin (IL)-1β and IL-10 were all

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BAHS, bacteria-associated haemophagocytic syndrome; BCC, *Burkholderia cepacia* complex; CGD, chronic granulomatous disease; CRP, C-reactive protein; CSA, cyclosporin A; CT, computed tomography; HPS, haemophagocytic syndrome; IL, interleukin; LDH, lactate dehydrogenase; PSL, prednisolone; TNF-α, tumour necrosis factor alpha; WBC, white blood cells.

Fig. 1. Clinical course. Changes in body temperature, number of WBC (white bars), serum levels of ferritin (black bars) and CRP ($\blacktriangle$), as well as medication are shown. CEZ, Cefazolin; MEPM, meropenem; MINO, minocycline; TS, trimethoprim–sulfamethoxazole.

within the normal ranges, while increases in gamma interferon (27.4 IU ml^{-1} , normal range $<0.5 \text{ IU ml}^{-1}$), IL-2 (3.0 U ml^{-1} , normal range $<0.8 \text{ U ml}^{-1}$) and IL-6 (38.4 pg ml^{-1} , normal range $<4 \text{ pg ml}^{-1}$) were observed.

We suspected haemophagocytic syndrome (HPS) associated with a bacterial infection and a tendency toward disseminated intravascular coagulation on the basis of the results of blood culture and peripheral blood tests, and performed bone marrow analysis. The diagnosis of HPS was made as the result of a bone marrow aspiration, which revealed macrophages that were engulfing erythroblasts (Fig. 2a), platelets (Fig. 2b) and lymphocytes. Meropenem and human IgG were used to treat the underlying infection, and heparin was administered for hypercoagulability. On the 9th hospital day, serum levels of AST, ALT, LDH and ferritin were further elevated to 256 U l^{-1} , 171 U l^{-1} , 1103 U l^{-1} and $5346.6 \text{ ng ml}^{-1}$, respectively. We primarily used corticosteroid therapy with prednisolone (PSL) in a daily dose of 50 mg for HPS after a blood culture for bacteria was found to be negative. Although the PSL therapy transiently suppressed the fever, the fever returned to the previous untreated level and her clinical condition worsened with progressive pancytopenia ($3280 \text{ WBC } \mu\text{l}^{-1}$; $9.0 \text{ g haemoglobin dl}^{-1}$; $133\,000 \text{ platelets } \mu\text{l}^{-1}$). A second bone marrow aspiration was performed and failure of the corticosteroid therapy to suppress the haemophagocytic process was confirmed on the 15th hospital day. To

inhibit the activities of HPS, a treatment regime of cyclosporin A (CSA) in combination with PSL was decided upon. We judged that the BCC infection was improved by meropenem because the CRP reached an almost negative level and HPS was the principal disease at this point. Therefore, meropenem was changed to an oral administration of levofloxacin before CSA treatment. Meanwhile the level of CRP elevated slowly and meropenem treatment was consequently resumed, simultaneous with the CSA treatment. CSA suppressed the high grade fever dramatically and improved the pancytopenia. We therefore concluded that CSA was highly effective in the treatment of HPS. However, the patient was still febrile and had a new complaint of a non-productive cough with rapidly elevated levels of CRP (121 mg l^{-1}). A chest radiograph and computed tomography (CT) scan and repeated blood cultures were performed to clarify the cause of this on the 21st hospital day. A chest radiograph showed diffuse, bilateral, poorly defined opacities (Fig. 2c) and the chest CT scan demonstrated multiple, wedge-shaped nodular opacities in both lungs (Fig. 2d). Some of the nodules were located at the end of feeding vessels and abutted the pleura. Blood cultures again identified BCC and these findings resulted in a diagnosis of septic pulmonary embolism. Sepsis occurred in spite of treatment with meropenem, and we selected an intensive treatment with minocycline, oral administration of trimethoprim–sulfamethoxazole and micafungin sodium as a preventive treatment for the emergence of the fungus. Her clinical condition gradually

Fig. 2. Bone marrow and lung features. Mature histiocytes with haemophagocytosis were revealed in bone marrow (Wright–Giemsa staining, $\times 1000$). The phagocytes engulfed red erythroblasts (a) and platelets (b). Images of a chest X-ray (c) and CT scan (d) showed numerous small nodular opacities in whole lung fields.

improved and the high grade fever disappeared on the 23rd hospital day, and serum levels of CRP and ferritin returned to the normal range around the 54th hospital day. Complete improvement in the lung lesion was confirmed 2 months later following serological improvement.

Discussion

CGD is a primary immunodeficiency characterized by recurrent life-threatening infections with catalase-positive bacteria, including *Staphylococcus aureus* and *Enterobacteriaceae*, and *Aspergillus* species. The disease is due to mutations in any one of the four genes encoding the subunits of phagocyte NADPH oxidase. This enzyme catalyses the monovalent reduction of O_2 to superoxide (O_2^-), which is necessary for the microbicidal activity in phagocytic cells (Lekstrom-Himes & Gallin, 2000). Winkelstein *et al.* (2000) reported a registry of 368 CGD patients in the USA. The most prevalent infection was pneumonia (79%), followed by suppurative adenitis (53%), subcutaneous abscess (42%), liver abscess (27%), osteomyelitis (25%) and sepsis (18%). In the case reported here, on admission, the patient had already suffered from BCC sepsis, the source of which was thought to be the uterus. A chest radiograph showed no pneumonia, and other characteristic findings such as lymphadenopathy, subcutaneous abscess and liver abscess were not observed on physical and ultrasound examination. BCC is an opportunistic pathogen and does not usually infect healthy

individuals. In CGD patients, an infection with BCC has a dramatic impact on morbidity and mortality (Mahenthalingam *et al.*, 2005), and pneumonia and/or sepsis due to BCC has been reported to be the second most common cause of death (Winkelstein *et al.*, 2000). Although BCC is susceptible to meropenem, the patient remained afebrile, and presented unexplained progressive cytopenia and a tendency toward disseminated intravascular coagulation. We confirmed the diagnosis of HPS based on a persistent high fever, progressive cytopenia and bone marrow findings. HPS is characterized by exaggerated histiocytic proliferation and activation. The clinical features of HPS include a high grade fever, hepatosplenomegaly, lymphadenopathy, cytopenia and coagulopathy. The progression of cytopenia is a diagnostic finding based on peripheral blood tests, and supporting characteristic findings are liver dysfunction symptoms such as elevations in AST, ALT and LDH, hyperferritinaemia caused by haemophagocytosing macrophages/histiocytes, and coagulation abnormalities (Tsuda, 1997). Following supportive care and treatment of the underlying infection, we first started PSL and then used it in combination with CSA for HPS treatment. However, the patient subsequently developed recurrent sepsis with BCC and a severe septic pulmonary embolism, which were probably worse in the immunosuppressed state. While corticosteroid and immunosuppressive therapy had to be continued for HPS, we performed a triple antibiotic combination therapy with meropenem, minocycline and trimethoprim–sulfamethoxazole. Finally, we were successful in treating both the underlying infection and HPS.

The causal factors of HPS fall into two main groups: inherited/primary and reactive/secondary. The latter includes infection (viruses, bacteria, fungi and parasites), malignancy, autoimmune disease and drugs. The increased production of various cytokines may be related to the pathogenesis of HPS. In infection-associated HPS, high levels of gamma interferon and $TNF-\alpha$ as a result of proliferating T cells following infection contribute to macrophage activation with haemophagocytosis and the formation of a cytokine network (Fisman, 2000; Larroche & Mouthon, 2004). However, reports of HPS caused by nonviral pathogens, including BAHS, are much fewer than those of virus-associated HPS, and the immunopathological mechanism in BAHS is less well understood. Our hypothesis on the facilitation of HPS due to BCC infection in a CGD patient is that the inability of phagocytic cells to kill BCC may stimulate the proliferation and phagocytic activity of macrophages, and allow overactivation of haemophagocytes as the first immune response to the infection. Another potential mechanism is that the hypercytokinemia observed in the systemic inflammatory response syndrome, which was proposed as a broad definition of sepsis, may participate in macrophage activation. Stephan *et al.* (1997) reported that 12 out of 20 mechanically ventilated patients with sepsis syndrome or septic shock and thrombocytopenia were identified as

having HPS. Francois et al. (1997) studied 50 patients who had been diagnosed with both sepsis syndrome and thrombocytopenia of undetermined origin, and reported that HPS was diagnosed in 32 of these patients. They concluded that the presence of high serum cytokine production (such as TNF- α , IL-1 and IL-6) seen in both the sepsis syndrome and HPS may play a role in the pathophysiological mechanisms.

An encounter with HPS due to a bacterial infection in daily practical work is rare and the strategy for HPS is inconsistent treatment of the causative infection. We felt it was necessary to take HPS into consideration when treating a CGD patient with systemic inflammatory response syndrome, and note the importance of careful observation with orderly and assiduous treatment for HPS in a CGD patient.

References

- Fisman, D. N. (2000).** Hemophagocytic syndromes and infection. *Emerg Infect Dis* **6**, 601–608.
- Francois, B., Trimoreau, F., Vignon, P., Fixe, P., Praloran, V. & Gastinne, H. (1997).** Thrombocytopenia in the sepsis syndrome: role of hemophagocytosis and macrophage colony-stimulating factor. *Am J Med* **103**, 114–120.
- Larroche, C. & Mouthon, L. (2004).** Pathogenesis of hemophagocytic syndrome (HPS). *Autoimmun Rev* **3**, 69–75.
- Lekstrom-Himes, J. A. & Gallin, J. I. (2000).** Immunodeficiency diseases caused by defects in phagocytes. *N Engl J Med* **343**, 1703–1714.
- Mahenthiralingam, E., Urban, T. A. & Goldberg, J. B. (2005).** The multifarious, multireplicon *Burkholderia cepacia* complex. *Nat Rev Microbiol* **3**, 144–156.
- Stephan, F., Thiolier, B., Verdy, E. & Tulliez, M. (1997).** Role of hemophagocytic histiocytosis in the etiology of thrombocytopenia in patients with sepsis syndrome or septic shock. *Clin Infect Dis* **25**, 1159–1164.
- Tsuda, H. (1997).** Hemophagocytic syndrome (HPS) in children and adults. *Int J Hematol* **65**, 215–226.
- Winkelstein, J. A., Mariko, M. C., Johnston, R. B., Jr, Boyle, J., Curnutte, J., Gallin, J. I., Malech, H. L., Holland, S. M., Ochs, H. & other authors (2000).** Chronic granulomatous disease. Report on a national registry of 368 patients. *Medicine* **79**, 155–169.