CASE REPORT

Intestinal tuberculosis complicated with perforation during anti-tuberculous treatment in a 13-year-old girl with defective mitogen-induced IL-12 production

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Primary immunodeficiency

Interleukin-12 (IL-12) is a cytokine which is secreted by activated phagocytes and dendritic cells and promotes cell-mediated immunity to intracellular pathogens, by inducing type 1 helper T cell (TH1) responses and interferon-\( \gamma \) (IFN-\( \gamma \) ) production. Defects in the IL-12 may cause selective susceptibility to intracellular pathogens, such as mycobacteria. We herein report on a 13-year-old girl with defective mitogen-induced IL-12 production, who developed intestinal tuberculosis with wide dissemination involving the lung and urinary tract. She improved gradually, but developed terminal ileal perforation approximately 6.1 months following initiation of anti-tuberculous treatment. The paradoxical response phenomenon was suspected. The girl subsequently underwent surgical resection of the affected bowel segment with a temporary double barrel stoma, and ileocolonic anastomosis was performed after the completion of the anti-tuberculous therapy. The patient remained well, with no evidence of recurrent tuberculosis in the past 5 years. This case illustrates the possibility of underlying primary immunodeficiency in a patient with disseminated tuberculosis; delayed tuberculous intestinal perforation can develop during chemotherapy for tuberculosis.

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Introduction

Primary immunodeficiency diseases are attributable to a deficiency in cellular, humoral, or molecular functions of the immunity systems. Patients with primary immunodeficiency diseases usually present clinically with an increased frequency, severity, or persistence of infections, or with an occurrence of infections which are attributable to opportunistic pathogens. We report a patient with defective mitogen-induced interleukin-12 (IL-12) production, who was diagnosed with intestinal tuberculosis with wide dissemination and who experienced free intestinal perforation during anti-tuberculous treatment.

Case report

A 13-year-old girl was admitted to our hospital with a 2-year history of chronic abdominal pain of increasing severity. The pain was poorly localized, colicky in nature and partially relieved after defecation. She had nausea, malaise, reduced appetite with weight loss of two pounds in 2 months. Despite regular dietary intake, she was noted to have "poor growth" for a few years. Otherwise, her past health was unremarkable. Her parents had consanguineous marriage and the patient had two elder sisters aged 18 and 20 years, with good past health. On examination, she was pale with a body weight of 27 kg (5 kg < third percentile) and had bilateral ankle edema. Right pleural effusion and ascites were detected. Baseline laboratory investigation was as follows: hemoglobin = 8.7 g/dL (normal = 11.6–15.5); mean cell volume = 71.5 fl (normal = 83–98); white blood cell count = 8.0/mm³ (normal = 3.9–10.7); neutrophil = 5.4/mm³ (normal = 2.1–7.8); lymphocyte = 0.6/mm³ (normal = 1.2–3.4); platelet count = 387/mm³ (normal = 152–358); sodium = 133 mmol/L (normal = 136–145); potassium = 3.6 mmol/L (normal = 3.5–5.1); erythrocyte sedimentation rate = 47 mm/hour (normal < 31); C-reactive protein = 43.7 mg/L (normal < 8); urea = 3.7 mmol/L (normal = 1.4–5.4); creatinine = 24 umol/L (50–77); albumin = 13 g/L (normal = 29–42); globulin = 27 g/L (normal, no reference); total bilirubin = 9 umol/L (normal = 5–20); alanine aminotransferase = 12 IU/L (normal = 5–20); alkaline phosphatase = 85 IU/L (normal = 50–162); calcium = 2.12 mmol/L (normal = 2.10–2.55 mmol/L); phosphate = 0.84 mmol/L (normal = 0.9–1.55); spot glucose = 8.6 mmol/L (normal, no reference). Chest radiography upon admission showed a right pleural effusion. Ultrasound (US)-guided thoracentesis with biopsy revealed exudative lymphocytic pleural effusion, with non-specific histology in pleural tissue. Computed tomography (CT) scan of the thorax, abdomen and pelvis revealed that there was bilateral pleural effusion, bowel wall thickening with increased stranding of mesenteric fat and ascites. The liver, spleen, pancreas, both kidneys and ureters appeared unremarkable. No thoracic and intra-abdominal lymphadenopathy was demonstrated in CT imaging. A technetium-99m-labeled human serum albumin scan showed protein leakage in the ascending colon (Fig. 1A, 1B and 1C). Colonoscopy without terminal ileum intubation showed multiple ulcers with nodular edges present at the ileocecal valve and histological examination revealed the ulcers were infiltrated by neutrophils, lymphocytes and histiocytes, with the presence of numerous acid-fast bacilli on Ziehl-Neelsen staining (Fig. 2A, 2B and 2C). Culture of the sputum, early morning urine and pleural fluid subsequently yielded a positive growth of Mycobacterium tuberculosis (MTB), which was sensitive to isoniazid (INH), rifampicin, ethambutol, and streptomycin. Testing for HIV antibodies was negative. Thus, the patient had a diagnosis of intestinal tuberculosis, causing protein-losing enteropathy, with wide dissemination, involving the lung and urinary system. Treatment with once daily INH 150 mg, rifampicin 300 mg, pyrazinamide 1 g, and ethambutol 750 mg was commenced. The anti-tuberculous drug regimen was interrupted for 5 days because of impaired renal function, which was thought to be due to acute tubular nephritis caused by rifampicin, with features including: serum creatinine = 151 umol/L, glomerular filtration rate = 29 mL/min/1.73m², glycosuria, and increased urinary loss of sodium and potassium. Anti-tuberculous treatment with INH, pyrazinamide, ethambutol and levofloxacin was restarted upon normalization of the renal function. The patient’s condition improved, with resolution of abdominal pain and pleural effusion. One year of anti-tuberculous therapy was planned including an initial 3 months of INH, pyrazinamide, ethambutol and levofloxacin, followed by 9 months of INH, levo- floxacin and ethambutol. She was evaluated for primary immunodeficiency. The lymphocyte subset profile was performed by flow cytometry and the results were: lymphocytes = 644/μL (normal = 1900–3200); B-cells = 86/μL (normal = 300–500); T-cells = 488/μL (normal = 1300–2200); CD4 T cell = 375/μL (normal = 600–1100); CD8 T cells = 129/μL (normal = 500–1000); and NK cells = 41/μL (normal = 300–500). The patient had lymphopenia affecting all lymphocyte subsets. The proliferation response of the patient’s lymphocytes to antigens stimuli [phytohemagglutinin (PHA), purified protein derivative (PPD) and Cryptococcus] was suboptimal. The mitogen-stimulated cytokine profile of the patient, performed by the Enzyme-linked immunospot (ELISPOT) assay, revealed that PHA- and conA-stimulated IL-12 were seriously low (Table 1). This was compatible with IL-12 deficiency.

Eight weeks following discharge (128 days after commencement of anti-tuberculous therapy), the patient presented again with acute-onset right lower quadrant colicky pain of increasing severity and diarrhea for 3 days. There were signs of bilateral pleural effusion, ascites and vague non-tender right lower quadrant mass on physical examination. Routine blood tests revealed anemia (hemoglobin = 6.7 g/dL), lymphopenia (0.5/mm³), and hypoalbuminemia (albumin 11 g/L). A CT scan of the thorax and abdomen revealed similar findings as compared with the previous imaging. The peritoneal fluid by US-guided diagnostic paracentesis showed the presence of viable acid-fast bacilli, without emergence of a resistant strain. The sputum and early morning urine had no growth of MTB. The working diagnosis was the underlying defective mitogen-induced IL-12 production, which made the disseminated MTB infection not well-controlled. The anti-tuberculous regimen was intensified to include intravenous amikacin and levofloxacin was switched from the oral to the intravenous route. Her clinical condition gradually improved. About 4 weeks afterwards (182 days after the anti-tuberculous therapy had been started), the patient...
presented with acute-onset of generalized abdominal pain and vomiting. There were signs of peritonitis on physical examination. The relevant laboratory investigation was as follows: white blood cell count $= 3.4$ mm$^3$ (normal $= 3.9-10.7$); lymphocyte $= 1.1$ mm$^3$ (normal $= 1.2-3.4$); normal renal function; albumin $= 25$ g/L (normal $= 29-42$); globulin $= 29$ g/L (normal, no reference). An urgent CT scan of the abdomen revealed free intraperitoneal gas, a markedly dilated terminal ileum, gross mural thickening and mucosal enhancement along the terminal ileum down to the transverse colon. Multiple intra-abdominal lymphadenopathy was detected. An exploratory laparotomy, which was carried out immediately afterwards, revealed there was a partially concealed perforation over the terminal ileum, with dense adhesion at the right side of the abdomen extending down to the right fallopian tube and a moderate amount of turbid peritoneal fluid. A limited right hemicolecotomy with double barrel stoma (ileostomy and colostomy) was performed. The postoperative course was uneventful, and the previous anti-tuberculous regimen was restarted 1 week afterwards. Histological examination of the resected segment of the terminal ileum revealed there were several ill-formed granulomas with numerous acid-fast bacilli in the submucosa and subserosa accompanied with transmural inflammation and multiple mucosal erosions. Caseating necrosis was not present. The peritoneal fluid did not reveal any viable mycobacterial growth.

A total of 18 months of anti-tuberculous therapy (6 months of oral INH, pyrazinamide, ethambutol, plus intravenous amikacin and levofloxacin, followed by 12 months of oral INH, pyrazinamide, ethambutol and moxifloxacin) was given to the patient. The closure of the stoma with ileocolonic anastomosis was performed 3 months after

Figure 1.  (A) Mildly focal increased tracer activity at the right lower quadrant of the abdomen was seen at 2 hours. (B) The lesion became more prominent and moved slightly upward at 7 hours. (C) At 24 hours, prominent curvilinear tracer activity was seen at the lower part and left side of the abdomen.
the completion of the anti-tuberculous therapy. The patient remained well with no evidence of recurrent MTB in the past 5 years.

Discussion

Our patient had intestinal tuberculosis with wide dissemination into the respiratory and urinary system, which was complicated with delayed free perforation during anti-tuberculous treatment. Although the patient did not have any recurrent sepsis in the past, there are several features suggesting a probable underlying primary immunodeficiency. The lymphocyte count on admission was extremely low and there were no cell-mediated immune responses (i.e., absence of lymphadenopathy and granuloma formation) in the CT scan and pathological specimens. Patients with primary immunodeficiency diseases usually present clinically with an increased frequency, severity or persistence of infections, or occurrence of opportunistic infections in which our patient belonged to the first category. Cells of normal immune systems respond to pathogens by producing a variety of cytokines and our patient showed the defective IL-12 production in the mitogen-stimulation test. IL-12 is a heterodimeric cytokine which consists of two disulfide-linked subunits, p40 and p35, and is produced by activated antigen presenting cells (dendritic cells, macrophages), particularly upon infection with intracellular pathogens such as mycobacteria and salmonella. IL-12 promotes the development and activity of cytotoxic T-lymphocytes, natural killer cells, and macrophages.

<table>
<thead>
<tr>
<th>Stimulant</th>
<th>IFN-γ P Normal range</th>
<th>IL-2 P Normal range</th>
<th>IL-6 P Normal range</th>
<th>TNF-α P Normal range</th>
<th>IL-12 P Normal range</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHA</td>
<td>680 450–3500</td>
<td>3400 3000–6900</td>
<td>48,800 19,000–105,000</td>
<td>73,800 27,000–108,000</td>
<td>35 144–1072</td>
</tr>
<tr>
<td>ConA</td>
<td>3400 2000–10,900</td>
<td>1360 1300–6500</td>
<td>47,200 19,000–97,000</td>
<td>73,800 19,000–97,000</td>
<td>10 79–558</td>
</tr>
</tbody>
</table>

ConA = concanavalin A; P = patient; PHA = phytohemagglutinin.
Results are given as cytokine secreting cells per million peripheral blood mononuclear cells.
is also a powerful inducer of IFN-γ production by T cells and natural killer (NK) cells. Both IL-12 and IFN-γ appear to be essential for the development of protective cell-mediated immunity in human. Defects in the IL-12-IFN-γ circuit will increase the susceptibility of individuals to infections caused by intracellular pathogens, like poorly pathogenic mycobacteria [non-tuberculous mycobacteria (NTM)] and salmonella species; these patients have subsequently been classified as part of the syndrome Mendelian susceptibility to mycobacterial disease (MSMD). IL-12 deficiency can be a risk factor for acquisition of severe mycobacterial and salmonella infection, as reported by de Jong et al. In fact, the IL-12-IFN-γ circuit is essential for controlling resistance to these pathogens and no other redundant protective immune mechanism can compensate for this deficiency.

Germline mutations in five genes involved in the IL-12-IFN-γ circuit have been found in genetic analysis of affected kindreds: (1) IFN-γR1 and IFN-γR2, encoding the ligand-binding chain R1 and R2 of the IFN-γ receptor, respectively. These first two categories comprise partial or complete recessive null mutations of the receptor signaling chains for IFN-γ. This causes either a defect in receptor expression or formation of non-functional receptors; (2) STAT1, encoding the signal transducer and activator of transcription-1 (stat-1) in IFN-γ receptor signaling pathway. Stat-1 is a downstream signaling molecule for IFN-γ. This category comprises partial or complete defects in the signal transduction molecule STAT1, which is required for signaling via the IFN-γ receptor; (3) IL-12B, encoding the p40 subunit shared by IL-12 and IL-23. This type of defect results in an inability to produce IL-12 and IL-23, due to deletion within the gene encoding the inducible chain of IL-12 (IL-12B), which is shared by the two cytokines, IL-12 and IL-23; (4) IL-12 RB1, encoding the β1 subunit shared by IL-12 and IL-23 receptors. This last category is the null recessive mutation of the IL-12 RB1 gene encoding the IL-12 receptor chain; this results in an inability to respond to IL-12 and IL-23.

The various types of mutations in these five genes may be associated with partial or complete deficiency of the gene product and thus define up to 10 distinct inherited disorders. The severity of the clinical phenotype depends on the genotype. Complete IFN-γR1 and IFN-γR2 deficiencies predispose patients to overwhelming infections with impaired granuloma formation in early childhood, whereas partial IFN-γR1, IFN-γR2 and STAT-1 deficiencies predispose patients to curable infections with mature granulomas at various ages. Although the technique of genetic sequencing is not available in our institution, the overwhelming clinical course of our patient was compatible to the former entity. Moreover, our patient did not develop fulminant sepsis with other viral, bacterial or fungal pathogens, which suggests that IL-12 may be dispensable for protection to pathogens other than intracellular bacteria. There are anecdotal reports of the beneficial effects of IFN-γ supplementation in patients with IL-12B, IL-12Rβ1, and dominant partial IFN-γ deficiency. IFN-γ is of no use in complete IFN-γR1 or -R2 deficiency, where the outcome is poor despite anti-mycobacterial chemotherapy.

Free intestinal perforation is a rare but serious complication of intestinal TB, with the reported incidence ranging from 1 to 15%. It may be solitary or multiple and usually happens in the distal ileum, which may be due to its common involvement in gastrointestinal TB. There are two kinds of perforation as related to the institution of anti-tuberculous treatment: early and delay type. The former occurs shortly after the initiation of anti-tuberculous therapy and thus it may represent the natural progression of the disease process or the impaired ulcer healing and reduced reinforcement of mesentery caused by a reduced inflammatory response, as a result of anti-tuberculous treatment. The other type of perforation is a paradoxical response, as these patients usually feature with clear documentation of initial improvement with anti-tuberculous treatment before the occurrence of intestinal perforation, like our case. The pathogenesis of this phenomenon is not fully understood. Possible mechanisms include a regain of the host’s delayed hypersensitivity response, and an increased exposure to mycobacterial antigens released from killed bacilli.

Although anti-tuberculous therapy had been interrupted for a short period and the anti-tuberculous regimen had a borderline low dose of INH without rifampicin, the clinical improvement of our patient was accompanied BY a rise of serum albumin level and lymphocyte count, and supported the diagnosis of paradoxical response rather than treatment failure. Previous studies reported that the perforation occurred between 2 days and 4 months after the initiation of anti-tuberculous therapy.

Cheng et al reported that this paradoxical response occurred after an upsurge in the lymphocyte count and sometimes an exaggerated tuberculin skin reaction might be observed. Our patient had severe lymphopenia and had a delayed recovery of her lymphocyte count, which accounted for the longer delay of the intestinal perforation. The treatment of choice for perforation in intestinal TB is resection of the affected segment of the bowel, followed by an end-to-end anastomosis.

Simple closure of the lesion is not advised, as it is associated with a high incidence of leakage and fistula formation. Previous studies reported a high mortality ranging from 25 to 100%. Factors associated with mortality included delayed operation, multiple perforation sites, primary closure of the lesions, leakage from the operation site and steroid therapy. Anti-tuberculous therapy should be started as soon as possible.

This case highlights the importance of finding any immunodeficiency in those with tuberculosis or NTM infection in a disseminated, fatal or sometimes in a familial distribution, and the need to maintain a high index of suspicion of intestinal perforation when patients present with acute abdominal pain while receiving treatment for TB.

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References


