JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 1996, p. 227–229 Vol. 34, No. 1
0095-1137/96/$04.00+0

Microbiological Findings about Pulmonary Cryptosporidiosis in Two AIDS Patients

C. DUPONT,[1]* M. E. BOUGNOUX,[2] L. TURNER,[1] E. ROUVEIX,[1] AND M. DORRA[1]

Departments of Internal Medicine[1] and Microbiology,[2] Hôpital Ambroise-Paré, 92100 Boulogne, France

Received 25 April 1995/Accepted 14 September 1995

There is no known treatment for pulmonary cryptosporidiosis, a rare complication of intestinal cryptosporidiosis in AIDS patients. We report two cases of cryptosporidiosis which were unusual because (i) extracellular invasive forms of the parasite were found in the bronchoalveolar lavage and (ii) the outcome was favorable in one patient after treatment with azithromycin.

Cryptosporidium organisms, which are intestinal protozoa, are a well known cause of enteritis in AIDS patients. Pulmonary or respiratory cryptosporidiosis is a rare complication of intestinal infection. Fewer than 30 cases have been reported in the literature since the first report for an AIDS patient in 1984 (1). However, the prevalence of lung involvement may be underestimated through lack of systematic investigation. One study has reported a prevalence of 17% in human immunodeficiency virus (HIV)-positive patients with respiratory symptoms (6). Current diagnosis of pulmonary cryptosporidiosis is based either on the identification by pathological findings of any forms of the intracellular cycle of the parasite in the epithelium cell or on the detection of thick-walled oocysts on smears of infected pulmonary fluids. Up to now, no effective therapy for intestinal and extraintestinal cryptosporidiosis has been found.

In this report, we describe two new cases of respiratory cryptosporidiosis treated with azithromycin in HIV-positive patients. Furthermore, we describe an unusual microbiological diagnosis of extracellular invasive forms of the parasite (e.g., sporozoite or merozoite) which were found in the bronchoalveolar lavage (BAL).

Patient 1. A twenty-eight-year-old man who was an intravenous drug user was admitted to the hospital in December 1993. He complained of productive cough and fever. He had been HIV positive since 1987 and had no concurrent opportunistic infections. The $CD4^+$ lymphocyte count was low (50/mm^3). Azidothymidine therapy and medication for primary prophylaxis against *Pneumocystis carinii* and *Toxoplasma gondii* were not regularly taken. He had hypoxemia, and chest X-rays showed right interstitial pneumonia. BAL specimens were negative for *P. carinii*, *T. gondii*, cytomegalovirus, herpesvirus, mycobacteria, and legionellae, and cultures were negative for bacteria and fungi. Despite treatment with 3 g of amoxicillin-clavulanate and 3 g of pristinamycin per day, fever and cough were persistent when the patient was discharged. In January 1994, this patient was again admitted for persistent cough and watery diarrhea. The chest X-ray was unchanged. A second BAL was performed, and no opportunistic pathogens, including *P. carinii*, *T. gondii*, cytomegalovirus, herpesvirus, mycobacteria, legionellae, and fungi, were found. However, smears stained with May-Grunwald-Giemsa stain (MGG) showed the presence in alveolar macrophages of cryptosporidia at various stages of the life cycle and the presence of numerous extracellular invasive forms of the parasite (e.g., sporozoite or merozoite). These were unicellular comma-shaped bodies, with one anterior spiky end and one round posterior end, and the nucleus was visible inside the third posterior end, as shown in Fig. 1. Only oocysts were evident after auramine staining of BAL. A few *Cryptosporidium* oocysts were also identified in stool specimens. Abdominal echography was normal. The patient was first treated with 2 g of paromomycin per day for 6 days and then with 1.2 g of azithromycin per day for 1 month, and respiratory symptoms improved. Ten months after treatment, he had no respiratory symptoms and a new BAL was found to be negative.

Patient 2. A 35-year-old female who was an intravenous drug user was admitted to the hospital in May 1994 for abdominal pain, diarrhea, and vomiting. She was known to have been HIV positive since July 1993, when she developed toxoplasmic encephalitis associated with suspected HIV vasculitis. Hepatitis C virus serology was positive. On physical examination, weight loss was noted. Leukocytes were normal, CD4 lymphocyte counts were low (9/mm^3), and liver enzyme levels were elevated. (γ-Glutamyl transpeptidase, alanine aminotransferase, and aspartate aminotransferase levels were, respectively, four, two, and two times the normal level.) Stool specimens showed the presence of a large number of *Cryptosporidium* oocysts. Despite treatment with 2 g of paromomycin per day for 20 days, the patient had persistent diarrhea and began to have a productive cough, shortness of breath, and hypoxemia. Chest X-rays showed moderate bilateral interstitial pneumonia. BAL revealed the presence of *Cryptosporidium* oocysts after MGG and auramine staining. No other pathogenic agents (viral, bacterial, mycobacterial, or parasitologic) were found in the BAL specimens. Paromomycin was replaced by azithromycin (1.2 g/day); pulmonary symptoms improved, but there was vomiting and persistent diarrhea requiring morphine injections. After 2 months of treatment, the patient's condition worsened, with increasing cough and hypoxemia. At that time, stool examination still showed the presence of a large number of *Cryptosporidium* oocysts. In July 1994, the patient complained of right hemiparesis, and a computed tomography scan showed possible vasculitis. Worsening neurological disorders were responsible for death 2 days later.

Discussion. The diagnosis of respiratory cryptosporidiosis is based on pathological findings in tracheal or bronchial tissues (5). After being stained with MGG, hematoxylin and eosin,

* Corresponding author. Mailing address: Department of Internal Medicine, Hôpital Ambroise-Paré, 9 Avenue du Général de Gaulle, 92100 Boulogne, France. Phone: 33 1 49095660. Fax: 33 1 49095649.

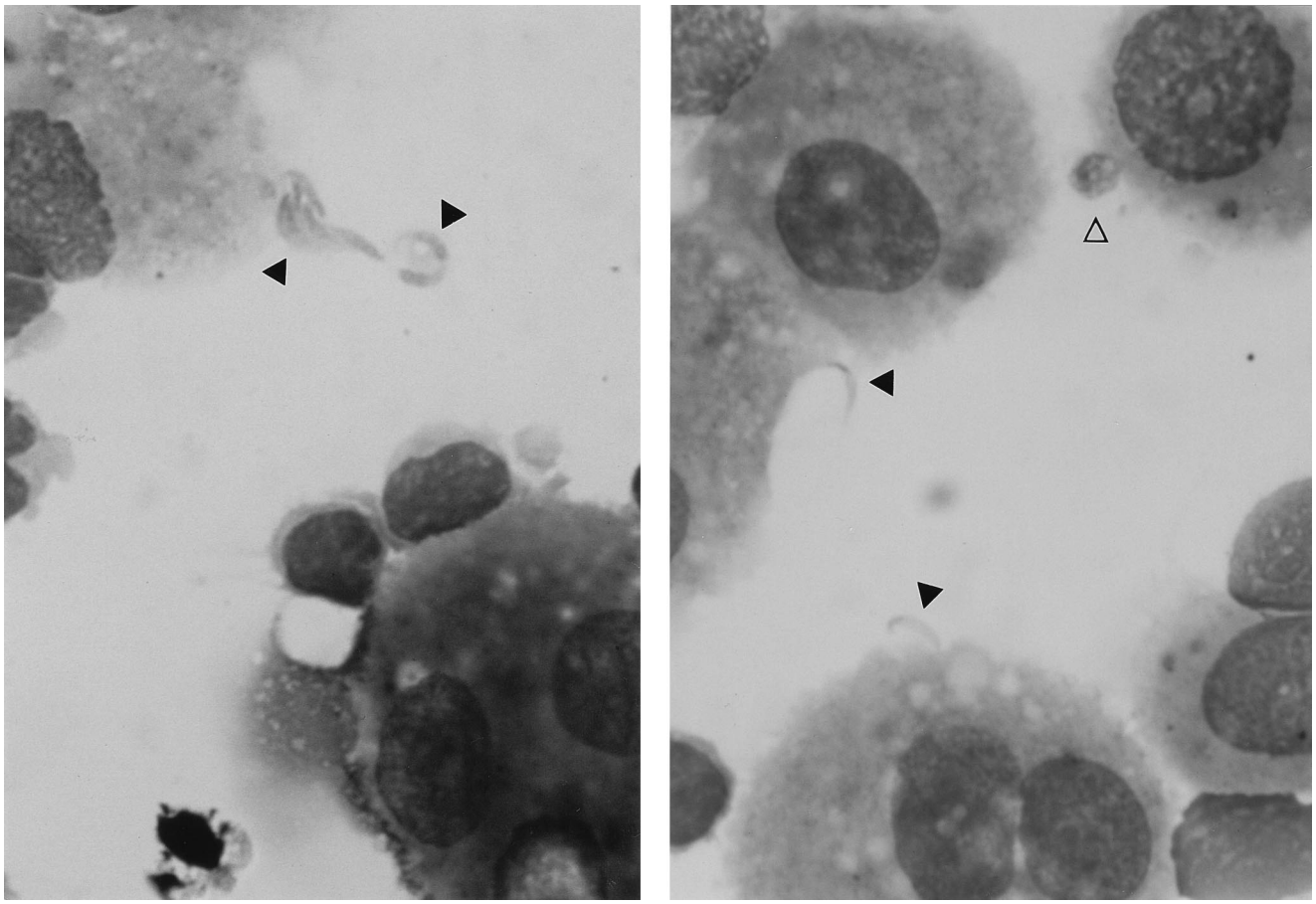

FIG. 1. Giemsa-stained smear of BAL showing extracellular invasive forms of *Cryptosporidium* organisms (black arrowheads) and intracellular development form of *Cryptosporidium* organisms on the surface of an alveolar macrophage (white arrowhead). Original magnification, ×1,250.

periodic acid-Schiff stain, or Gomori silver, all of the stages in the life of *Cryptosporidium* organisms have been observed in the microvillus border of unciliated epithelial cells and within the bronchial mucus glands (9, 10). However, histologic specimens are often not available. On smears of infected fluids, including sputum, broncheal aspirate, or BAL, only thick-walled oocysts are easily recognized after various staining techniques, such as several modifical versions of the acid-fast stain (Kinyoun and Zielh-Neelson), fluorescent auramine-rhodamine stain, or indirect immunofluorescence (6, 7). However, these infectious forms are often scattered, which may lead to false-negative results, and it is useful to recognize forms at stages other than the oocyst stage.

For our patients, we stained BAL smears with MGG, which revealed all of the stages in the life of *Cryptosporidium* organisms, even though oocysts were very rare. Furthermore, in patient 1, extracellular invasive forms of the parasite (sporozoites or merozoites) were also present in the BAL, which has apparently never been reported before. We postulated that this resulted either from massive tracheal or bronchial proliferation of the parasite contaminating the alveolar sample during the lavage process or from excystation of oocysts in the BAL sample after its collection. The latter possibility seems unlikely in patient 1, since the sample was examined very quickly after harvesting. The invasive forms of *Cryptosporidium* organisms may cause problems during differential diagnosis with other opportunistic protozoa such as (i) *T. gondii* tachyzoites, which are about as long as *Cryptosporidium* sp. tachyzoites but are curved and wider, and (ii) the amastigote forms of *Leishmania* spp., which are rarely seen in BAL and have an ovoid shape and a kinetoplast easily seen next to the nucleus when MGG is used as the stain.

In the two present cases of pulmonary or respiratory cryptosporidiosis, clinical and radiologic findings were nonspecific. Lung cryptosporidiosis preceded the onset of diarrhea in patient 1, which underlined the need for good diagnostic tools. Concomitant pathogens, such as cytomegalovirus, *P. carinii*, *Mycobacterium avium-M. intracellulare*, or *Mycoplasma pneumoniae*, may be present in such patients (2). However in these two patients, there were no pathogens other than *Cryptosporidium* organisms.

The pathogenesis of *Cryptosporidium* lung infection is still unclear. It was recently shown that *Cryptosporidium* organisms could develop in the bronchial epithelium of calves (9). Infection can result from the inhalation of oocysts after vomiting or the hematogenous spread of the oocysts. Although intestinal *Cryptosporidium* sp. organisms are not usually invasive, oocysts have been found inside macrophages, which can have defective phagocyte killing ability (7). In fact, *Cryptosporidium* organisms can multiply in macrophages in vitro (8), suggesting that extraintestinal parasites might spread via circulating phagocytes. This hypothesis is further supported by a case of intestinal and pulmonary cryptosporidiosis in which postmortem examination disclosed the presence of *Cryptosporidium* organisms inside the lumen of submucosal blood vessels (5).

Up to now, there has been no curative treatment for cryptosporidiosis. Current therapy is limited to macrolides (spiramycin) or aminosides (paromomycin). Paromomycin has proved effective for treating intestinal cryptosporidiosis (12, 13) but not for treating extraintestinal forms of the disease, probably because of the lack of intestinal absorption of the drug (12). Inhaled paromomycin was recently proposed for treatment of respiratory cryptosporidiosis, with encouraging results (4). Azithromycin, a new macrolide antibiotic, appeared to be effective in immunosuppressed rats with cryptosporidiosis (11). Its use has been proposed for treatment of intestinal cryptosporidiosis in humans (3), and it was given to the patients reported here. Patient 1 improved after 1 month of treatment and was still free of respiratory symptoms 10 months later. In contrast, the physical state of patient 2 worsened despite treatment, and large numbers of *Cryptosporidium* oocysts were still present in the feces just before death. Part of this treatment failure might be explained by the poor absorption of azithromycin because of the digestive intolerance present in this patient. Further studies to evaluate the efficacy of azithromycin in treating disseminated cryptosporidiosis are needed.

REFERENCES

1. **Brady, E. M., M. L. Margolis, and O. M. Korzeniowski.** 1984. Pulmonary cryptosporidiosis in acquired immune deficiency. JAMA **252:**89–90.
2. **Brea-Hernando, A. J., E. Bandres Franco, J. D. Mosquera Lozano, M. Lantero Benedito, and M. Ezquerra Lezcano.** 1993. Pulmonary cryptosporidiosis and AIDS. Presentation of a case and review of the literature. Ann. Med. Interne **10:**232–236.
3. **Dionisio, D., M. Meli, S. Carbonai, A. Farese, P. Corsi, A. Orsi, and F. Leoncini.** 1994. Decreased diarrhea and *Cryptosporidium* excretion in AIDS patients treated with paromomycin, abstr. 208. AIDS Congress Milan.
4. **Fujita, H., H. Mohri, T. Inayama, T. Amano, and T. Okubo.** 1994. Successful paromomycin inhalation therapy for respiratory cryptosporidiosis in AIDS patients, abstr. PB0645. AIDS Congress Yokohama.
5. **Gentile, G., L. Baldassari, A. Caprioli, G. Donelli, M. Venditti, G. Avvisati, and P. Martino.** 1987. Colonic vascular invasion as a possible route of extraintestinal cryptosporidiosis. Am. J. Med. **82:**574–575.
6. **Holjyng, N., and B. N. Jensen.** 1988. Respiratory cryptosporidiosis in HIV-positive patients. Lancet **1:**590–591.
7. **Ma, P., T. G. Villanueva, D. Kaufman, and J. F. Gillooley.** 1984. Respiratory cryptosporidiosis in the acquired immune deficiency syndrome. JAMA **252:** 1298–1301.
8. **Martinez, F., C. Mascaro, M. J. Rosales, J. Diaz, J. Cifuentes, and A. Osuna.** 1992. In vitro multiplication of *Cryptosporidium parvum* in mouse peritoneal macrophages. Vet. Parasitol. **42:**27–31.
9. **Mascaro, C., T. Arnedo, and J. Rosales.** 1994. Respiratory cryptosporidiosis in a bovine. J. Parasitol. **80:**334–336.
10. **Moore, J., and J. Frenkel.** 1991. Respiratory and enteric cryptosporidiosis in humans. Arch. Pathol. Lab. Med. **115:**1160–1162.
11. **Rehg, J. E.** 1991. Activity of azithromycin against cryptosporidia in immunosuppressed rats. J. Infect. Dis. **163:**1293–1296.
12. **Rehg, J. E.** 1994. A comparison of anticryptosporidial activity of paromomycin with other aminoglycosides and azithromycin in immunosuppressed rats. J. Infect. Dis. **170:**934–938.
13. **Wallace, M. R., M. T. Nguyen, and J. A. Newton.** 1993. Use of paromomycin for the treatment of cryptosporidiosis in patients with AIDS. Clin. Infect. Dis. **17:**1070–1071.