Demonstration of *Isospora belli* by Acid-Fast Stain in a Patient with Acquired Immune Deficiency Syndrome

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*Isospora belli*, like *Cryptosporidium* sp., a sporozoan parasite of the small intestine, has been isolated from both immunodeficient and immunologically normal persons. In immunocompetent persons the infection may be asymptomatic; it frequently causes mild and self-limited diarrhea. The spectrum of the disease in persons with acquired immune deficiency syndrome is not well established. *I. belli* did not stain well with the methods usually used for intestinal protozoa and was difficult to see unstained. However, it stained well with the acid-fast technique used for *Cryptosporidium* sp. and was easily identified by such stains. A case of combined infection with *Cryptosporidium* sp. and *I. belli* is reported.

An increasing number of infections, many of them zoonoses seldom or never seen in immunocompetent persons, are found to be associated with acquired immune deficiency syndrome (AIDS) (3). Of the protozoal diseases, *Pneumocystis carinii* pulmonary and retinal infections (4, 7), toxoplasmal encephalitis (13), and intestinal infection by *Cryptosporidium* sp. (1, 5, 8, 11, 12) are common. *Entamoeba histolytica* and *Giardia lamblia* infections are also frequently obscured. On the basis of published data, *Isospora belli* infections seem to be uncommon, since we can find only one indirect reference in the literature (3). However, we know of several cases in which this parasite was found in the stools of patients with known or presumptive AIDS. Moreover, we suspect that *I. belli* may often escape diagnosis because of the technical difficulties associated with its laboratory identification. We report here the fortuitous discovery that this parasite stained readily with the modified acid-fast stain used for identification of *Cryptosporidium* sp.

CASE REPORT

The patient in whom we were initially able to show the presence of *I. belli* by means of acid-fast stains was a 34-year-old homosexual man. He was first admitted to the Kaiser Foundation Hospital, San Francisco, in May 1983 with a temperature of 40°C after 2 weeks of low-grade fever, nonproductive cough, and dyspnea on exertion. His symptoms may have begun some 4 months earlier, when episodic night sweats developed.

Results of a physical examination were normal except for diffuse shotty lymphadenopathy and a palpable spleen. The lung fields were clear, as was a chest X-ray film. The leukocyte count was 4.8 × 10⁹/liter, with a differential count of 61 polymorphonuclear leukocytes, 24 lymphocytes, 14 monocytes, and 1 eosinophil. Measurement of arterial blood gases while he was breathing room air showed a pH of 7.44, a carbon dioxide partial pressure of 38 mmHg, and an oxygen partial pressure of 83 mmHg. The initial impression was of AIDS, with probable pneumocystis pneumonia. Bronchoscopy did not show the presence of *P. carinii*, and his fever responded to oral erythromycin.

In July 1983, low-grade fever, dyspnea on exertion, and night sweats again developed. He also complained of abdominal cramps and diarrhea. Stool examinations at this time showed *E. histolytica*, which was treated with conventional doses of metronidazole and diiodohydroxyquin; this led to an improvement in his condition. In October 1983, abdominal cramps, diarrhea, and low-grade fever again developed. Stool culture showed the presence of *Shigella* group B strains; *Cryptosporidium* sp. was found by acid-fast stain. No other intestinal parasites were identified at that time. He was treated with trimethoprim-sulfamethoxazole, resulting in symptomatic improvement. Several weeks later, he was again admitted to the hospital because of weakness, persistent fever, and dry cough. The leukocyte count was 9.7 × 10⁹/liter, with a differential count of 68 polymorphonuclear leukocytes, 2 stab cells, and 30 lymphocytes. Measurement of arterial blood gases while he was breathing room air showed a pH of 7.45, partial pressure of CO₂ of 25 mmHg, and partial pressure of O₂ of 65 mmHg. Again, the chest was clear to auscultation, and results of X-ray evaluation and bronchoscopy examination were normal. Stool examinations showed the presence of *G. lamblia*; he was treated with quinacrine, which provided some improvement, and discharged. However, in December 1983, diarrhea again increased; he was again intermittently febrile, and the adenopathy was noted to have increased. Results of conventional stool studies were negative, but acid-fast stains once again showed the presence of *Cryptosporidium* sp. and *I. belli*. No treatment was attempted because the diarrhea once again became episodic.

In January 1984, biopsy of a submandibular lymph node showed the presence of Kaposis's sarcoma.

MATERIALS AND METHODS

The Permanente Medical Group Regional Laboratory in Berkeley, Calif., performs stool examinations for all Kaiser Foundation Hospitals and associated medical clinics in Northern California. Routine stool examination for parasites is done on specimens preserved in polyvinyl alcohol fixative and in Formol-saline solution. Upon request, stool specimens from patients with AIDS are examined for *Cryptosporidium* sp. by a modified acid-fast technique (2). We exam-
ined 56 specimens from 37 patients. Seven specimens from five patients were found to contain oocysts of Cryptosporidium sp.; one of the specimens also contained oocysts of I. belli. Portions of specimens containing unusual parasites of any kind are routinely saved for reference purposes.

RESULTS

Oocysts of Cryptosporidium sp. were identified in acid-fast stained preparations, along with small numbers of oocysts of I. belli in a stool specimen from a patient with AIDS who was hospitalized at the Kaiser Foundation Hospital in San Francisco. I. belli was readily recognized by its size (ca. 30 by 12 μm) and the contained, rounded mass of germinal material, or sporoblast. This mass, which in older specimens divides to form two sporoblasts, stains a deep red, and a precipitate of this stain in most cases outlines the oocyst wall, which itself does not stain. Examination of an unstained concentrate of the stool specimen also showed oocysts of I. belli (Fig. 1). Five other stool specimens in which I. belli had been identified at various times during the preceding 4 years were still available. They were stained by the same modified acid-fast technique, and in three of them the I. belli took the stain well, although not generally as intensely as in the fresh specimen. Oocysts were extremely rare in the other two specimens and could not be found in the stained preparations.

DISCUSSION

I. belli, like Cryptosporidium, is a sporozoan. Unlike Cryptosporidium, which occurs in a variety of animal hosts and is probably an accidental parasite of humans, I. belli occurs only in humans. Both parasites occur in the small intestine; Cryptosporidium appears to multiply only in the brush border of the intestinal epithelial cells, whereas I. belli invades those cells. I. belli is worldwide in distribution but is seldom reported from most areas. It is seen frequently in the tropics (14). In persons with an intact immune mechanism, infection is often asymptomatic or marked by a mild and self-limited diarrhea; protracted infections are, however, reported. Whether the infection in immunodeficient persons differs in severity from that in immunocompetent persons is not known, since immunocompromised persons regularly suffer from concurrent infections.

The patient discussed above, like many other AIDS victims, was found to be infected with a number of different bacterial and protozoal pathogens. We can only speculate about the contribution of any single agent to his clinical condition, and there is considerable uncertainty as to the sequence in which they were acquired. It is certainly possible that the I. belli was present but undetected at the time of the initial examinations of the patient’s stool samples by acid-fast stains. Review of those slides failed to reveal I. belli, but it is frequently present in small numbers. In the interim, he had received trimethoprim-sulfamethoxazole, although at a smaller dosage and for a shorter duration than those used for the treatment of isosporiasis.

Whether I. belli is more common in immunocompromised hosts or whether its apparent increased occurrence is merely the result of more careful examination is not clear. That I. belli infection may be common in certain situations, in which at least one factor is poor sanitation, is shown by its presence in 5 of 103 civilian naval personnel and their families returning to the United States after World War II internment in the Philippines (10). Anal-oral transmission of other intestinal protozoa has been shown to lead to strikingly increased occurrences of these organisms among homosexual men. A similar mechanism may apply to I. belli.

Isosporiasis, unlike cryptosporidiosis at present, can be successfully treated. Combinations of pyrimethamine and sulfadiazine, as well as trimethoprim-sulfamethoxazole, have been found curative (14). We hope that the availability of a simple staining technique will lead to easier identification of this parasite.

LITERATURE CITED