Alga Associated with Diarrhea in Patients with Acquired Immunodeficiency Syndrome and in Travelers

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Spherical bodies resembling coccidian oocysts and measuring 8.0 to 9.0 μm in diameter were seen in the stools of eight persons with explosive, watery diarrhea. Seven had recently traveled to tropical countries, mostly in the Caribbean, and four had acquired immunodeficiency syndrome. The structures were easily discernible in wet mounts by light microscopy and contained variable numbers of granular inclusions, but were refractory to, or stained partially with, 12 commonly used laboratory stains. Electron microscopy revealed an outer fibrillar coat, a thin cell wall, granules, and organelles which were not surrounded by membranes. One type of organelle was similar to the thylakoid photosynthesizing organelles of blue-green algae (cyanobacteria). These findings indicate that the bodies may be a species of blue-green algae.

In 1986, Soave et al. (R. Soave, J. P. Dubey, L. J. Ramos, and M. Tummings, Clin. Res. 34:533A, 1986) reported large numbers of a coccidian-like body seen in diarrheic stools from four persons who had traveled to Haiti and Mexico. These patients were immunologically competent and complained of nausea, vomiting, anorexia, weight loss, and explosive, watery diarrhea. It was concluded that the organism resembled a coccidian body or a fungal spore.

Over the past 2 years, similar organisms have been seen in eight stool specimens sent to the Centers for Disease Control, Atlanta, Ga., and to the New York City Department of Health. In each case the organism was described as resembling a coccidian oocyst or a "large Cryptosporidium." All of the patients had symptoms similar to those mentioned above. Four suffered from acquired immunodeficiency syndrome (AIDS), and seven had recently visited countries in the Caribbean, Mexico, South America, India, or Southeast Asia. Only one patient's stools contained another organism that may have been responsible for his diarrhea; this patient had AIDS and was infected with Entamoeba histolytica and Dientamoeba fragilis. In most of the patients, the intestinal symptoms cleared within 2 weeks along with a decrease in the number of cystlike bodies. One patient who traveled frequently to South America reported several episodes of acute diarrhea over the course of a year, and organisms with similar appearances were observed in his stool on these occasions.

One smear from the unconcentrated stool of an AIDS patient showed as many as 20 organisms per oil immersion field.

MATERIALS AND METHODS

All stool specimens were submitted in 10% Formalin, and the organisms were concentrated by Formalin-ethyl acetate sedimentation (1). The deposits were further cleared of fecal debris through six layers of wet cotton gauze, followed by sedimentation for 30 min and centrifugation at 500 × g for 2 min. As the organisms collapsed within 2 min in hypertonic solutions, they could not be concentrated by zinc or sucrose flotation or by sucrose density gradient centrifugation. Portions of the sediment were used for electron microscopy, and the remainder was suspended and used to make stained smears for light microscopy.

Specimens were examined as wet mounts and were stained with Giesma, Gram, Gridley-fungus, hematoxylin and eosin, Lugol's iodine, methylene blue, modified acid-fast, periodic acid-Schiff, safranin, and trichrome by bright-field microscopy at ×400 and ×1,000 magnifications. Auramine O-stained specimens were examined by fluorescence microscopy.

For electron microscopy, pellets from centrifuged suspensions were suspended in molten 2.0% agarose at 45°C. The agar pellets were cut into 1.0-μm3 blocks, postfixed with osmium tetroxide for 45 min, dehydrated through an alcohol series, embedded in Ultra-Low Viscosity resin (Poly- sciences Inc., Warrington, Pa.), and polymerized overnight at 70°C. Sections were cut on a Reichert Ultracut E microtome with a diamond knife, stained with uranyl acetate, and examined in a Philips 410 transmission electron microscope at 60 kV.

RESULTS

Light microscopy. In clean wet mounts, the organisms were easily seen as nonrefractile spheres measuring 8.0 to 9.0 μm in diameter. Most contained three to nine granular inclusions measuring 3.0 μm or less. The inclusions were highly refractile and had a greenish tinge (Fig. 1a). A few spheres were empty or had collapsed into crescents.

The organisms were refractory to all but two stains, modified acid-fast and safranin, and these stains showed great variability, with many organisms remaining as unstained glassy, wrinkled spheres. Modified acid-fast stained the organisms faint pink to deep red, and some appeared to have retained granules of stain while others had a bubbly appearance (Fig. 1b). Safranin stained the organisms orange. Unlike Cryptosporidium oocysts, which tend to wrinkle during dehydration, most of the bodies retained their shape and symmetry. No technique we used showed the contents of the spheres. Some organisms stained a faint pink with periodic acid-Schiff, and the outer walls of the spheres

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FIG. 1. Bright-field micrograph of coccidian-like organisms in unstained wet mount (a) and organisms in fecal smear stains with a modified acid-fast method (b).

FIG. 2. Electron micrograph of the organism. CW, Cell wall; DG, dark granule; F, fibrillar coat; LG, light granule.
FIG. 3. Electron micrograph of Chlorella sp. showing thylakoid (T) and associated granules.

stained moderately with Gomori methenamine-silver and more weakly with methylene blue so that the organisms looked like brown or blue rings. In contrast to the strong lasting fluorescence seen with Isospora belli and Cryptosporidium controls, auramine O fluorescence was too weak for photography.

Ruptured spheres showed a peculiar break that resembled a tear in a membrane rather than a crack in a rigid cell wall.

**Electron microscopy.** The organisms were seen as roughly spherical objects, 6.0 to 7.0 μm wide (probably shrunken during processing), with a 100-nm fibrillar outer coat and a 50-nm-thick cell wall. A cell membrane, 17 nm wide, surrounded the cytoplasm, which contained light and dark granules (Fig. 2). The similar appearance to laboratory-grown Chlorella spp. was evident from Fig. 3. The dark granules appeared to be electron-dense disks with a concave area on one side, while the lighter granules, which were much larger, were not covered by a membrane. No nucleus, mitochondria, or endoplasmic reticulum was seen. Other specimens showed bands of wavy, lamellar structures associated with the large, electron-dense granules (Fig. 4). These were similar to the chloroplasts of photosynthesizing organisms.

**DISCUSSION**

On initial examination, the spherical organisms seen in eight diarrheic stool specimens strongly resembled coccidian oocysts. Unlike the latter, however, the organisms were refractory to most stains. They could best be seen in wet mounts. Their inclusions were highly variable in size and number and appeared to be composed of amorphous materials. There were no structures with the complexity of coccidian sporozoites.

The spheres had membranous walls that were thinner and more flexible than the thick, rigid walls of fungal blastospores or conidia. Ruptured organisms tended to collapse. The organisms differed further from yeast cells by staining weakly with Gomori methenamine-silver, Gram, periodic acid-Schiff, or Gridley-fungus stain.

A fibrillar outer slime coat, cytoplasmic granules, lamellar bodies resembling thylakoids with alternating light and dark bands, the absence of a membrane-bound nucleus, and the procaryotic appearance indicated a resemblance to the blue-green algae (cyanobacteria) (3, 4, 7-9). Unlike the chloroplasts of higher green plants, the photosynthetic thylakoids composed of flattened membranous sacs are not contained within an envelope. The cytoplasm of the blue-green algae also contains numerous storage granules. Similar structures were seen in the organisms studied (Fig. 4).

We were unable to obtain high-resolution electron micrographs of the organisms because stool specimens were not ideally preserved for electron microscopy. Our observations strongly suggest that the unknown organism was a blue-green alga, similar in structure to Chlorella spp. There have been reported outbreaks of diarrheal illness in humans and other animals associated with drinking water containing large numbers of several species of blue-green algae (5, 11). If further studies support this conclusion, it is possible that the organisms were ingested with improperly filtered water. Many blue-green algae produce endotoxin-like substances (6, 7) and other factors that inhibit the growth of organisms in in vitro culture (7). These may have been the cause of the
diarrhea in these patients either directly or by dysfunction of the normal gut flora.

Although the blue-green algae are generally autotrophic, some are capable of growth in the dark when supplied with sugars, albumin, peptone, casein, and various amino acids (7). This may explain the persistence of the organism in the stool of some patients for up to 2 weeks.

The morphology of the organism in wet mounts, its acid-fast staining properties, its presence in watery stools, and its association with AIDS patients could easily lead to its misidentification as a Cryptosporidium sp. or Blastocystis hominis or E. histolytica cysts. None of the experienced parasitologists who saw the organism could recall having seen it before, either in diarrheic or in normal stools.

Studies are in progress to determine the specific identity of this organism and its geographic distribution, epidemiology, and pathogenic potential. There is a strong possibility that it is a blue-green alga capable of causing severe diarrheal illness in both immunodeficient and immunocompetent individuals. We therefore suggest that the possible role of blue-green algae be considered during investigations of outbreaks of diarrheal illness when another causative agent is not found (10).

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LITERATURE CITED


