MODIFICATION OF THE CLINICAL COURSE OF INTESTINAL MICROSPORIDIOSIS IN ACQUIRED IMMUNODEFICIENCY SYNDROME PATIENTS BY IMMUNE STATUS AND ANTI-HUMAN IMMUNODEFICIENCY VIRUS THERAPY

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Abstract. The clinical course of 37 Enterocytozoon bieneusi–infected acquired immunodeficiency syndrome patients with diarrhea was studied. Parasite clearance was seen in 15 patients (40.5%). Clearance of E. bieneusi resulted in a 25–100% reduction in episodes of diarrhea, suggesting that microsporidia are true pathogens. Univariate and multivariate proportional hazards analyses revealed that peripheral blood CD4 cell counts ≥ 100/mm³, the use of two or more antiretroviral medications, and use of a protease inhibitor were statistically associated with decreased time to clearance of E. bieneusi. Specific anti-microsporidial therapy (albendazole) was not associated with parasite eradication. Factors related to immunocompetence and human immunodeficiency virus suppression appeared to be important in the clearance of E. bieneusi.

The clinical entity of Enterocytozoon bieneusi–related diarrheaa first characterized by Modigliani and others and Desportes and others was originally considered to be a relentless, progressive, wasting condition with significant morbidity and mortality seen in severely immunosuppressed patients with CD4 cell counts generally less than 100 cells/mm³. Parasite eradication using metronidazole, albendazole, and thalidomide was considered problematic. Recent observations suggest that the clinical course of E. bieneusi–associated gastrointestinal infection has changed from its original description. A four-year evaluation of the yearly and seasonal prevalence of microsporidiosis in southern California by our group revealed a large, and statistically significant, decrease in yearly prevalence in 1995 and 1996 as compared to 1993 and 1994 (Conteas CN, OGW Berlin, unpublished data). Closer scrutiny of acquired immunodeficiency syndrome (AIDS) patients with E. bieneusi–associated diarrhea revealed patients with higher CD4 cell counts than previously considered to predispose to E. bieneusi infection. Parenthetically, intact cellular immunity correlated well with spontaneous clearance (in the absence of antiprotozoal therapy) in two documented cases of self-limited E. bieneusi–related diarrheaa in human immunodeficiency virus (HIV)–negative, nonimmunosuppressed individuals with normal cell–mediated immunity. The goal of this study was to evaluate the clinical course of E. bieneusi–associated diarrheaa with special attention directed to major factors related to immune competence; specifically, the status of the patient’s cell mediated immunity and the number and types of antiretroviral drugs used.

MATERIALS AND METHODS

Acquired immunodeficiency syndrome patients with diarrheaa were selected from the hospitals and clinics of the Southern California Permanente Medical Group. Patients were enrolled between 1993 and 1996 and screened initially by stool examination for microsporidial spores using both a modified chromotropic trichrome stain (Weber) and spore epifluorescence with Fungi Fluor stain. Specimens were fluoresced under a 330–380 nm UV filter to reveal characteristic brilliant blue spores. Speciation was performed by electron microscopy. Presence of the parasite was confirmed by upper gastrointestinal endoscopy using a pediatric colonoscope (PCF100; Olympus, Long Beach, CA) and jejunal biopsy. Histologic examination of fixed specimens was performed on spores using the modified chromotropic trichrome and the fluorescences stains. Touch preparations were prepared from endoscopic biopsies smeared over a standard microscopic slide and ethanol-fixed. Modified chromotropic trichrome and Fungi Fluor staining were performed as previously described. The T cell values (CD4, CD8, and CD4:CD8 ratios) were taken at the time of initial documentation of microsporidial infection. Antiretroviral medications and coinfections at the time of diagnosis of microsporidial infection were determined for all patients. Documentation of clearance was evaluated endoscopically using the same methodologies as previously described.

Informed consent was obtained from all study participants, and this study was approved by the Kaiser Permanente Medical Care Program, Southern California Region, Institutional Review Board for the Protection of Human Subjects. The time to resolution of the infection was defined as time from onset of clinical symptoms to the clearance of the organism from the intestinal tract for patients who cleared this infection. Patients were followed until either the end of the study (December 1996) or death.

The Cox proportional hazards model was used to estimate hazard ratios. When available, HIV serum viral loads were obtained from the patients at the time of clearance of microsporidia. Blood plasma HIV RNA levels were quantified using the Chiron Quantiplex 2.0 branched chain DNA (bDNA) assay (Chiron Corporation, Emeryville, CA). The lower limit of detection of this assay is 500 copies of HIV RNA/ml of plasma.

RESULTS

Table 1 describes characteristics of the subjects. Thirty-seven male subjects were observed for an average of 8.5 months (range = 1–27 months). Median age was 37 years (range = 29–55 years), and median CD4 count was 47/mm³.
Of the 37 subjects evaluated for microsporidiosis, 15 (40.5%) had stool and endoscopic resolution of their infection. Stool and endoscopic resolution was documented in 15 (40.5%) subjects. Among these patients, mean time to clearance of microsporidiosis was 2.5 months (range 1±12 months). Thirteen (77.3%) of 17 patients who resolved their infection had ever taken albendazole. Total normalization of stool character and frequency was noted in nine (60.0%) of 15 patients with significant improvement noted in the remaining six (40.0%). Stool frequency decreased by 25±75% from diarrheal baseline in nine (60.0%) of 15 patients with clearance of the parasite. In general, plasma viral loads were low among these patients (range $5,013$ copies/ml of plasma). Enteric coinfections with another enteric pathogen was not significantly associated with time to clearance of microsporidiosis (RH $3.8$, $P = 0.01$). There was a suggestion that use of two antiretroviral medications was associated with reduced time to clearance, but this association was not statistically significant in the third approach. Subjects were classified as using either a low (0, 1, or 2) or a high (3 or 4) number of antiretroviral medications. Compared with subjects taking zero or one antiretroviral medication, subjects who took three or four medications had significantly decreased time to clearance of microsporidiosis (RH $7.1$, $P = 0.02$). There was a suggestion that use of two antiretroviral medications was also associated with reduced time to clearance, but this association was not statistically significant. In the third approach, subjects were classified as either taking or not taking saquinavir mesylate, ritonavir, or indinavir sulfate (protease inhibitors). This analysis showed that use of a protease inhibitor significantly decreased time to parasite clearance (RH $5.5$, $P = 0.001$).

**Multivariate analyses.** We selected antiretroviral therapy use (two, or three-four medications versus none or one medication) as the initial variable to be placed in the multivariate model. Additional variables were added one by one to test their independent association with time to clearance. Of the additional factors tested, only high CD4 count was also independently associated with time to clearance (RH for CD4 count $\geq 100$ mm$^3$ was 2.7, $P = 0.22$; RH for use of two antiretroviral medications was 2.7, $P = 0.22$; RH for use of three or four medications was 7.4, $P = 0.02$). Concomitant infection with another enteric pathogen was not significantly associated with time to clearance in two factor models.

**DISCUSSION**

A review of the clinical course and outcome of 37 *E. bieneusi*-infected AIDS patients with diarrhea in the Southern California Permanente Medical Group demonstrated parasitic clearance in 15 patients (40.5%) with attendant resolution of diarrhea in nine patients and improvement in six patients within an average 2.5 months. Clearance of the five strata were then collapsed into three levels: up to one (zero or one antiretroviral medications), exactly two (two antiretroviral medications), and three or more (three or four antiretroviral medications). Compared with subjects taking zero or one antiretroviral medication, subjects who took three or four medications had significantly decreased time to clearance of microsporidiosis (RH = 7.1, $P = 0.02$). There was a suggestion that use of two antiretroviral medications was also associated with reduced time to clearance, but this association was not statistically significant. In the third approach, subjects were classified as using either a low (0, 1, or 2) or a high (3 or 4) number of antiretroviral medications. In this analysis, high antiretroviral therapy use was associated with significantly decreased time to parasite clearance (RH = 3.4, $P = 0.02$). Finally, subjects were classified as either taking or not taking saquinavir mesylate, ritonavir, or indinavir sulfate (protease inhibitors). This analysis showed that use of a protease inhibitor significantly decreased time to parasite clearance (RH = 5.5, $P = 0.001$).
parasite occurred infrequently in the presence of specific anti-microsporidal therapy with albendazole, since 72.7% of the patients who failed to resolve their infection were treated with that medication. In addition, patients who took albendazole but failed to clear microsporidiosis tended to be completely refractory to the drug, with little or no attendant resolution of diarrheal symptoms. Resolution and clearance correlated with specific parameters of the patients’ immune status and with the application of more intensive anti-HIV therapy.

Univariate and multivariate analyses revealed that peripheral blood CD4 cell counts ≥ 100/mm³, the use two or more anti-HIV medications and use of a protease inhibitor were associated with an increased likelihood of clearing microsporidiosis, as well as a decrease in time to clearance of the parasite among subjects whose infection was self-limited. Factors pertinent to cellular immunity and inhibition of HIV replication were more effective in parasite clearance than antiprotozoal therapy with albendazole, the drug-of-choice for microsporidal clearance or suppression. None of the reverse transcriptase inhibitors or protease inhibitors used in HIV suppression have known antimicrosporal activity. More significant, most of the 15 patients who cleared their infection did so in a self-limited fashion in the absence of antiparasitic therapy or any change in their anti-HIV medications. Only two of the patients were on albendazole at the time of remission. The parasite appeared and disappeared without special antiparasitic treatment in 10 of 12 patients. The three other patients who did clear their microsporidiosis were each completely refractory to albendazole. This is consistent with recent reports that albendazole treatment does not eradicate microsporida in the majority of cases. All three patients added protease inhibitors (two added saquinavir and one added indinavir) to their medication regimens and subsequently cleared the parasite. Parasite clearance was accompanied by increased CD4 counts in only one of these three patients.

Review of several studies revealed CD4 cell counts in the same range as noted in our patient population who failed to clear E. bieneusi (55.0 ± 77.9/mm³ [mean ± SD]). The CD4 cell counts among patients in this study who resolved their infection were generally higher than those described in the literature (161.1 ± 133.9/mm³). Even studies somewhat skeptical of the role of E. bieneusi in the production of diarrhea in HIV-positive patients demonstrated a similarly elevated CD4 count (average = 168/mm³) in groups of infected patients, many of which were considered asymptomatic. Kotler and Orenstein posited the possibility of a relationship between parasite burden and immune status that may, in part, be responsible for the patient’s overall minimally symptomatic clinical status. Extrapolation from the previously described self-limited intestinal E. bieneusi infection in the two immunologically competent HIV-negative patients to the more immunologically intact HIV-positive patients who cleared their infection (as opposed to the less immunologically conserved HIV-positive patients who did not clear their infection) suggests the importance of preserved cell-mediated immunity in clearance of E. bieneusi. Antiretroviral therapy with two or more drugs and the use of a protease inhibitor in the therapeutic regimen correlated with decreased time to clearance of E. bieneusi. Historically, the majority of microsporidial diarrhea studies were completed prior to 1995, when the first protease inhibitor was approved by the Food and Drug Administration (saquinavir) and combination antiretroviral therapy became popularized. The extensive use of combination antiretroviral therapy and the protease inhibitors used in this study stand in marked contrast to the other studies, which used monotherapy with a reverse transcriptase inhibitor. The low viral loads measured in six of the subjects at time of microsporidial clearance provides suggestive evidence of the relationship between HIV suppression and parasite clearance. More data, especially with proper controls, is needed to fully establish this relationship.

Clearance of microsporidiosis was associated with complete resolution of diarrhea in nine patients and a 25–75% decrease in stool frequency with improvement in stool consistency in the remaining six patients. No recurrent disease was noted. The role of E. bieneusi as a pathogen is highly suggested by these data. This study has demonstrated that the previous therapeutically unresponsive infection of the gastrointestinal tract by E. bieneusi may in fact be a self-limited disease in the proper clinical setting. Preservation of cell-mediated immunity (CD4 cell counts) and viral burden suppression by combination antiretroviral therapy, especially with protease inhibitors, appears to be of vital importance in the clearance of E. bieneusi. Further investigation is needed to define the mechanisms involved in these clinical observations to optimize E. bieneusi clearance, and perhaps even prevent infection by this parasitic pathogen.

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