Lymphoproliferative and Cytokine Responses to Cryptosporidium parvum in Patients Coinfected with C. parvum and Human Immunodeficiency Virus

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We compared the lymphoproliferative and cytokine responses to Cryptosporidium parvum in human immunodeficiency virus (HIV)-seropositive and -seronegative patients. The lymphoproliferative and cytokine responses (interleukin-2 [IL-2], IL-4, IL-5, IL-10, gamma interferon, and tumor necrosis factor alpha) were assessed for 11 HIV-seropositive, Cryptosporidium-positive (group I) patients; 20 HIV-seronegative, Cryptosporidium-negative (group II) patients; 10 HIV-seronegative, Cryptosporidium-positive (group III) patients, including four post-renal transplant (group IIIa) and 6 presumably immunocompetent (group IIIb) patients; and 20 HIV-seronegative, Cryptosporidium-negative healthy individuals (group IV). No significant difference was observed in the number of patients showing positive lymphoproliferative responses in group I compared to group III (post-renal transplant [group IIIa] or immunocompetent [group IIIb]) patients, while a comparison of the median stimulation indices shows that responses were significantly lower in Cryptosporidium-infected, immunosuppressed (group I and IIIa) patients than in immunocompetent (group IIIb) patients. The number of patients showing positive responses and median stimulation indices was significantly higher for Cryptosporidium-infected (HIV-seropositive and -seronegative) individuals than for uninfected individuals, suggesting that Cryptosporidium induces significant in vitro lymphoproliferative responses in infected individuals. Cytokine levels, except for that of IL-5, were significantly higher in HIV-seropositive and -seronegative patients infected with Cryptosporidium; HIV-seronegative and Cryptosporidium-negative patients, and apparently healthy individuals and to correlate the responses with CD4 counts and history of diarrhea in HIV-seropositive patients to shed further light on the role of cell-mediated immune responses to Cryptosporidium in leading to symptomatic or asymptomatic infection in immunocompromised patients.

MATERIALS AND METHODS

Subjects. Two hundred six HIV-seropositive, 153 HIV-seronegative, and 50 healthy individuals were enrolled in a previous study for the detection of Cryptosporidium by stool examination with modified Ziehl-Neelsen staining (4), safranine methylene blue staining (3), antigen detection enzyme-linked immunosorbent assay (Ridascreen Cryptosporidium; R-Biopharm, Germany), and a nested PCR targeting the small subunit rRNA gene (30). Based on the results of our previous study (18), out of the subjects detailed above, 11 HIV-seropositive and Cryptosporidium-positive (group I) individuals, 20 HIV-seronegative and Cryptosporidium-negative (group II) individuals, 10 HIV-seronegative and Cryptosporidium-positive (group III) individuals, and 20 HIV-seropositive and -parvum

Cryptosporidiosis is self-limiting in immunocompetent hosts but can be life threatening in immunocompromised hosts. The duration of diarrheal illness and the ultimate outcome of intestinal cryptosporidiosis depend on the immune status of the patient (1). Chronic cryptosporidiosis in AIDS patients correlates with a decrease in T-cell function. Patients with CD4 counts of >180 cells/μl usually have a self-limiting infection, whereas most patients with counts of <140 cells/μl develop severe and persistent infection (12). Gamma interferon (IFN-γ) has a central role in protective immune responses against Cryptosporidium infection in mouse models (23). Studies demonstrate the importance of T cells, in particular CD4+ T cells, in clearing and providing protection against cryptosporidiosis in mice (6). Most of the evidence has come from studies done on animal models. However, reports regarding the lymphoproliferative and cytokine responses to C. parvum in infected human subjects are scarce. In an earlier study (14), lymphocyte proliferation in response to Cryptosporidium antigen was found in both immunocompetent patients with a history of cryptosporidiosis and 75% of healthy individuals, while no proliferation was observed in human immunodeficiency virus (HIV)-seropositive (only three studied) patients. In the other study (15), significant proliferation in Cryptosporidium-infected, immunocompetent individuals and no proliferation, or very little proliferation, in HIV-seropositive individuals (both Cryptosporidium infected and uninfected) were observed. The same study reported the production of interleukin-2 (IL-2), high levels of IFN-γ and IL-10 in HIV-seronegative and Cryptosporidium-positive patients, and low levels of IFN-γ and IL-10 in HIV-seropositive and Cryptosporidium-positive patients in response to Cryptosporidium. The present study was aimed to evaluate and compare the lymphoproliferative and cytokine immune responses to C. parvum in HIV-seropositive and -seronegative patients infected with Cryptosporidium, HIV-seropositive and Cryptosporidium-negative patients, and apparently healthy individuals and to correlate the responses with CD4 counts and history of diarrhea in HIV-seropositive patients to shed further light on the role of cell-mediated immune responses to Cryptosporidium in leading to symptomatic or asymptomatic infection in immunocompromised patients.
negative Cryptosporidium-negative healthy individuals without any history of cryptosporidiosis (group IV) were selected for the study from the Immunodeficiency Clinic, the inpatient and outpatient departments of Nehru Hospital attached to the Post-Graduate Institute of Medical Education and Research, Chandigarh, a tertiary-care hospital in north India. The diagnosis of HIV was established as per National AIDS Control Organisation (NACO) guidelines (WHO criteria adopted by NACO) (24). After obtaining informed consent from each individual, demographic characteristics, such as sex, age, history of diarrhea, and any other relevant symptoms, were recorded on a preplanned pro forma document. HIV patients receiving antiretroviral therapy were excluded from the study, as it might affect HIV status and immune response and thereby may affect the results. About 10 ml venous blood was collected from each patient in vials containing heparin.

**Antigen and mitogen.** Cryptosporidium parvum crude soluble antigen (CCA) was prepared by the sonication of oocysts (15). Brieﬂy, Cryptosporidium parvum oocysts (Iowa strain) obtained from NIH AIDS Research and Reference Reagent program were washed thrice with phosphate-buffered saline (PBS) (15,000 rpm, 15 min), suspended in PBS, freeze-thawed 20 times, sonicated (12 cycles of 30 s), and centrifuged (15,000 rpm, 15 min, 4°C). The supernatant was used as the CCA after estimating the protein level by Lowry’s method. Phytohemagglutinin (PHA) (Bangalore Genie, India) was used as mitogen.

**Lymphocyte proliferation assay.** Peripheral blood mononuclear cells (PBMCs) were separated by mixing heparinized blood with an equal volume of PBS and layering it over Histopaque, followed by centrifugation at 1,800 rpm for 30 min. Buffy coat cells were collected, washed thrice with RPMI 1640 medium (Gibco, Grand Island, NY) and suspended in RPMI 1640. The PBMCs were adjusted to a concentration of 1 × 10^6 cells/ml of RPMI 1640. The cells were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum and antibiotics in the presence of CCA and the nonspeciﬁc antigen PHA. The cells were incubated at 37°C in the presence of 10% CO_2_. Different concentrations of antigens (1, 2, 5 and 10 pg/ml) were initially used, and the optimum proliferative responses were achieved with 2 pg/ml CCA and 5 pg/ml PHA stimulation for 3 days. At the end of the incubation period, the culture supernatant of the cells was collected and kept at −80°C for the cytokine assay. A total of 1 μCi [3H]thymidine was added, followed by overnight incubation. The cells were harvested after centrifugation at 3,000 rpm for 10 min, and the pellet was washed twice with normal saline, placed in scintillation fluid, and counted on a β-scintillation counter.

The stimulation index (SI) was calculated by using the following formula: SI = counts per minute in stimulated culture/counts per minute in unstimulated culture (control).

Positive responses were considered when the SI was greater than or equal to 2 in response to CCA and greater than or equal to 20 in response to PHA (29).

**Cytokine assay.** The IFN-γ, tumour necrosis factor alpha (TNF-α), IL-2, IL-4, IL-5, and IL-10 levels were measured in the PBMC culture supernatant (in response to CCA) by commercial enzyme-linked immunosorbent kits (Immunotech, Beckman Coulter, France) as speciﬁed by the manufacturers. Brieﬂy, 50 µl of calibrator (standard) or test sample was added per well (coated with monoclonal antibody), followed by 2 h of incubation at 18 to 25°C while shaking at 350 rpm. After washing, 50 µl of biotinylated monoclonal antibody and 100 µl of streptavidin-horseradish peroxidase conjugate were added. After incubation and washing, 100 µl of substrate was added, followed by the addition of stop solution. The absorbance value was read at 450 nm. The test sample results were calculated by interpolation from a standard curve with absorbance values on the vertical axis and cytokine concentrations on the horizontal axis (pg/ml). The sensitivities of each in the assay were as follows: 1 pg/ml for IL-5, 4 pg/ml for IFN-γ, and 5 pg/ml for TNF-α, IL-2, IL-4, and IL-10.

**Ethical clearance.** This study was approved by the Institutional Ethics Committee.

**Statistics.** Percentages of positives between the groups were compared by Fisher’s exact test. SI values and cytokine levels of the groups were compared by the Mann-Whitney test. A P value of <0.05 was interpreted as indicative of a statistically significant difference.

**RESULTS**

**Demographics.** Demographic characteristics of the individuals in the study are detailed in Table 1. Out of 11 HIV-seropositive, Cryptosporidium-positive (group I) and 20 HIV-seronegative individuals (group II) patients, 5 (45.5%) and 9 (45%), respectively, had diarrhea. All 10 HIV-seronegative, Cryptosporidium-positive (group III) patients had diarrhea. Out of these, 4 (40%) had undergone kidney transplantation (group IIIa), and the remaining six were presumably immunocompetent (group IIIb) (Table 1).

**Lymphoproliferative response.** For analyzing lymphoproliferative response data, two methods were used. According to the first method (29), subjects showing SI of >2 in response to CCA and >20 in response to PHA were considered subjects showing positive responses. In another method (13, 25, 26, 27, 29), median SI values between the different groups were compared as reported earlier for various infections including parasites.

**Number of patients showing positive lymphoproliferative responses.** The numbers of patients showing positive responses to CCA (SI > 2) and PHA (SI > 20) in the different groups are detailed in Fig. 1. Positive responses to CCA were found in significantly higher numbers of Cryptosporidium-infected (group I and III) individuals than in Cryptosporidium-uninfected (group II and IV) individuals (P < 0.05). No significant difference (P > 0.05) was observed for HIV-seropositive, Cryptosporidium-positive (group I) patients compared to HIV-seronegative, Cryptosporidium-positive (group III) patients (Fig. 1).

Positive responses to PHA (SI > 20) were seen in significantly higher numbers (100%) of healthy individuals than other groups (P < 0.05) (Fig. 1).

### Table 1. Demographic profiles of the individuals included for the study of lymphoproliferative and cytokine responses

| Group | No. of subjects | Mean age (yr) ± SD* (range) | No. (%) of males | No. (%) of females | No. of subjects postrenal transplantation | No. (%) of subjects | Mean CD4 count (cells/μl) ± SD (range) |
|-------|----------------|-----------------------------|------------------|-------------------|----------------------------------------|---------------------|---------------------------------------|
| I (HIV+, Crypto+) | 11 | 34.1 ± 6.4 (25–46) | 7 (62.6) | 4 (36.4) | 5 (45.5) | 0 (0) | 182.5 ± 119.3 (46–379) |
| II (HIV+, Crypto-) | 10 | 34.2 ± 10 (25–64) | 15 (75) | 5 (25) | 9 (45) | 0 (0) | 198.6 ± 143 (30–583) |
| III (HIV+, Crypto+) | 10 | 26.2 ± 16.5 (3.5–46) | 6 (60) | 4 (40) | 10 (100) | 4 (40) | Not available |
| IV (HIV+, Crypto [healthy]) | 20 | 26.9 ± 3.2 (23–35) | 10 (50) | 10 (50) | 0 (0) | 0 (0) | Not available |
| Total | 61 | 30.5 ± 10.1 (3.5–64) | 38 (62.3) | 23 (37.7) | 24 (39.3) | 4 (40) | |

* HIV+, HIV positive; Crypto+, Cryptosporidium positive; HIV−, HIV negative; Crypto−, Cryptosporidium negative.

b SD, standard deviation.
Median SI. Median SI (range) values in different groups in response to CCA and PHA are detailed in Fig. 2. The median SI in response to CCA was significantly higher for Cryptosporidium-infected (group I and III) individuals than for Cryptosporidium-uninfected (group II and IV) individuals ($P < 0.01$). However, no significant difference ($P > 0.05$) was observed for HIV-seropositive, Cryptosporidium-positive (group I) patients compared to HIV-seronegative, Cryptosporidium-positive (group III) patients. On further analysis of group III, the median SI was found to be significantly lower for HIV-seropositive, Cryptosporidium-positive (group I; $P < 0.05$) patients and HIV-seronegative, Cryptosporidium-positive patients who had renal transplantation (group IIIa, $P < 0.01$) than for HIV-seronegative, Cryptosporidium-positive immunocompetent patients (group IIIb). There was no significant difference between the two immunosuppressed groups, i.e., HIV-seropositive, Cryptosporidium-positive (group I) patients and HIV-seronegative, Cryptosporidium-positive patients with renal transplants (group IIIa) (Fig. 2).

The median SI in response to PHA was significantly higher for healthy (group IV) individuals than for Cryptosporidium-infected [group I ($P < 0.001$) and group III ($P < 0.0001$)] individuals. No significant difference in median SI was observed for HIV-seropositive, Cryptosporidium-positive (group I) patients compared to HIV-seronegative, Cryptosporidium-positive (group III) patients ($P > 0.05$). There was no significant difference between the HIV-seronegative, Cryptosporidium-positive (group I) patients and the HIV-seronegative, Cryptosporidium-positive patients with renal transplants (group IIIa) (Fig. 2).

Cytokine response. The median levels (ranges in parentheses) of IFN-γ, IL-2, TNF-α, IL-4, IL-5, and IL-10 in the different groups are detailed in Table 2.

The median IFN-γ, IL-2, and TNF-α (Th1 cytokines) and IL-4 and IL-10 (Th2 cytokines) levels were found to be significantly higher for Cryptosporidium-infected (group I and III) individuals than for Cryptosporidium-uninfected (group II and IV) individuals. No significant difference was observed for HIV-seropositive, Cryptosporidium-positive patients (group I) compared to HIV-seronegative, Cryptosporidium-positive patients (group III) either with transplants (group IIIa) or presumably immunocompetent (group IIIb).

HIV-seropositive, Cryptosporidium-negative patients (group II) showed significantly higher levels of IFN-γ, IL-2, and TNF-α (Th1 cytokines) compared to healthy individuals (group IV). Very low levels of IL-5 were found in our study in all the groups (Table 2).

Proliferative and cytokine responses versus diarrhea. The median SI in response to CCA and PHA and the cytokine levels in response to CCA in the group I and II patients with and without a history of diarrhea are detailed in Table 3. No significant difference ($P > 0.05$) was observed in the median SI in response to CCA and PHA or the cytokine levels in both groups I and II between patients with or without diarrhea.

Proliferative and cytokine responses versus CD4 counts. The median SI in response to CCA and PHA and the cytokine levels in response to CCA in group I and II patients with CD4 counts of $<200$ or $>200$ cells/μl are detailed in Table 4.

No significant difference ($P > 0.05$) was observed in the median SI in response to CCA or PHA or the cytokine levels in both groups I and II between patients with CD4 counts of $<200$ or $>200$ cells/μl (Table 4).

DISCUSSION

In the present study, positive proliferation in response to stimulation with CCA ($SI > 2$) was observed in 82% of HIV-seropositive, Cryptosporidium-positive patients and 100% of HIV-seronegative, Cryptosporidium-positive patients, including renal transplant and presumably immunocompetent patients, but in only 15% of HIV-seropositive, Cryptosporidium-negative subjects and 20% of healthy subjects. Positive responses were found in significantly higher numbers of Cryptosporidium-infected individuals than in Cryptosporidium-uninfected individuals. Positive responses to PHA were found in 27% of individuals.
HIV-seropositive and Cryptosporidium-positive, 55% of HIV-seropositive and Cryptosporidium-negative, and 30% of HIV-seronegative and Cryptosporidium-positive patients compared to 100% of healthy individuals (P < 0.05). The successful induction of lymphocyte proliferation by CCA but not by PHA in sensitized individuals showed that the response was specific for Cryptosporidium. No significant difference was observed in the numbers of patients showing positive responses to CCA between HIV-seropositive, Cryptosporidium-positive patients and HIV-seronegative, Cryptosporidium-positive (posttransplant or immunocompetent) patients. In contrast, a previous study (14), based on a similar type of analysis, showed the proliferation to Cryptosporidium in both immunocompetent patients with cryptosporidiosis and 75% of healthy individuals, while PBMCs from HIV-seropositive patients (only three studied) did not proliferate in response to Cryptosporidium.

In the present study, on comparing the SI in response to CCA between different groups, the median SI was significantly higher for Cryptosporidium-infected individuals than for Cryptosporidium-uninfected individuals, indicating specific proliferative responses in sensitized individuals after stimulation with Cryptosporidium antigen. There was no significant difference between the proliferative responses in HIV-seropositive, Cryptosporidium-positive (group I) patients and HIV-seronegative, Cryptosporidium-positive (group III) patients. However, on further analysis of HIV-seronegative, Cryptosporidium-positive (group III) patients (posttransplant or immunocompetent), the median proliferation was significantly lower for HIV-seronegative, Cryptosporidium-positive (group I) patients and HIV-seronegative but posttransplant Cryptosporidium-positive (group IIIb) patients (immunocompromised) than for HIV-seronegative, Cryptosporidium-positive immunocompetent (group IIIb) patients, suggesting that the immune status of the host appears to play a significant role in modulating proliferative responses to Cryptosporidium antigen. This is in agreement with a previous study (15), whereby significant proliferation in Cryptosporidium-infected immunocompetent individuals and no proliferation, or very little proliferation, in HIV-seropositive individuals both Cryptosporidium-infected and -uninfected were reported. In the present study, no significant difference was observed in the median lymphoproliferative response between the two immunosuppressed groups (HIV seropositive [group III] and HIV seronegative but with renal transplants [group IIIa]) of patients

| Group or comparison | No. of subjects | Median cytokine level (pg/ml) (range) | P value |
|--------------------|----------------|--------------------------------------|---------|
| I (HIV+, Crypto+)  | 11             | 301.1 (166.2–342.8)                  | <0.0001 |
| II (HIV+, Crypto-) | 20             | 176 (116.3–244.4)                   | <0.0001 |
| III (HIV+, Crypto-) | 10          | 320.6 (165.1–342.2)                 | <0.0001 |
| IIIa (HIV-, Crypto-, renal transplant) | 4             | 318.9 (209.5–337.2)                 | <0.0001 |
| IIIb (HIV-, Crypto-, immunocompetent) | 6             | 321.7 (165.1–342.2)                 | <0.0001 |
| IV (HIV-, Crypto-) [healthy] | 20             | 104.1 (26.4–175.1)                  | <0.0001 |

* HIV+, HIV positive; Crypto+, Cryptosporidium positive; HIV-, HIV negative; Crypto-, Cryptosporidium negative.
* NS, not significant.

The values for comparisons are P values.
infected with *Cryptosporidium*. Both of these groups showed significantly lower responses than HIV-seronegative, *Cryptosporidium*-positive immunocompetent patients, which indicates lower responses due to immunosuppression induced by either HIV infection or immunosuppressive drugs in transplant patients.

We found significantly higher levels of IFN-γ, IL-2, TNF-α, IL-4, and IL-10 in *Cryptosporidium*-infected individuals than in *Cryptosporidium*-uninfected individuals, irrespective of HIV-immune status. This is in agreement with an earlier study (14) which reported higher levels of IL-10 and IFN-γ in HIV-seronegative patients with a history of cryptosporidiosis but comparatively low levels of IL-10 and no production of IFN-γ in healthy individuals. In contrast, other results have failed to detect IFN-γ in cryptosporidiosis patients' PBMCs in response to *Cryptosporidium* (19, 20). In the present study, no significant difference was observed in the cytokine responses to *Cryptosporidium* between immunosuppressed and immunocompetent infected individuals with *Cryptosporidium*. In contrast, in an earlier study (15), IL-2, high levels of IFN-γ and IL-10 in HIV-seronegative and *Cryptosporidium*-positive patients, and low levels of IFN-γ and IL-10 in HIV-seropositive and *Cryptosporidium*-positive patients were reported in *Cryptosporidium*-stimulated PBMCs.

In the present study, significantly higher levels of IFN-γ, IL-2, and TNF-α (Th1) and IL-4 and IL-10 (Th2) cytokines were observed in *Cryptosporidium*-infected individuals than in uninfected individuals, suggesting the production of both Th1 (IFN-γ, IL-2, and TNF-α) and Th2 (IL-4 and IL-10) cytokines in infected individuals. In an earlier study (15), IFN-γ and IL-2 production was observed in a significant number of infected immunocompetent individuals which suggested mainly a trend toward a Th1 response, while the production of Th2-type cytokines (IL-5 and IL-10) was also observed in a small number (20%) of immunocompetent individuals with *Cryptosporidiosis*. The study showed parallel increases in both IFN-γ (inflammatory cytokine) and IL-10 (anti-inflammatory cytokine), which was similar to our findings. Gomez Morales et al. (15) suggest the existence of a balance between these two cytokines that could shift to Th2 when there is a deficiency in IFN-γ production (15) or a condition involving high levels of IL-10, such as in HIV infection (2, 5, 21). Another study (1) suggests that the most-effective Th response to control cryptosporidial infection may be a dynamic one in which there is a strong early Th1 response but the later maturation of a more-balanced response with a Th2 component may facilitate parasite removal. Experimental studies have produced contrasting reports regarding the roles of the Th1 and Th2 cytokines. A few studies (8, 9, 11, 22, 28) support the role of Th1 cytokines, and a few others (10, 23, 28) suggest that Th2 cytokines may also play a part in protection from cryptosporidiosis. In our study, low levels of IL-5 were found in all the groups. In support of our observation, in an earlier study (15), the induction of IL-5 in response to *Cryptosporidium* antigen was observed in PBMCs from a small number (20%) of immunocompetent individuals with prior cryptosporidiosis and from none of the HIV-positive individuals.

No significant difference was observed in proliferative and cytokine responses to *Cryptosporidium* antigen in the HIV-seropositive, *Cryptosporidium*-positive patients with diarrhea compared to the HIV-seronegative, *Cryptosporidium*-positive patients without diarrhea. However, as the numbers of subjects in symptomatic and asymptomatic groups are small in the present study, more studies with high numbers of subjects are required to shed further light on the role of lymphoproliferative and cytokine responses in cryptosporidiosis. Though the pathogenesis of diarrhea in *Cryptosporidium* infection is not well understood, important factors may include the disrupted mucosal architecture and intestinal dysfunction resulting from the infection, the host response to the infection, and *C. parvum*-associated apoptotic epithelial-cell death besides other factors (7, 16). Hernandez et al. suggest that most of the pathogenesis associated with cryptosporidiosis could be due to physiological changes that are induced by the elevated substance P, a neuropeptide and pain transmitter located in areas of inflammation including the gastrointestinal tract, which is known to stimulate inflammatory cells, thus inducing proinflammatory cytokines and enhanced Cl⁻ secretion (17).

In our study, no significant difference (P > 0.05) was observed in the median SI in response to CCA or PHA and in the median cytokine levels of HIV-seropositive patients with CD4 counts of <200 or >200 cells/μl. An earlier study (15) showed that PBMCs from HIV-seropositive patients with cryptosporidiosis and CD4 counts of >500 cells/μl did not proliferate, whereas PBMCs from only one HIV-positive subject with cryptosporidiosis and a CD4 count of >200 but <500 cells/μl proliferated in response to *Cryptosporidium*. The same study reported no differences in cytokine responses among patients with different CD4 counts, which is in agreement with the present study.

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**TABLE 4. Lymphoproliferative (median SI) and cytokine responses to CCA in HIV-seropositive patients with respect to CD4 counts**

| Group and CD4 count (cells/μl) | Median SI (range) | IFN-γ | Median cytokine response to CCA (pg/ml) (range) |
|------------------------------|-------------------|-------|-----------------------------------------------|
|                              | CCA | PHA | IL-2 | TNF-α | IL-4 | IL-5 | IL-10 |
| I (HIV⁺, *Cryptosporidium*⁺) |     |     |       |       |       |       |       |
| (n = 11)                     |     |     |       |       |       |       |       |
| <200 (n = 7)                 | 3   | 1.9–6.8 | 16.7 (2.6–22.2) | 317.2 (170.7–339.4) | 67.7 (64.4–85.9) | 794.6 (416.6–970.9) | 271 (0–63.8) | 0 (0–34.4) | 813.1 (607.7–1,842.2) |
| >200 (n = 4)                 | 4.7 | 0.6–13.2 | 19.2 (19.1–36.1) | 239 (166.2–342.8) | 70 (69.3–81.2) | 796.5 (416.3–810.9) | 17 (0–39.1) | 0.8 (0–14.7) | 1,135.5 (720.5–1,577.1) |
| II (HIV⁺, *Cryptosporidium*⁻) |     |     |       |       |       |       |       |
| (n = 17)                     |     |     |       |       |       |       |       |
| <200 (n = 10)                | 1.1 | 1–1.3 | 8.1 (5–29.1) | 174 (119.1–244) | 23.5 (13.2–37.5) | 123.5 (0–272.3) | 0 (0–31.2) | 0 (0–24.1) | 430.4 (337.7–811.5) |
| >200 (n = 7)                 | 1.2 | 1.1–2.8 | 27.2 (13.3–30.5) | 181.2 (116.3–234) | 19.8 (17.1–40.9) | 112 (0–347.2) | 14.4 (0–33.9) | 0 (0–25.9) | 473.1 (343.4–715.6) |

* HIV⁺, HIV positive; *Cryptosporidium* positive; *Crypto*⁻, *Cryptosporidium* negative. For all values for patients with CD4 counts <200 versus values for those with CD4 counts >200, P is >0.05.
In conclusion, the present study indicated significantly lower lymphoproliferative responses in immunocompromised patients than in immunocompetent patients infected with Cryptosporidium, while no difference was observed in cytokine response. The study suggests that these responses may not be the only factors associated with protection from infection. Protective immune responses in cryptosporidiosis appear to involve multiple aspects of the immune system which may not be fully described by proliferative and cytokine responses. The limitation of the present study is that we have used CCA, which may contain a variety of proteins and nonprotein components, both with and without antigenic properties, and this may be the reason for the differences seen in the findings. Moreover, few conclusions can be drawn because of the small number of subjects in the study with the symptomatic cryptosporidiosis. It will be worthwhile to study and compare the responses of more subjects with the use of more-specific antigenic fractions to ascertain the present findings.

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