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Cytokine Responses to *Plasmodium falciparum* Liver-Stage Antigen 1 Vary in Rainy and Dry Seasons in Highland Kenya

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Seasonal epidemics of malaria occur in highland areas of western Kenya where transmission intensity varies according to rainfall. This study describes the seasonal changes in cytokine responses to *Plasmodium falciparum* liver-stage antigen 1 (LSA-1) by children (≤17 years old) and adults (≥18 years old) living in such a highland area. Fourteen- to 24-mer peptides corresponding to the N- and C-terminal nonrepeat regions of LSA-1 stimulated production of interleukin-5 (IL-5), interleukin-10 (IL-10), gamma interferon (IFN-γ), and tumor necrosis factor alpha (TNF-α) by peripheral blood mononuclear cells (PBMC) from 17 to 73% of individuals in both age groups in both seasons. IL-10 and TNF-α responses were more frequent during the high-transmission, rainy season than during the low-transmission, dry season (73 and 67% versus 17 and 25% response rates, respectively). In contrast, there was no seasonal change in the proportion of LSA-1-driven IFN-γ and IL-5 responses. Children produced less IFN-γ than adults, but IL-5, IL-10, and TNF-α levels were similar for both age groups. Depletion of $CD8^+$ cells from PBMC decreased IFN-γ but increased IL-10 production. Individuals with LSA-1-stimulated IL-10 responses in the dry season were less likely to become reinfected in the subsequent rainy season than those without IL-10 responses (25% versus 49%; $P = 0.083$). These data support the notion that maintenance of LSA-1-driven IL-10 and TNF-α responses requires repeated and sustained exposure to liver-stage *P. falciparum*. In contrast, IFN-γ responses increase slowly with age but persist once acquired. $CD8^+$ T cells are the major source of IFN-γ but may suppress production or secretion of IL-10.

Epidemics of malaria in the highlands of Uasin Gishu district of Kenya have been reported intermittently since 1902 (1, 3, 8, 12, 17, 18). These outbreaks usually occur during the rainy season (generally between April and September), when the number of *Anopheles* mosquitoes increases (17). Since the late 1980s, highland malaria epidemics have occurred more frequently and caused significant morbidity and mortality during the rainy season (37). The partial immunity to malaria that develops in adults living in areas where malaria is holoendemic is associated with repeated and frequent exposure to infective mosquitoes (36). In highland areas, prolonged periods of low or no exposure to infective mosquitoes during the dry season presumably results in reduction in the number of parasites that become established in the liver. This may in turn lead to diminished antigen-specific immunity to pre-erythrocytic and blood-stage *Plasmodium falciparum* with increased susceptibility to malaria infection when transmission rises during the rainy season.

To date, the only longitudinal study of immune responses in epidemic highland malaria has been done in Madagascar and was concerned with a blood-stage antigen. Examination of adult residents during an epidemic in 1986 to 1987 showed that antibody levels and lymphocyte proliferation to Pf155 ring-infected erythrocyte surface antigen peptides decreased after the outbreak was controlled. Cytokine responses were not reported (27). Studies on immune responses in areas of Sudan with unstable malaria transmission have focused primarily on antibody responses (4, 9, 10, 39). Prospective studies of seasonal changes in malaria antigen-specific immune responses in areas where malaria is holoendemic have also focused primarily on antibody and lymphocyte proliferation responses (31, 32). The importance of cytokines in mediating resistance against pre-erythrocytic malaria infection has been documented in animal models (21, 34, 35, 38) and suggested by observations of naturally infected and irradiated-sporozoite-immunized humans (23, 25, 26). We therefore examined antigen-specific cytokine responses of residents of a village in the highlands of western Kenya during the rainy and dry seasons.

This study focused on *P. falciparum* liver-stage antigen 1 (LSA-1), an ~200-kDa molecule expressed exclusively during hepatic schizogony (11, 42). Previous studies of cytokine responses to LSA-1 have been conducted in residents of areas of Africa and Papua New Guinea where malaria is holoendemic. These have documented gamma interferon (IFN-γ), tumor necrosis factor alpha (TNF-α), interleukin-10 (IL-10), and cytotoxic T-lymphocyte (CTL) responses to polypeptides encoded by the N- and C-terminal nonrepeat regions of LSA-1 (5, 7, 13, 23, 25, 26). Recent observations of Gabonese children have shown a decreased rate of reinfection in individuals whose peripheral blood mononuclear cells (PBMC) made IFN-γ in response to LSA-1 (25). In addition, examination of residents of an area of Kenya where malaria is holoendemic, located approximately 50 miles from the current study site, showed that IL-10 responses to recombinant LSA-1 proteins correlated with a delayed rate of reinfection following radical cure with chemotherapy (23). These and other studies of CTL responses to LSA-1 (13) support its inclusion in a multistage malaria vaccine.

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MATERIALS AND METHODS

Human subjects. Volunteers were residents of the village of Kabobo in Uasin Gishu district of Kenya. Kabobo is situated at an elevation of approximately 7,000 feet (2,134 m). The nearest paved road is located 10 miles from the village. *P. falciparum* transmission is unstable, and epidemics of malaria with high morbidity and mortality have been described (37). The regular outbreaks of malaria that occur in this area every rainy season may be indicative of a gradual change in malaria endemicity in this area from epidemic to seasonally endemic. Only *P. falciparum* and *Plasmodium malariae* species have been documented. The great majority of malaria is due to *P. falciparum* (17).

To minimize the effects of travel and imported malaria from surrounding areas, only volunteers who lived year-round in Kabobo were recruited. Adults were defined as persons ≥18 years old, and children were defined as persons ≤17 years old. The cytokine responses of 80 individuals (25 children, age range of 1 to 17 years; 55 adults, age range of 18 to 80 years) were investigated during the rainy season in August 1996. One hundred twelve individuals (36 children [age range, 2 to 17 years] and 76 adults [age range, 18 to 80 years]) were studied during the dry season in March 1997. Thirty-seven subjects were tested for IFN-γ production at both time points. Twelve subjects were tested for IL-10 production at both time points.

Signs and symptoms of malaria (fever, headache, vomiting, chills, fatigue, joint pains, splenomegaly, hepatomegaly, jaundice, pallor, altered mental status) were noted at the time of enrollment. Prior use of antimalarial medications was also ascertained and recorded. Blood samples were collected from adults (10 to 20 ml) and children (5 ml) by venipuncture. Thick and thin smears were stained and examined for *Plasmodium* species by trained microscopists from the Division of Vector Borne Diseases, Ministry of Health, Kenya. Symptomatic individuals whose blood smears were positive for *P. falciparum* were treated with a single dose of sulfadoxine-pyrimethamine in accordance with the policy of the Kenya Ministry of Health. Two blood smears were positive for *P. malariae*; these individuals were treated with chloroquine. No mixed infections were noted. Blood was also obtained from 14 North American adults who had never traveled to areas where malaria is endemic.

At the end of the dry season, thick and thin blood smears were obtained weekly for 10 weeks from 98 of 112 individuals whose PBMC cytokine responses had been evaluated in the dry season. These subjects were first given a single dose of sulfadoxine-pyrimethamine to clear blood-stage infection. A blood smear was obtained 2 weeks later to ensure that they were smear negative for *Plasmodium* species prior to evaluation of the time to reinfection. Sulfadoxine-pyrimethamine eliminated blood-stage malaria in all but one of the study subjects, who was treated with quinine and doxycycline and excluded from the follow-up study. If study subjects had symptoms of malaria between the weekly regular visits to obtain a blood smear, they reported to a local health clinic, where the results of blood smears were recorded. Symptomatic subjects with malaria parasites detected by blood smear were treated with sulfadoxine-pyrimethamine.

Informed consent was obtained from all subjects and/or their guardians. Ethical approval for this study was granted by the Kenya Medical Research Institute National Ethical Review Committee and the Institutional Review Board for Human Studies at University Hospitals of Cleveland, Case Western Reserve University.

Seasonal changes in malaria prevalence. Thick and thin smears of fingerprick blood samples from 6- to 12-year-old schoolchildren were examined for *P. falciparum* in October 1996, at the end of the rainy season, and in March 1997, at the end of the dry season. One hundred eighty children were tested in October, and 201 children were tested in March.

Preparation of PBMC and cytokine assays. Blood was anticoagulated with heparin and transported from the field to the laboratory within 4 h of venipuncture. PBMC were separated from whole blood by Hypaque-Ficoll density gradient centrifugation. For cytokine assays, 10^6 PBMC with or without peptide, antigen, or mitogen were incubated for 5 days in culture medium, and supernatants were stored at −70°C before transport to Cleveland. IL-5, IL-10, IFN-γ, and TNF-α were measured by two-site enzyme-linked immunosorbent assay as previously described (19). IFN-γ production was measured prior to testing for other cytokines. The limited amount of supernatant precluded testing all samples for all cytokines from each time period. Cytokine concentrations in supernatants of unstimulated (control) cultures were subtracted from the values of peptide or antigen/mitogen-stimulated cultures.

To evaluate the cellular sources of IL-10 and IFN-γ, $CD8^+$ cells were removed from PBMC with immunomagnetic beads as described elsewhere (20). The depleted cell population was then stimulated with LSA-1 peptides as described above. Depletion with immunomagnetic beads removed >98% of $CD8^+$ cells as detected by immunofluorescent staining with anti-CD8 antibody.

LSA-1 peptides, antigens, and mitogens. Five peptides, one from the N-terminal and four from the C-terminal nonrepeat regions of LSA-1 (NF54 strain of *P. falciparum*, GenBank accession no. X56203) were used. The amino acid sequences were residues 84 to 107 (LTMSNVKNVSQTNFKSLLRNLGVS), 1742 to 1760 (HTLETVNISDVNDFQISKY), 1813 to 1835 (NENLDDLDEGIE KSSEELSEEKI), 1836 to 1849 (KKGKKYEKTKDNNF), and 1888 to 1909 (DN EILQIVDELSEDITKYFMKL). Peptides were synthesized by 9-fluorenylmethoxy carbonyl chemistry (15) (kindly supplied by Nga Nguyen, Food and Drug Administration) and used at a concentration of 10 μg/ml with the exception of peptide 1836-1849, which was used at 2 μg/ml. Peptides 84-107, 1813-1835, and 1888-1909 have been shown to stimulate proliferation of PBMC from North Americans inoculated with irradiated *P. falciparum* sporozoites (22) and IFN-γ production by adults living in an area of Papua New Guinea where malaria is holoendemic (5). Phytohemagglutinin (1 μg/ml) was used as a mitogen control, and streptolysin O (10 μg/ml) and/or *Mycobacterium tuberculosis* purified protein derivative (10 μg/ml) served as nonmitogen antigen controls. Only those PBMC preparations that produced cytokines in response to these three controls were included in the analysis.

Statistics. Differences in the frequency of positive cytokine responses to LSA-1 peptides were compared by the χ^2 test. Quantitative differences in the level of cytokine production were evaluated by the nonparametric Mann-Whitney U test. The correlation between the levels of two cytokines was assessed by the Spearman rank test. Association of positive responses between two cytokines was assessed by contingency table analysis (χ^2 test). Quantitative differences in the level of cytokine production for the paired PBMC and $CD8^+$ cell-depleted PBMC samples were evaluated by the nonparametric Wilcoxon matched-pairs signed-rank test.

Differences in the prevalence of parasitemia among various groups were compared using the χ^2 test. Cytokine responders were compared to nonresponders for time to appearance of parasitemia, the percentage of individuals who had a positive blood smear for *P. falciparum* within the 10 weeks of chemotherapy-induced cure, and the mean level of parasitemia. Time to appearance of parasitemia was assessed by Kaplan-Meier survival analysis (with differences compared by the log-rank test) and Cox proportional hazards. The percentage of blood smear-positive individuals in each group was compared using two-way contingency table (χ^2) analysis. The mean levels of asexual parasitemia were compared by Student's *t* test.

RESULTS

Prevalence of blood-stage *P. falciparum* infection during the rainy and dry seasons. In October 1996, near the end of the rainy season, 82 of 180 schoolchildren (45.5%) had *P. falciparum* parasitemia. In March 1997, at the end of the dry season, only 18 of 201 schoolchildren (8.9%) were parasitemic ($P < 0.001$). No other *Plasmodium* species were observed. Among the study subjects, 47 of 80 (58.7%) and 18 of 112 (16.1%) individuals were parasitemic in the rainy and dry seasons, respectively ($P < 0.001$).

Cytokine responses by North Americans. PBMC from North Americans were examined because some malaria antigens have been found to stimulate lymphocyte responses by persons who have never been infected or exposed to the parasite (41). Cytokine production of >20 pg/ml in response to one or more LSA-1 peptides was observed for PBMC from 3 of 14 individuals for IL-5, 5 of 13 for IL-10, 5 of 14 for IFN-γ, and 6 of 14 for TNF-α. The cutoff value for a positive response by Kenyan study subjects was defined as greater than the mean plus 2 standard deviations (SD) of the North American controls. Positive responses were defined as follows: for IL-5, >72 pg/ml; for IL-10, >132 pg/ml; for IFN-γ, >214 pg/ml; and for TNF-α, >188 pg/ml.

Frequency of PBMC cytokine responses to various LSA-1 peptides by residents of the Kenyan highlands. IL-5, IL-10, IFN-γ, and TNF-α were produced in response to one or more LSA-1 peptides (Table 1). IL-10 responses to the N-terminal 84-107 peptide were less frequent than to any of the C-terminal peptides ($P < 0.01$). Similarly, a lower frequency of TNF-α responses to the N-terminal 84-107 than the C-terminal 1836-1849 peptide was observed ($P = 0.01$). The frequencies of responses to the other C-terminal peptides were similar to that of the N-terminal peptide. No significant differences between the rates of responses to the N-terminal and C-terminal peptides were noted for IFN-γ or IL-5.

IL-10 and TNF-α responses were more common during the rainy than dry season (PBMC from 73.2 and 66.7% of subjects made each cytokine to at least one peptide during the rainy season, compared with 17.0 and 24.6% during the dry season; $P < 0.001$). The decreased IL-10 and TNF-α response rates during the dry season were most striking for the C-terminal

TABLE 1. Frequency of PBMC cytokine responses to various LSA-1 peptides

LSA-1 peptide	No. (%) with positive response to LSA-1 peptides[a]							
	IL-5		IL-10		IFN-γ		TNF-α	
	Rainy (n = 41)	Dry (n = 84)	Rainy (n = 41)	Dry (n = 94)	Rainy (n = 80)	Dry (n = 112)	Rainy (n = 33)	Dry (n = 69)
84-107	11 (26.8)	14 (16.7)	5 (12.2)	6 (6.4)	11 (13.7)	22 (19.6)	6 (18.2)	7 (10.1)
1742-1760	6 (14.6)	17 (20.2)	9 (22.0)	10 (10.6)	20 (25.0)	24 (21.4)	6 (18.2)	8 (11.6)
1813-1835	9 (21.9)	12 (14.3)	21 (51.2)	11 (11.7)	8 (10.0)	24 (21.4)	8 (24.2)	6 (8.7)
1836-1849	5 (12.2)	11 (13.1)	19 (46.3)	8 (8.5)	10 (12.5)	20 (17.9)	14 (42.4)	6 (8.7)
1888-1909	5 (12.2)	23 (27.4)	16 (39.0)	8 (8.5)	16 (20.0)	28 (25.0)	11 (33.3)	12 (17.4)
Any peptide	21 (51.2)	30 (35.7)	30 (73.2)	16 (17.0)	37 (46.2)	52 (46.4)	22 (66.7)	17 (24.6)

[a] A positive response is defined as greater than the mean + 2 SD of North American PBMC stimulated with the same peptide.

1813-1835, 1836-1849, and 1888-1909 peptides (Table 1). The frequencies of IL-5 and IFN-γ responses were similar during both seasons ($P > 0.10$).

Thirty-seven individuals (age range, 6 to 54 years) donated blood in both rainy and dry seasons. There was a sufficient amount of supernatants from PBMC at both time points to measure only two cytokines. We first measured IFN-γ since earlier studies of pre-erythrocytic immunity have focused on this mediator (5, 7, 25). In the rainy season, 20 individuals had PBMC that produced IFN-γ when stimulated with one or more LSA-1 peptides. Fourteen of twenty subjects (70%) who responded at this time continued to do so during the dry season. Of the 17 individuals whose PBMC did not produce IFN-γ in the rainy season, 8 (47%) had responses during the dry season. The levels of IFN-γ for these individuals were similar across seasons (median IFN-γ level in rainy season = 240 pg/ml; median in dry season = 286 pg/ml; $P > 0.05$). Enough supernatant remained to measure IL-10 levels in both seasons for 12 of 37 individuals. Nine of the twelve individuals had a decrease in IL-10 level in response to LSA-1, and the median IL-10 level in response to LSA-1 in these individuals decreased from 289 to 64 pg/ml ($P = 0.049$).

The frequency of cytokine responses was similar when the study subjects were grouped according to the presence or absence of blood-stage *P. falciparum* on thick smear, use of antimalarial medication within the previous 2 weeks, or clinical symptoms and signs of malaria (data not shown).

Relationship of age to cytokine responses. Table 2 describes the frequencies and levels of cytokine responses for persons ≤17 and those ≥18 years old. A pattern similar to that of the entire population was observed; i.e., for both children and adults, the frequencies and median levels of IL-10 and TNF-α responses were greater in the rainy than dry season, whereas IL-5 and IFN-γ responses did not change. For example, the median levels of IL-10 produced by children during the rainy and dry seasons were, respectively, 201 and 37 pg/ml ($P < 0.001$). The frequencies and levels of cytokine responses were similar in children and adults for all cytokines except IFN-γ, for which the frequency of responses was lower in children than adults (e.g., during the dry season, 31% of children responded, compared with 53% of adults; $P = 0.028$).

Cytokine production and resistance to reinfection with *P. falciparum*. Kaplan-Meier survival analysis demonstrated a trend toward prolonged time to reinfection for IL-10 responders to LSA-1 compared with nonresponders (Fig. 1). The difference did not reach statistical significance at the 10-week follow-up ($P = 0.15$). A lower percentage of IL-10 responders than nonresponders also developed blood-stage infection detectable by inspection of thick smears (25.0% versus 49.2%). This difference approached statistical significance ($P = 0.083$)

TABLE 2. LSA-1 peptide-driven cytokine production according to age and season

Cytokine	Children (≤17 yr old)			Adults (≥18 yr old)		
	Rainy	Dry	P	Rainy	Dry	P
IL-5						
Median (range)[a]	65 (0–558)	44 (0–792)	NS	84 (0–1,184)	30 (0–1,345)	NS[b]
No. positive/total[c]	6/13 (46.1)	9/28 (32.1)	NS	15/28 (53.6)	21/56 (37.5)	NS[d]
IL-10						
Median (range)	201 (21–1,875)	37 (0–663)	<0.001	205 (0–1,562)	56 (0–1,088)	<0.001
No. positive/total	14/17 (82.3)	6/28 (21.4)	<0.001	16/24 (66.7)	10/66 (15.1)	<0.001
IFN-γ						
Median (range)	76 (0–8,000)	6 (0–2,572)	NS	275 (0–3,690)	245 (0–8,000)	NS
No. positive/total	8/25 (32.0)	12/36 (33.3)	NS	29/55 (52.7)	40/76 (52.6)	NS
TNF-α						
Median (range)	266 (0–8,681)	44 (0–792)	0.002	344 (0–3,500)	37 (0–1,345)	<0.001
No. positive/total	8/14 (57.1)	4/24 (16.7)	<0.001	14/19 (73.7)	13/45 (28.9)	0.001

[a] Median amount of cytokine produced in picograms per milliliter.
[b] Mann-Whitney U test; NS, not significant.
[c] A positive response is defined as greater than the mean + 2 SD of North American controls. Values in parentheses represent percent positive.
[d] χ^2 test.

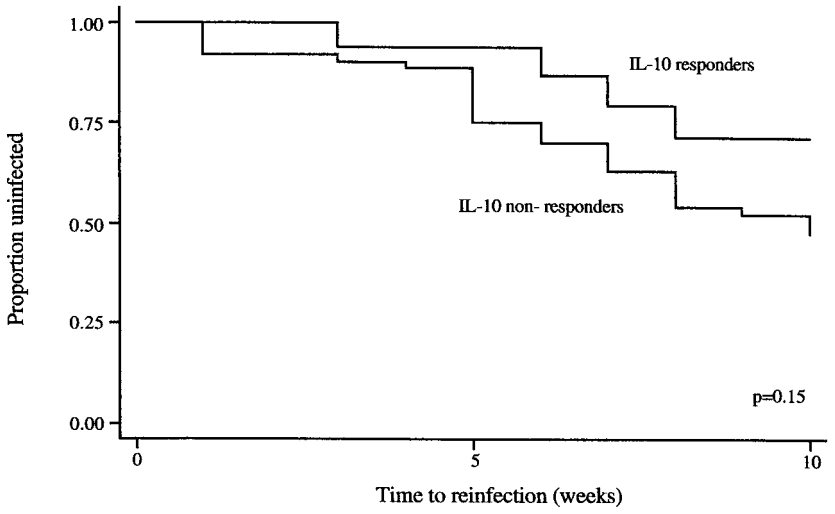

FIG. 1. LSA-1-induced IL-10 production and time to reinfection with *P. falciparum* (Kaplan-Meier analysis).

(Table 3). There was no difference in the period of time to development of *P. falciparum* parasitemia when subjects were grouped according to whether or not their PBMC produced IL-5, IFN-γ, or TNF-α in response to LSA-1 peptides (data not shown). The percentages of IL-5, IFN-γ, and TNF-α responders and nonresponders who developed parasitemia in the 10-week follow-up period also did not differ significantly (Table 3). There was no difference in the level of parasitemia between responders and nonresponders for any cytokine tested (data not shown).

Correlation between production of various cytokines. LSA-1-stimulated PBMC production of all four cytokines was measured in 56 subjects during the dry season. (This correlation was not examined during the rainy season since PBMC from only 18 subjects studied at this time had a sufficient amount of culture supernatant to measure all four cytokines.) A positive correlation was observed between all pairs of cytokines evaluated except TNF-α and IFN-γ (Table 4).

Contribution of $CD8^+$ cells to LSA-1-stimulated IFN-γ and IL-10 production. The effect of $CD8^+$ cell depletion on LSA-1 peptide-stimulated IFN-γ production was studied in six individuals whose PBMC produced this cytokine in response to one or more LSA-1 peptides. The level of IFN-γ decreased by 85% following depletion of $CD8^+$ cells (median level for nonfractionated PBMC = 266 pg/ml [range, 224 to 2,184 pg/ml] versus 40 pg/ml [range, 1 to 944 pg/ml] for $CD8^+$ cell-depleted PBMC; $P = 0.028$) (Fig. 2). The effect of $CD8^+$ cell depletion on IL-10 production was studied in 11 individuals. IL-10 production increased following depletion of CD8 cells from PBMC of nine subjects (Fig. 3). The median level of IL-10 for the depleted population was 66 pg/ml (range, 25 to 170 pg/ml) versus 15 pg/ml (range, 1 to 70 pg/ml) for nonfractionated PBMC ($P = 0.007$).

TABLE 3. LSA-1-driven cytokine responses and frequency of reinfection

Cytokine	No. with positive smear/total (%)[a]		*P*
	Responder[b]	Nonresponder	
IL-5	11/25 (44.0)	23/44 (52.3)	NS[c]
IL-10	4/16 (25.0)	30/61 (49.2)	0.083
IFN-γ	20/43 (46.5)	24/51 (47.1)	NS
TNF-α	6/14 (42.9)	20/40 (50.0)	NS

[a] Blood smear positive for *P. falciparum* at any time in the 10 weeks following treatment.
[b] A responder is defined as an individual with a cytokine level greater than the mean + 2 SD of North American control subjects.
[c] χ^2 test; NS, not significant.

DISCUSSION

Malaria epidemics in the highlands of Kenya are characterized by abrupt and transient increases in infection and morbidity that coincide with periods of heightened transmission following prolonged dry spells. The epidemiology of highland malaria differs from that in areas where malaria is holoendemic in that adults as well as children appear to be affected (3, 8, 37). This study describes the temporal changes in antigen-specific T-cell cytokine responses that are believed to contribute to elimination of liver-stage malaria. LSA-1-driven IL-10 and TNF-α responses, unlike IFN-γ and IL-5 responses, were observed to be weaker in the dry, low-transmission season than in the rainy, high-transmission season. The data suggest that lack of frequent and repeated exposure to liver-stage *P. falciparum* during the dry season leads to decreased immunologic boosting and waning of LSA-1-specific IL-10 and TNF-α responses. It is not yet clear why antigen-specific T-cell IFN-γ and IL-5 responses are not affected in the same way. The results also suggest a trend toward an association between protection from *P. falciparum* reinfection and LSA-1-driven IL-10 but not TNF-α, IFN-γ, or IL-5 responses. Although

TABLE 4. Correlation between production of various cytokines[a]

Cytokine	IFN-γ		IL-5		IL-10	
	r^{2b}	*P*	r^2	*P*	r^2	*P*
IFN-γ			0.354	0.007	0.382	0.004
IL-5	0.354	0.007			0.349	0.009
IL-10	0.382	0.004	0.349	0.009		
TNF-α	0.118	NS[c]	0.368	0.005	0.282	0.035

[a] PBMC from 56 individuals were studied for production of all four cytokines during the dry season.
[b] Spearman's rank correlation coefficient.
[c] NS, not significant.

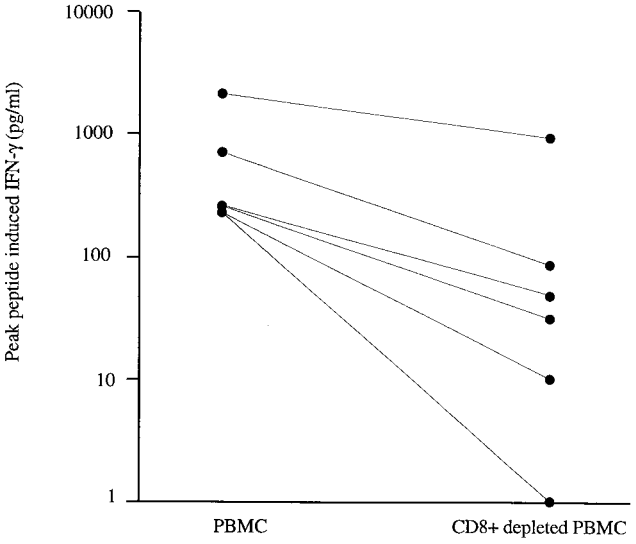

FIG. 2. Peak IFN-γ production by nonfractionated PBMC and $CD8^+$ cell-depleted PBMC in response to one or more LSA-1 peptides. Lines connecting two points correspond to values for cells from one person. IFN-γ values are expressed on a logarithmic scale.

these data were obtained from repeated cross-sectional studies of different groups of individuals living in the same area where malaria is endemic, the patterns were similar for IFN-γ and IL-10 responses of a subset of the same persons evaluated in both seasons. Data comparing the frequency and magnitude of cytokine responses by children and adults suggest that acquisition of T-cell IFN-γ responses to LSA-1, unlike the other cytokines examined, was related to cumulative and long-term exposure to infection. Once acquired, IFN-γ responses appear to persist.

Two recent studies have highlighted an association between LSA-1-driven IL-10 responses and *P. falciparum* infection and morbidity. Luty et al. (26) performed a case-control study of Gabonese children with mild and severe malaria. Parasite clearance times in children with mild malaria were more rapid in those with PBMC IL-10 responses to LSA-1 peptides than in those without IL-10 responses. IL-10 production correlated with higher-level acute-phase antibody responses, which were also associated with rapid parasite clearance. Kurtis et al. (23) demonstrated that IL-10 production in response to recombinant LSA-1 proteins correlated with resistance to reinfection in adults living in an area of western Kenya where malaria is holoendemic. These observations, together with the present data showing that LSA-1-driven IL-10 production diminishes during the dry season, suggest that IL-10 mediates or indirectly contributes to elimination of liver-stage *P. falciparum*. Ho et al. have suggested that IL-10 down-regulates proinflammatory cytokines such as IFN-γ and TNF-α in acute malaria (14), and the former cytokine has been shown to decrease antigen-specific T-cell cytokine production in general (6). Our observations do not support the notion that IL-10 suppresses LSA-1-driven IFN-γ, TNF-α, or IL-5 since a negative correlation between IL-10 and the latter cytokines was not observed. Rather, the present findings are similar to those of Wenisch et al., who reported a positive correlation between serum IL-10 and IFN-γ in acute *P. falciparum* infection (40). More detailed examination of the regulatory role of IL-10 in pre-erythrocytic immunity requires measurement of production of various cytokines in the presence of neutralizing anti-IL-10 antibodies and a better understanding of whether IL-10 influences accumulation and activation of local effector cells, such as $CD8^+$ CTL and $CD8^+$ IFN-γ-secreting cells in the liver.

The role of TNF-α in malaria is complex and incompletely understood. Increased serum TNF-α levels have been reported in severe malaria (e.g., cerebral malaria) and uncomplicated morbidity (fever with parasitemia) (24, 28). The relationship between malaria antigen-specific T-cell TNF-α responses and liver-stage infection is less well studied. In the present study, the magnitude of the reduction in LSA-1-induced TNF-α from rainy to dry season was almost as marked as the decrease in IL-10 responses. The relatively more robust LSA-1-driven TNF-α response during the rainy season was not attributable to severe malaria since none of the study subjects had symptoms consistent with this illness. In addition, infection status documented by blood smear did not correlate with TNF-α responses, and unlike the case for LSA-1-induced IL-10 responses, there was no trend toward protection from infection with LSA-1-induced TNF-α responses. The lower number of individuals tested for TNF-α production ($n = 54$) than for IL-10 ($n = 77$) may have made correlation between protection and TNF-α responses more difficult to detect. Others have reported a lack of correlation between LSA-1-induced TNF-α and protection against infection (23, 25). It is possible that LSA-1-driven TNF-α responses correlate with relative resistance against severe morbidity rather than infection, although at present morbidity is thought to be associated primarily with immunity to blood-stage antigens.

IFN-γ is an important mediator of resistance to liver-stage malaria and the pathogenesis of disease in animal models (29, 30, 35), and LSA-1-specific T-cell IFN-γ responses develop in North American volunteers in whom sterile, transient immunity to *P. falciparum* infection has been induced by immunization with radiation-attenuated sporozoites (22). We observed no seasonal changes in the strength of LSA-1-induced IFN-γ responses in the highland study subjects and no correlation between LSA-1-induced IFN-γ responses and resistance to reinfection with *P. falciparum*. In a study conducted in a nearby area where malaria is holoendemic, the findings of Kurtis et al.

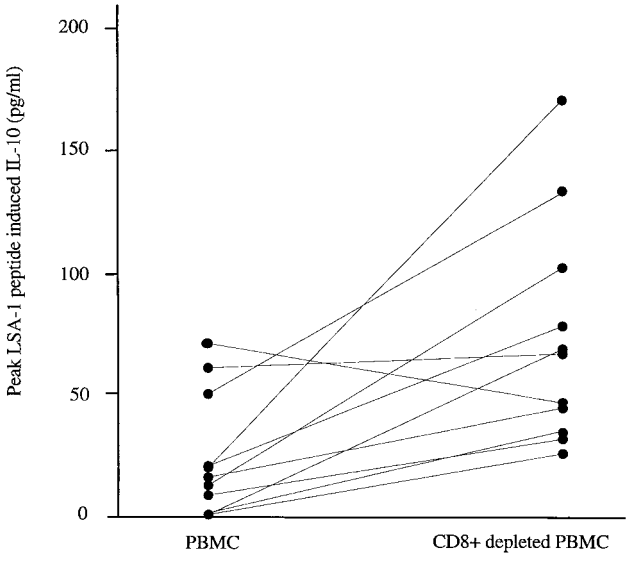

FIG. 3. Peak IL-10 production by nonfractionated PBMC and $CD8^+$ cell-depleted PBMC in response to one or more LSA-1 peptides. Lines connecting two points represent values for cells from one person.

were very similar: the proportion of persons who made IFN-γ in response to recombinant LSA-1 protein decreased at the end of the dry season, but there was no association between these responses and the time to reinfection (23). In contrast, Luty et al. observed that LSA-1-driven IFN-γ production was associated with delayed infection and reduction in the rate of reinfection in children (25). A previous study of adults in Papua New Guinea demonstrated that IFN-γ responses to the N-terminal 84-107 peptide were associated with repeatedly negative smears for *P. falciparum* over a 6-month period (5). Since we were able to document a trend toward protection from infection in individuals with IL-10 responses to LSA-1, and since our study numbers were even larger for IFN-γ (n = 94), the very similar rates of infection in those with and without IFN-γ responses to LSA-1 suggest that, at least in this population, LSA-1-induced IFN-γ production is not strongly protective against infection. As with TNF-α responses, it is possible that LSA-1-induced IFN-γ responses relate more to malarial morbidity than to infection.

The most remarkable age-associated difference observed in the present study was related to IFN-γ, the only cytokine for which there were fewer responses by children than by adults. One explanation is that multiple exposures and greater cumulative experience with liver-stage *P. falciparum* are required to induce IFN-γ but not IL-5, IL-10, or TNF-α responses to LSA-1. If this is so, the present findings suggest that once a given threshold is reached, LSA-1-induced IFN-γ responses persist even when transmission intensity decreases. In this context, it will be of interest to determine the phenotypes of the T-cell subsets that produce IFN-γ (see below), whether they have markers of the memory phenotype (e.g., CD45RO) (2), and whether they differ from those of T cells that secrete other cytokines when stimulated with LSA-1.

Because of limitations in the number of PBMC available from the study subjects, we were not able to perform detailed experiments to determine the subsets of T cells that made each of the cytokines. Based on the length of the peptides used to stimulate cytokine production (14 to 24 amino acids), both $CD4^+$ and $CD8^+$ T cells restricted by HLA class I and II molecules may have contributed. Results of experiments in which $CD8^+$ cells were depleted by immunomagnetic selection suggested that this subset is the major but not only source of IFN-γ. $CD8^+$ T cells were also the predominant source of IFN-γ following stimulation with the LSA-1 84-107 peptide in studies conducted in Papua New Guinea (K. Bucci, unpublished data). By contrast, LSA-1-driven IL-10 production was enhanced following depletion of $CD8^+$ cells (in 9 of 11 individuals). It is not clear whether this modest increase in cytokine production was due to removal of cells that secrete molecules which actively suppress production of IL-10 by the remaining monocytes and $CD4^+$ T cells or removal of a source for consumption of IL-10. Since IL-10 is a chemoattractant for $CD8^+$ (16), it is possible that this cytokine is involved in elimination of liver-stage parasites by virtue of its effects on the local accumulation of effector cells, such as IFN-γ-secreting CD8 cells.

Our study establishes that select LSA-1-driven cytokine responses in highland residents vary according to season and supports the idea that LSA-1-induced IL-10 production may have a role in protection from *P. falciparum* infection. Future studies will focus on determining whether seasonal changes in $CD4^+$ and $CD8^+$ T-cell immunity to LSA-1 (and other preerythrocytic or blood-stage malaria antigens) (29, 30, 33) are predictive of the rate and clonality of reinfection, high-density parasitemia, and uncomplicated malaria morbidity in children and adults.

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