

Impact of acute vivax malaria on the immune system and viral load of HIV-positive subjects

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Objective To explore the mechanisms of malariatherapy for human immunodeficiency virus (HIV)-infected patients and to identify which stage(s) of HIV infection is suitable for the treatment of malariatherapy.

Methods Therapeutic acute vivax malaria was induced and terminated after 10 fever episodes in 12 HIV-1-infected subjects : Group 1 (G1) had 5 patients with CD4 T-cell counts $\geq 500/\mu\text{l}$ at baseline , Group 2 (G2) had 5 patients with CD4 at $499-200/\mu\text{l}$ and Group 3 had 2 patients with CD4 $< 200/\mu\text{l}$ (not included in statistical analysis). Enzyme-Linked-Immuno-Sorbent Assay (ELISA) was used to measure plasma levels of cytokines and soluble activation markers. Flow cytometry was used to measure levels of lymphocyte subsets and phenotypes and CD4 cell apoptosis. Bayer bDNA assay was used to test plasma levels of HIV-1 RNA (viral load). Samples were taken and tested twice before malaria (baselines) , three times during malaria and seven times after termination of malaria (at day 10 and 1 , 3 , 6 , 12 , 18 and 24 months).

Results Levels of plasma tumor necrosis factor- α (TNF- α) , soluble TNF- α receptor-2 (sTNF-RII) , neopterin (NPT) and soluble IL-2 receptor (sIL-2R) significantly increased during malaria and sharply reduced to baselines post malaria in all groups. Stronger responses of the aforementioned factors were seen in G2 than in G1 during malaria ($P = 0.081, 0.001, 0.013, 0.020$). CD4 count and percentage ; CD4/CD8 ratio and CD25⁺ and CD4⁺ CD25⁺ percentages increased but HLA-DR⁺ percentage decreased either during or post malaria in G2. Most G2 patients experienced sustained increase but most G1 patients underwent natural history decline of CD4 counts and percentages during 2-year follow-up. Percentage of apoptotic CD4 cells decreased post malaria in all groups. G3 patients had weaker immune responses , however , one advanced AIDS patient in this group experienced clinical improvement after malariatherapy. Most of the 12 patients experienced increase of HIV viral load during malaria but the viral load returned to baseline levels 1-3 months after cure of malaria and remained near baseline levels for up to two years.

Conclusions Part of the mechanisms of malariatherapy is to induce high levels of cytokine activities and subsequently the changes of T-lymphocyte subsets and phenotypes in HIV-infected patients. These findings suggest that malariatherapy may treat HIV-1-infected patients whose CD4 baselines are in the range of 500-200/ μl .

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Infections of human immunodeficiency virus (HIV) and malarial parasite represent major public health problems in the developing world especially in sub-Saharan Africa. Immune suppression caused by HIV , leading to the emergence of other protozoan parasitic diseases ,¹ was expected to promote the prevalence of malaria or trigger more severe manifestation of the disease. Moreover , malaria was known to impair T-lymphocyte function ,²

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hence was anticipated to facilitate the progression of HIV infection to acquired immunodeficiency syndrome (AIDS). Therefore , a possible harmful interaction between both infections has been hypothesized in recent years. However , there is no evidence to support this hypothesis among over 30 available reports on this topic recently reviewed by Chandramohan and Greenwood.³ On the contrary , a hospital-based study conducted in Zaire⁴ indicated that none of 41 children with malaria and AIDS died but among the remaining 71 children with AIDS without malaria , 25 died ; there were no deaths in malaria patients with symptomatic HIV infection compared to 14% in non-HIV (but malarial) infected children. Furthermore , a well-controlled cohort study⁵ conducted in Uganda demonstrated that median survival of HIV-infected children with at least one documented malaria episode was significantly longer than that of those without a documented malaria episode. Moreover , a significantly greater proportion of children without malaria developed clinical symptoms of the United States Center for Disease Control and Prevention (CDC) AIDS category C than those with malaria and , there was a significantly decreased hazard to category C with each episode of malaria. Greenberg and his colleagues also observed in Zaire that patients with the dual infections who had more malarial episodes did not progress to AIDS as fast as those who had fewer episodes.⁶ These phenomena were consistent with the results from animal model studies⁷ in which HIV-like virus infection prevented death of animals from cerebral malaria and co-infection of malaria delayed the progression of the viral infection to animal AIDS. These findings paralleled the results of our previous clinical studies^{8,9} of malariotherapy (therapeutic acute vivax malaria) for HIV infection in which 8 HIV-1-infected persons who received malariotherapy experienced CD4 cells increase and neopterin (NPT) decrease in total trend and remained clinically well during 2-3 year follow up. All these findings suggest a beneficial (instead of harmful) interaction between HIV and malarial parasite in human.

This study stressed on the impact of therapeutic acute vivax malaria on the immune system and viral load of HIV-positive persons and , the potentially different immune responses among patient groups (stratified by CD4 cell baseline levels). This study was approved by the review board of Guangdong Provincial Committee of Science and Technology (China) , a scientific and ethic combination committee at the provincial level.

METHODS

Patients

Twelve Chinese HIV/AIDS patients (all were HIV-1

positive and confirmed by Western blot) who were naive of any kind of anti-retroviral therapy and stratified by CD4 T-cell count baselines were selected for this study. All patients signed informed consent. Patients were numbered according to descending order of CD4 cell count baselines. The 12 patients represented CD4 cell counts ranging from 1217 - 15/ μ L : Group 1 (G1) had 5 patients (cases 1-5) with CD4 counts \geq 500/ μ L (CDC category I) ;¹⁰ Group 2 (G2) had 5 patients (cases 6-10) with CD4 counts at 499-200/ μ L (CDC category II) and Group 3 had 2 patients (cases 11-12) with CD4 counts < 200/ μ L (CDC category III) but was not included in statistical analyses (Table 2). Cases 4 , 9 , 11 and 12 got HIV from sexual transmission , the others got HIV from drug injection (sharing needles). Ages were 22-35 years old at entry. Cases 9 and 12 are female and the others are male. Case 12 was a full-blown AIDS patient with complicated ulcer of external genitalia , pneumocystis carinii pneumonia (PCP , clinical diagnosis) with dyspnea and needed oxygen inhalation. There were no symptoms in the other 11 HIV-positive individuals (case 11 was classified as an AIDS patient according to 1993 CDC AIDS definition.)¹⁰ All patients had no history of malaria.

Therapeutic malaria induction

A blood donor with vivax malaria was tested negatively for HIV-1 , HIV-2 , hepatitis A , B , C , D , E , F , G and syphilis. Ten milliliters of heparinized whole blood containing 10⁷ Plasmodium vivax (Pv) infected red blood cells was intravenously injected into each HIV/AIDS patient to induce therapeutic malaria. Chloroquine was used to terminate malaria after 10 febrile episodes (except patients 4 and 12 whose malaria naturally disappeared before completion of the course of malariotherapy).

Laboratory measurements

Enzyme-Linked-Immuno-Sorbent Assay (ELISA) was used to measure plasma levels of cytokines and immune activation markers (procedures followed manufacturer 's instructions , results were calculated by AssayZa computer program) including tumor necrosis factor- α (TNF- α) , soluble TNF- α receptor-II (sTNF-RII) (both reagents from Genzyme Corporation , Cambridge , USA) , INF- γ (Coulter Immunotech , Marseille , France) , β -2-microglobulin (β -2M , tested only in cases 2 , 5 , 6 and 10) , neopterin (NPT) (both Diagnostica GmbH , Berlin , Germany) and soluble interleukin-2 receptor (sIL-2R , Endogen Incorporation , Woburn , USA). Flow cytometry Coulter EPICS ($\text{\textcircled{R}}$) ELITE cytometer and Coulter Immunotech reagents) was used to measure the percentages and absolute counts of CD4⁺ and CD8⁺

T-lymphocytes , percentages of CD25⁺ , CD4⁺ CD25⁺ , HLA-DR⁺ and CD8⁺ HLA-DR⁺ lymphocytes and of apoptotic CD4⁺ cells (propidium iodide stained and measured sub-G1 peak). All of these parameters in 20 HIV-negative age-matched healthy subjects were tested using the same methods for flow cytometry quality control. Data of the quality control are listed in Table 1. HIV viral load was measured by Bayer (formerly Chiron) bDNA assay (QUANTIPLEX™ HIV-1 RNA 3.0 machine and reagents , assay procedures strictly followed from manufacturer 's instructions). Samples (EDTA anti-coagulated) were taken and tested before , during and after malarial stages. More specifically , sample collection was conducted as follows : first baseline of pre-malaria (pre1) and second baseline of pre-malaria (pre2 , at least one week interval between pre1 and pre2) within a week ; first malarial fever episode (f1) , fifth fever episode (f5) and tenth fever episode (f10) were taken during the course of the infection and day 10 (pd10) , 1 month (m1) and 3 months (m3) after termination of malaria. Patients were extendedly followed for m3 CD4 cell counts and percentages and HIV viral load at 6 months (m6) , 12 months (m12) , 18 months (m18) and 24 months (m24).

Statistical analyses

Mean values of the two pre-malaria results for all parameters (except percentage of apoptotic CD4 cells) were calculated and compared with data from parameters gathered at other time points using paired-sample *t* test. Data were compared between subgroups using an independent-sample *t* test. SPSS 8.0 computer program was used for all statistical analyses. All significant tests were two-tailed. $P = 0.05$ was used as the statistically significant cut off value.

RESULTS

Clinical responses

Clinical incubation periods (from injection of Plasmodium to beginning of malarial fever) were 10 -17 days. All patients experienced febrile paroxysm every day or every other day with confirmed malaria parasitaemia. Malarial fever and parasitaemia naturally disappeared in case 4 (5 episodes) and case 12 (2 episodes) without using any anti-malarial drug. All other patients were cured of malaria without recrudescence or relapse after one standard course of chloroquine treatment. Karnofsky performance worsened only at malarial fever time in most patients but quickly recovered between febrile episodes and after termination of malaria. Case 12 (diagnosed with AIDS) experienced clinical improvement including : disappearance of ulcer of external genitalia , dyspnea and PCP (confirmed by chest X-ray) with recovery of

Karnofsky performance score from 20 to 90 and normal activities after two medium malarial fever episodes. This patient died 6 months after malariotherapy due to severe opportunistic infections including pneumonia and non-bacterial meningitis. The other 11 patients remained clinically well up to 24 months after malariotherapy.

Changes of plasma levels of cytokines and soluble activation markers

Levels of plasma TNF- α , sTNF-RII , sIL-2R and NPT significantly elevated during malaria and sharply declined to baseline levels in all patient groups. Interferon- γ (IFN- γ) at most testing time points in all 12 patients was undetectable either at baseline , during or post malaria. Level of β 2M increased during malaria and sharply returned to baseline post malaria in all tested patients (cases 2 , 5 , 6 and 10 , data not shown). Much stronger responses of plasma TNF- α ($P = 0.081$ at f5) , sTNF-RII ($P < 0.001$ at f5) , sIL-2R ($P = 0.040$, 0.020 at f5 and f10) and NPT ($P = 0.013$ at f10) were seen in G2 compared with G1 during malaria even though there were no differences in levels of these factors at baseline or post malaria between the two groups (Fig. 1A - D).

Changes of T-lymphocyte subsets

In G1 , the CD4 cell counts significantly declined during malaria ($P = 0.033$ at f5) , then recovered to around the baseline levels post malaria (Fig. 2A). Changed patterns of CD4 percentage (Fig. 2B) and CD4/CD8 ratio (Fig. 2C) were similar to that of CD4 counts (but not significant). CD8 counts decreased during malaria ($P = 0.013$, 0.012 at f5 and f10) then returned to baseline levels post malaria , but CD8 percentage increased at f1 ($P = 0.050$) and pd 10 ($P = 0.040$) then recovered to around baseline level at other time points (data not shown). Interestingly , in G2 , the percentage (Fig. 2B) and absolute count (Fig. 2A) of CD4 cells , as well as the CD4/CD8 ratio (Fig. 2C) , increased during malaria and remained at high levels post malaria (significantly higher in level of CD4 percentage at m3 , $P < 0.001$). Total trend of CD8 cell count decreased during or post malaria (not significant , data not shown). Total trend of CD8 percentage also reduced with the exception of a relatively higher level seen at pd 10 ($P = 0.313$, data not shown). Changed patterns of percentages for apoptotic CD4 cells (among total CD4 cells) were similar in all three study groups. Patterns included : relatively unchanged percentage at f5 ($P = 0.980$ for total group of the 12 patients) ; significant increase at f10 ($P = 0.015$) and apparent decrease to lower than baseline level post malaria (Fig. 2D , despite the fact that there was no statistical significance when compared with baseline observations (Fig. 2B) , there

was no significant difference in apoptotic CD4 percentage between the HIV-positive patients at post-malaria and the HIV-negative controls but there was significant difference between the two groups before the patients received malariatherapy , Table 1). Two-year follow-up data for CD4 cell counts and percentages are shown in Tables 2 and 3. Sustained increases or decreases in CD4 cell count levels are defined as increases or decreases of $\geq 100/\mu\text{L}$ in more than half of the available measurements between 6 and 24 months. of observation. By this definition , in G1 , four patients(cases 1-4) showed CD4 decreases and one patient (case 5) experienced no change in CD4 level. However , in G2 , three patients (cases 6-8) showed sustained CD4 cell increases by this criterion and two patients(cases 9 and 10) showed no changes in CD4 levels. Sustained increases or decreases in CD4 percentage levels are defined as increases or decreases of $\geq 4\%$ in more than half of the available measurements between 6 and 24 months of observation (Fahey JL , personal communication). By this definition , decreases in three patients (cases 1-3) , an increase in one (case 4) and no change in one (case 5) were seen among the G1 patients. In G2 , three patients (cases 7 , 8 and 10) showed increases and two patients (cases 6 and 9)

experienced no changes of CD4 percentages.

The total trends of lymphocyte counts and percentages decreased either during or post malaria in all groups. Total white blood cells (WBC) decreased during malaria then recovered to baseline levels post malaria in G1 and remained stable within the whole course in G2 (data not shown).

Changes of lymphocyte phenotypes

In G1 , percentages of $\text{CD}25^+$ and $\text{CD}4^+ \text{CD}25^+$ cells declined during malaria ($P=0.026$ at f5 and $P=0.033$ at f10 , respectively) and then rebounded to higher than baseline levels (not significant , Fig. 3A and B). $\text{HLA}^- \text{DR}^+$ percentage (Fig. 3C) remained relatively stable with the exception of a big variation at f10 and a higher level at pd10 ($P=0.039$). $\text{CD}8^+ \text{HLA}^- \text{DR}^+$ percentage increased either during or post malaria (not significant , Fig. 3D). In G2 , the total trends of $\text{CD}25^+$ and $\text{CD}4^+ \text{CD}25^+$ percentages increased but $\text{HLA}^- \text{DR}^+$ and $\text{CD}8^+ \text{HLA}^- \text{DR}^+$ percentages decreased either during or post malaria (not significant , Fig. 3 , A - D).

Change of HIV viral load

Viral load in all G1 and G2 patients increased during the

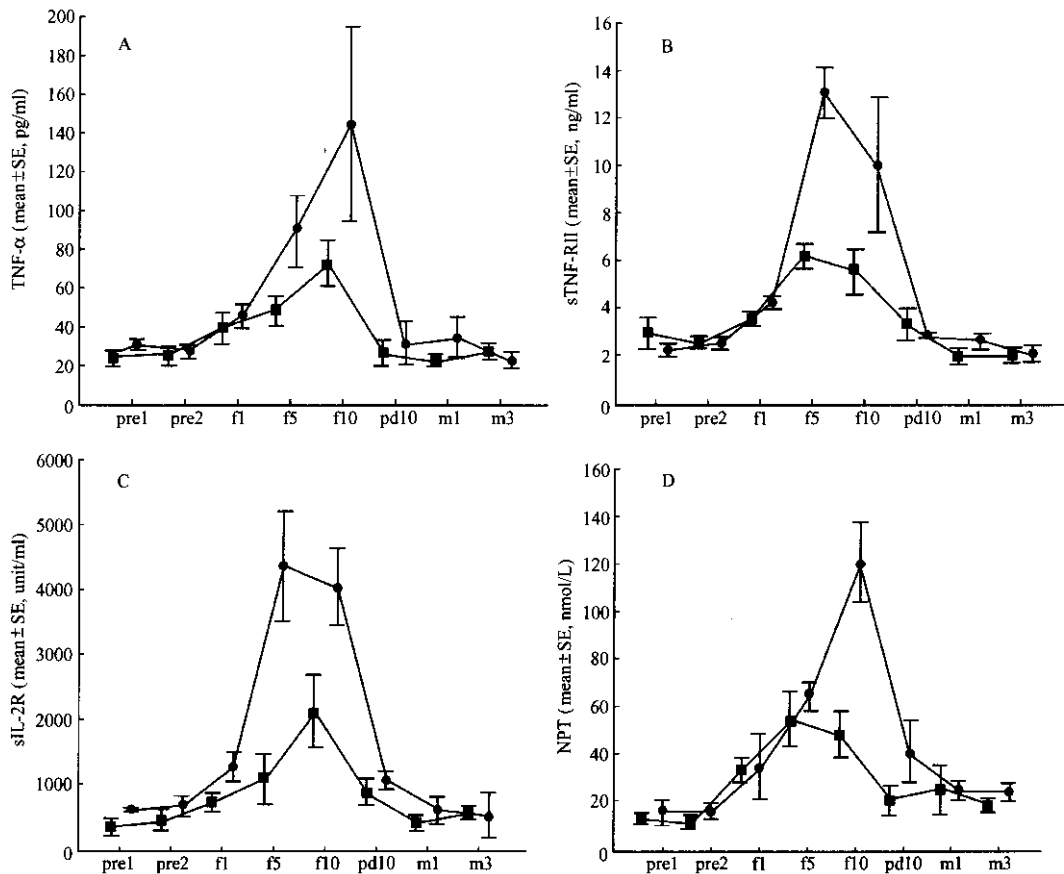


Fig. 1. Dynamics of plasma TNF - α and soluble immune activation markers (■ : G1 ; ● : G2).

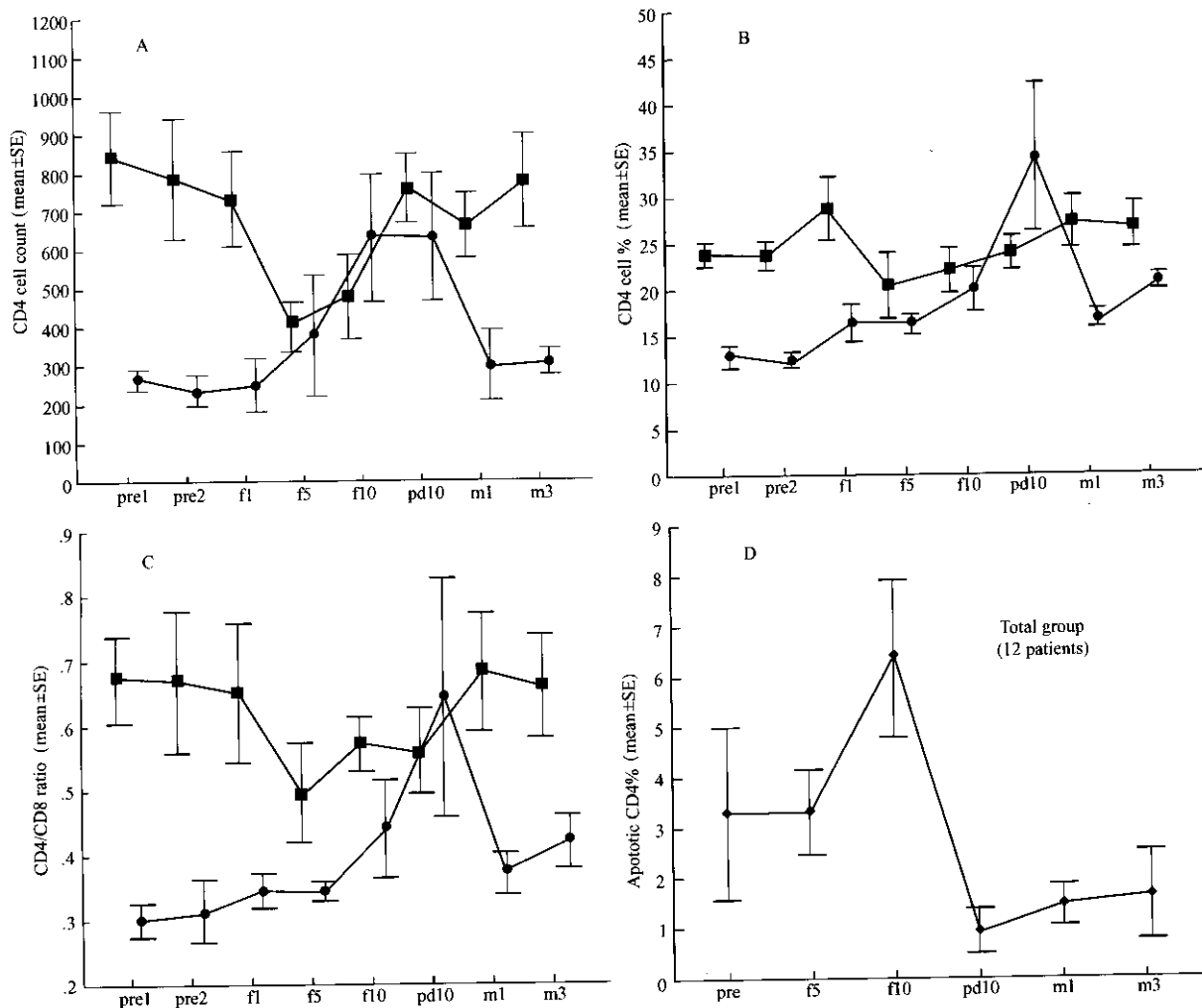


Fig. 2. Dynamics of CD4 cell count and percentage , CD4/CD8 ratio and apoptotic CD4 cell percentage (■ : G1 ; ● : G2).

malarial phase. In G1 , post malaria viral load returned to baseline levels in three patients (cases 3-5) during 1-3 months and decreased (but still remained higher) in two patients (cases 1 and 2). This parameter went up again in case 3 at m 12 and m 18 and in case 5 at m 18. Viral load in these patients either returned to baseline levels (cases 1 , 2 and 4) or lower than baseline (case 3 and 5) at m 24 (Fig. 4A). In G2 , viral load in four patients (cases 6 and 8-10) decreased to baseline levels 1-3 months post malaria (this decrease was observed in case 9 despite somewhat high levels at m 18 and m 24 (Fig. 4B). One patient (case 7) experienced a decrease in viral load at m 12 (Fig. 4B) unfortunately , the follow-up at most time points was missed in this case-he refused to come back to have his blood taken , but accepted interviews via the phone).

Information on G3 (cases 11 and 12)

In case 11 , the pattern of cytokine responses was similar to that of G2. Percentage of CD4 cells increased during malaria then returned to baseline level post malaria , but

CD4 cell count remained relatively unchanged throughout the whole course of malaria therapy. Furthermore , the percentage and number of CD8 cells and the CD4/CD8 ratio also remained relatively stable. Percentages of CD25⁺ and CD4⁺ CD25⁺ lymphocytes decreased and percentages of HLA-DR⁺ and CD8⁺ HLA-DR⁺ lymphocytes increased either during or post malaria. The pattern of response of apoptotic CD4 was similar to that of G1 and G2. This patient underwent apparent increment of viral load during malarial phase and decrement but remained at higher levels compared with the baseline post malaria up to m24 (data not shown) without sustained increases or decreases of CD4 counts and percentages (Tables 2 and 3). Case 12 , who was diagnosed with AIDS , experienced , between baseline and pd10 , increases in percentage and number of CD4 cells (1% and 15/ μ l to 4% and 41/ μ l -Table 2 and Table 3). Furthermore , a relative stabilization in levels of CD8 cells and lymphocyte phenotypes was also observed. Percentage of apoptotic CD4 cells sharply decreased from 22% at baseline to 6.5%-2% post malaria. Apparent

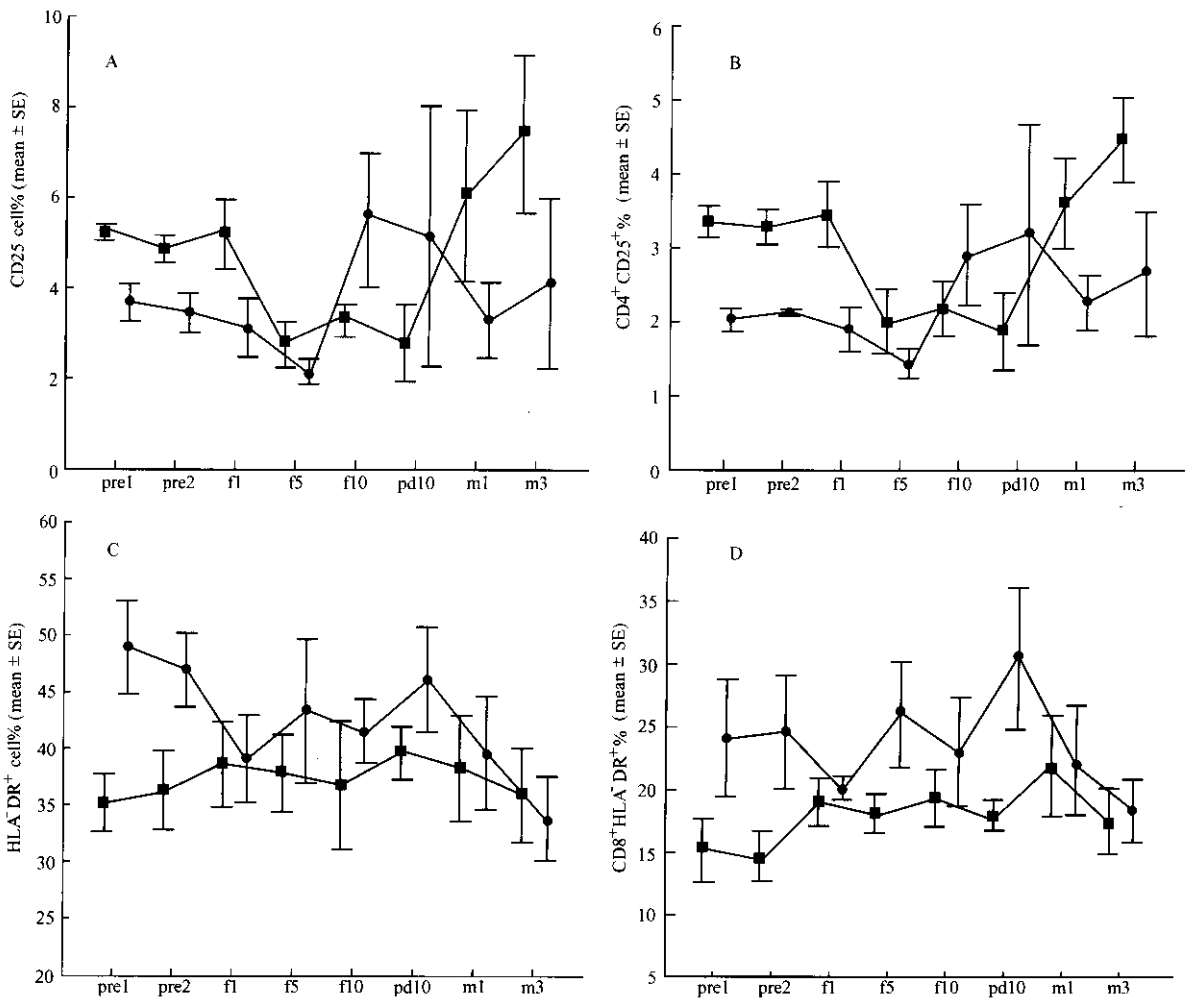


Fig. 3. Dynamics of percentages of CD25⁺, CD4⁺CD25⁺, HLA-DR⁺ and CD8⁺HLA-DR⁺ cells (■ : G1 ; ● : G2).

Table 1. Immunological parameters : data comparison of healthy persons against HIV-positive subjects in Guangzhou , China

Parameters	HIV (-) (n = 20)		HIV (+) (n = 12)		Change	P values
	Range	Mean ± SD	Range	Mean ± SD		
CD4 [#]	463 - 1658	805 ± 284	15 - 1217	475 ± 371	↓	<0.001
CD4 (%)	25.6 - 50.7	33.1 ± 5.7	1.2 - 27.6	16.7 ± 8.3	↓	<0.001
CD8 [#]	224 - 1261	814 ± 292	482 - 1584	1072 ± 381		0.127
CD8 (%)	21.5 - 39.1	32.5 ± 4.6	32.4 - 67.8	43.4 ± 9.6	↑	0.001
CD4/CD8 ratio	0.7 - 2.4	1.1 ± 0.4	0.03 - 0.8	0.4 ± 0.2	↓	<0.001
apoptotic CD4 (%)	0.1 - 0.8	0.3 ± 0.2	0.9 - 22.1	3.4 ± 5.9	↑	0.004
CD25 ⁺ (%)	3.2 - 6.7	4.5 ± 1.0	2.5 - 5.8	3.9 ± 1.1		0.442
CD4 ⁺ CD25 ⁺ (%)	2.2 - 5.8	3.5 ± 0.9	0.4 - 3.9	2.4 ± 1.0	↓	0.031
HLA-DR ⁺ (%)	13.6 - 35.2	21.7 ± 5.4	20.9 - 53.0	36.6 ± 10.5	↑	<0.001
CD8 ⁺ HLA-DR ⁺ (%)	1.5 - 7.5	4.0 ± 1.8	11.8 - 38.4	18.9 ± 8.4	↑	<0.001
Lymphocyte [#]	1041 - 4134	2478 ± 823	1300 - 4450	2546 ± 954		0.431
Lymphocyte (%)	20.0 - 53.0	35.0 ± 11.0	29.0 - 58.0	43.3 ± 9.2	↑	0.019
WBC [#]	2700 - 12800	7380 ± 2404	2900 - 12200	6048 ± 2566		0.170

: number ; % : percentage ; " ↑ " : parameter is higher in HIV-positive subjects than in HIV-negative subjects ; " ↓ " : parameter is lower in HIV-positive subjects than in HIV-negative subjects.

clinical improvements included : disappearance of PCP and ulcer of external genitalia and the normalization of

activities during the three-month follow-up. Very weak cytokine responses during malaria and relatively lower

Table 2. Dynamics of CD4 cell absolute number/ μ l

Grouping Case No.	G1					G2					G3	
	1	2	3	4	5	6	7	8	9	10	11	12
Baseline *	1217	1043	688	663	590	348	290	260	239	208	144	15
pd10	920	868	821	676	412	742	311	1159	262	687	119	41
m1	664	958	460	676	545			481	185	237	98	13
m3	1073	1083	493	672	552	377		350	235	257		16
m6	(-) 632	(-) 492	(-) 472		542			(+) 515	140	192	78	2
m12	(-) 521	(-) 524	(-) 503	(+) 767	564	(+) 499	(+) 412	298		206	66	
m18	(-) 569	(-) 561	(-) 524	(-) 552	509	(+) 470		(+) 466	178		106	
m24	(-) 516	(-) 334	(-) 441	(-) 403	(-) 335	278		(+) 360	169	181		

* Mean value of two pre-malariotherapy measurements (pre1 and pre2). Sustained increases or decreases in CD4 T cell levels are defined as : increases (+) or decreases (-) of at least 100/ μ l CD4 T cells in more than half of the available measurements observed between 6 and 24 months.

Table 3. Dynamics of CD4 cell percentage

Grouping Case No.	G1					G2					G3	
	1	2	3	4	5	6	7	8	9	10	11	12
Baseline *	28	27	26	24	20	13	13	12	16	14	8	1
pd10	22	24	22	31	21	37	17	53	14	49	8	4
m1	28	28	24	36	20			17	15	18	6	1
m3	26	23	24	36	25	31		19	22	20		2
m6	25	(-) 20	(-) 19		(+) 24			(+) 17	18	(+) 18	6	1
m12	(-) 20	(-) 19	(-) 19	(+) 29	(+) 27	(+) 25	(+) 17	(+) 18		(+) 18	5	
m18	(-) 22	(-) 21	(-) 21	(+) 32	21	15		(+) 20	(-) 12		7	
m24	26	(-) 21	(-) 21	(+) 29	18	15		(+) 19	18	(+) 19		

* Mean value of two pre-malariotherapy measurements (pre1 and pre2). Sustained increases or decreases in CD4 T cell levels are defined as : an increase (+) or decrease (-) of at least 4% in CD4 T cells in more than half of the available measurements observed between 6 and 24 months.

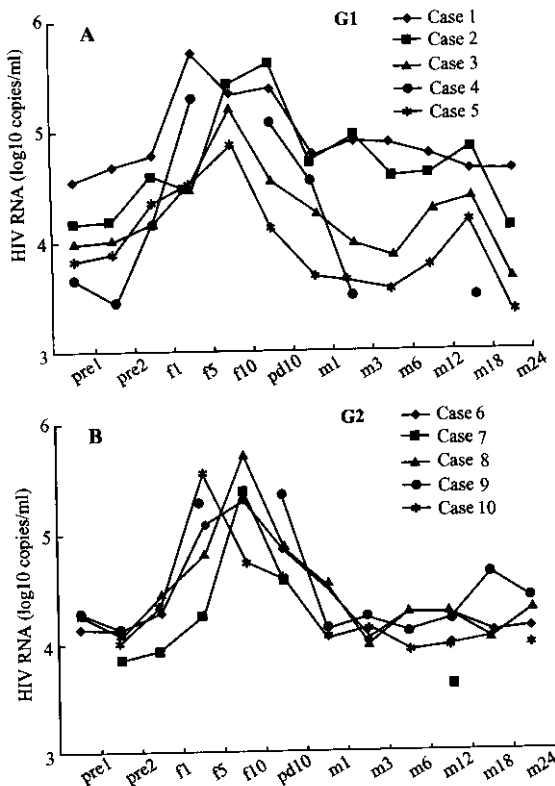


Fig. 4. Dynamics of HIV viral load. A : patients of group 1 ; B : patients of group 2.

levels of TNF- α , sIL-2R and NPT post malaria were observed in this patient (data not shown). Lastly , the

viral load decreased during malaria (only two medium fever episodes) but rebounded to higher than baseline post malaria until m 6 (data not shown).

DISCUSSION

The response patterns of T-lymphocyte subsets and phenotypes to acute vivax malaria are quite different between HIV-positive subjects with different CD4 cell baselines (G1 \geq 500/ μ l and G2 at 499 - 200/ μ l). In G1 patients , the down-regulation of CD4 cell number and percentage , CD4/CD8 ratio , CD25⁺ and CD4⁺ CD25⁺ cell percentages and up-regulation of CD8⁺ HLA⁺ DR⁺ cell percentage were observed during malaria even if these parameters recovered to (CD25⁺ and CD4⁺ CD25⁺ percentages to higher than baseline levels post malaria). In contrast , in G2 patients up-regulation of CD4 cell number and percentage , CD4/CD8 ratio , CD25⁺ and CD4⁺ CD25⁺ cell percentages and down-regulation of CD8 cell number , HLA⁺ DR⁺ and CD8⁺ HLA⁺ DR⁺ cell percentages were observed during , and remained the changed , post malaria. In a cross-sectional study ,¹¹ the percentages of CD4 and CD8 cells and the ratio of CD4/CD8 were similar between HIV-negative persons infected with Pv or Plasmodium falciparum (Pf) or dually infected with the both parasites and the control population without malaria ; however the absolute counts of CD4 cells , CD8

cells and total lymphocytes were generally lower and the percentages of CD25⁺ and HLA-DR⁺ cells were significantly higher (either Pv or Pf or dual Pv and Pf) in malaria patients than in the controls. These phenomena , when compared to our data for HIV⁺ patients , suggest very different responses of lymphocyte subsets and phenotypes to acute malaria among individuals with different HIV and immune status. We postulate that besides of damage or reproduction of the cells , these sharp responses during malarial phase at least partially represent redistribution¹² of lymphocytes such as CD4 cells transferred from lymph nodes and other lymphoid tissues into the peripheral blood stream in (at least most) G2 patients or from peripheral blood to lymphoid tissues in G1 patients.

It is known that plasma levels of sIL-2R reflect the activity of IL-2 *in vivo* and that IL-2 gene expression *in vivo* and IL-2 production of peripheral blood mononuclear cells (PBMCs) to stimulation of mitogens *in vitro* decrease in HIV-positive subjects.^{13,14} The findings in our present study indicates that HIV infected persons , especially G2 patients , have strong sIL-2R (reflecting IL-2) response to acute vivax malaria. This is consistent with the results noted in our previous report that therapeutic acute vivax malaria induced elevated plasma IL-2 level in HIV-infected individuals after cured of malaria.⁹ In a well-controlled *in vitro* study,¹⁵ complex malaria antigens , derived from Pf induced as effective cell proliferation and cytokine (IL-2 and IFN- γ) production from PBMCs from AIDS patients as those from HIV-negative healthy subjects. But single malaria antigen MSP-1 derived from Pf and other antigens and mitogens had none of these effects. Our results indicate a much stronger response of TH1-type cytokines (IL-2 and IFN- γ reflected by their activity markers sIL-2R and NPT which are easier for measurement than the cytokines proper)¹³ and one proinflammatory cytokine (TNF- α) to acute vivax malaria in patients with more advanced HIV infection (G2) than in those with relatively earlier infection (G1). Up regulation of CD4 levels in G2 patients appears to be due to the strong cytokine responses which may be sufficient enough to induce CD4 production. But we do not know why HIV-infected patients with CD4 baselines $\geq 500/\mu\text{l}$ or $< 200/\mu\text{l}$ are weaker responders to malariotherapy , in terms of increased cytokine and CD4 responses , compared with those patients with CD4 baselines at 200 – 499/ μl . These *in vivo* phenomena contrast with the *in vitro* measurements with other antigens or mitogens which indicate a greater impairment of response in advanced HIV patients. Our observations suggest that malarial parasite , which contains complete malaria antigens , induces effective

cytokine or immune response against the malarial infection in HIV/AIDS patients and this response may also be efficacious against infections by other organisms as observed in the patient with full-blown AIDS who experienced clinical improvement (disappearance of PCP and ulcer of external genitalia).

Multiple studies have shown that HIV infection not only causes death of infected cells but induces uninfected CD4 cell apoptosis.¹⁶⁻¹⁸ In HIV-negative patients with malaria , the increase of T-lymphocyte apoptosis was reported.^{19,20} We did observe the “ pile-up ” effect of CD4 cell apoptosis of HIV and Pv coinfection during malaria , but after the malaria was cured , the percentage of apoptotic CD4 cells sharply reduced from a high level to a nearly normal level ($0.7\% \pm 1.1\%$; the normal control level was $0.3\% \pm 0.2\%$). The decrease in the percentage of apoptotic CD4 cells post malaria may be attributed to strengthened IL-2 and IFN- γ activities. It is known that TH1-type cytokines (IL-2 and IFN- γ) prevent and TH2-type cytokines (IL-4 and IL-10) promote CD4 cell apoptosis.^{21,22} We have indicated found that acute vivax malaria provokes high activities of TH1-type cytokines in HIV-positive persons. Studies by other researchers have indicated that malaria promotes activities of TH1 , some TH2-type cytokines (IL-10 but not IL-4) and proinflammatory cytokines (TNF- α , IL-1 and IL-6) in HIV-negative subjects.²³⁻²⁸ We demonstrate that malariotherapy effectively stimulates HIV-infected persons to produce all or most these cytokines (reflected by elevation of $\beta 2\text{M}$ level during malaria). These cytokines act on the immune systems which are at different levels of impaired immune function induced by HIV. This may lead to a new balance of the immune systems , such as resulting increase of IL-2 levels after termination of malaria in patients whose IL-2 levels are relatively lower before malarial infection (this was demonstrated in our previous study). The increase of IL-2 levels may block apoptosis and stimulates production of CD4 cells , which is reflected by elevated counts and percentages of CD4 cells and of CD25⁺ (IL-2 receptor α chain) and CD4⁺ CD25⁺ cells as well as declined percentage of apoptotic CD4 cells in G2 patients in our present study. In this study , the increase of CD4 cell percentage was more obvious than the increase in the number of CD4 cells because of the apparent reduction of lymphocyte (which was due to the decrease of percentage since WBC number returned to baseline level post malaria , data not shown). The decrease of lymphocyte percentage (from $43.3\% \pm 9.2\%$ at baseline to $32.7\% \pm 8.8\%$ at m3 post malaria in the 12 patients) seemed to normalize the parameter which was significantly higher at baseline than the control (Table 1).

HLA-DR significantly increased and CD25 significantly decreased in HIV-infected individuals²⁹ (the same phenomena were also found in our present study , Table 1) . Several reports^{30,31} have indicated that prognostically significant immune markers of HIV infection include decreased CD4 number and percentage ; decreased CD4/CD8 ratio and CD25⁺ total lymphocytes ; increased percentage of HLA`DR⁺ total lymphocytes and increased sIL-2R , NPT , TNF- α , sTNF-RII and β 2M. In HIV-positive subjects with CD4 baselines at 200 – 499/ μ l we observed the following : increases in the mean level of CD4 number and percentage and CD4/CD8 ratio , an increase in CD25⁺ and CD4⁺ CD25⁺ percentages , decreases in apoptotic CD4 percentage and CD8 number , decreases in HLA`DR⁺ and CD8⁺ HLA`DR⁺ percentages and stable levels in plasma activation markers including sIL-2R , NPT , TNF- α , sTNF-RII and β 2M after therapeutic malaria was terminated. It should be noted that no complications were observed in 20 HIV-infected patients who received malariotherapy despite a marked rise in plasma activation marker levels during the 10-30 day malarial phase.^{9,32} Furthermore , patients with progressively lower CD4 T-cell baseline levels experienced milder malarial symptoms and parasitemia (Chen et al. Chin Public Health 2001 ,17 :1071-1072). All these phenomena further support the hypothesis that for humans , there is a beneficial interaction between HIV and the malarial parasite (this is consist with the findings in animal model studies).

This study confirmed that therapeutic vivax malaria did transiently increase HIV viral load as reported by Hoffman and colleagues.³³ Xiao and colleagues³⁴ have demonstrated , *in vitro* , that Pf antigen-induced HIV-1 replication is mediated through TNF- α activity. Other groups of researchers have observed a rapid increase in plasma HIV RNA levels immediately after extracorporeal whole-body hyperthermia (EWBH , the highest body temperature 41 or 42°C -similar to that of malariotherapy) with negative HIV cultures , indicating a heat-induced release of nonviable HIV from cellular and/or tissue reservoirs.³⁵⁻³⁷ In G2 patients , our present study found that the HIV viral load quickly returned to baseline levels 1-3 months after malaria was cured , however the increase of CD4 cell levels induced by therapeutic malaria in some of these patients lasted much longer (at least 12 – 24 months). This time difference seems to be one of the basic principles of malariotherapy for HIV infection. The G1 patients appear to follow a natural history decline in CD4 level and a relatively unchanged HIV viral load. A transient clinical improvement including the disappearance of opportunistic infections ; temporal increase of CD4 levels post malariotherapy and a decrease

in HIV viral load during the malarial phase (only two medium fever episodes) were observed in a patient diagnosed with AIDS (case 12). These observations were noted without the use of any antiretroviral drugs or antibiotics. Our results suggest that patients with CD4 baselines < 200/ μ l may receive little benefit from malariotherapy since cases 11 and 12 experienced elevation of viral load after termination of malaria without apparent or sustained increase of CD4 levels (there appears to be no harmful effects in these patients , because case 12 did get transient clinical improvement and case 11 , as indicated via telephone interview , remained clinically well for 36 months after malariotherapy).

In conclusion , malariotherapy benefited some of the HIV-infected patients diagnosed in CDC category II (CD4 count levels at 200 – 499/ μ l)¹⁰ as they experienced increases of CD4 cell counts and percentages and their HIV viral load remained relatively unchanged for at least 1-2 years after therapy. Therefore a well-controlled trial involving CDC category II patients should be carefully conducted to confirm if the use of malariotherapy is a null or valid approach. We do not advocate the routine use of this therapy for treatment until further scientific research has been conducted. It has been shown that highly active antiretroviral therapy (HAART) cannot eradicate HIV *in vivo* because it cannot clear HIV in the resting memory CD4 cells or in other viral reservoirs (including lymph nodes and other lymphoid tissues).³⁸ Studies conducted by other researchers have demonstrated that a mixture of TNF- α , IL-2 and IL-6 activated resting T-cells including CD4 cells *in vitro* this sentence is unclear).³⁹ Our present data and other data²⁸ indicate that malaria , or therapeutic malaria , provokes high levels of the aforementioned cytokines *in vivo* and an acute attack of malaria may induce the redistribution of CD4 T-lymphocytes (i. e. the transfer of cells from lymphoid tissues into the peripheral blood stream in G2 patients). This further suggests that malariotherapy may empty HIV viral reservoirs while under the treatment of HAART. Other studies have found that there was cross-reactivity of anti-malaria antibodies and HIV tests including shared antibodies against p17 , p24 , p31 , p51 , p55 , p66 and p160 of HIV-1.^{40,41} Therefore , we hypothesize that HAART plus malariotherapy may eradicate HIV *in vivo*. We recently did an animal model study to test if drugs in HAART regimens inhibit malaria parasites (or not). We found that none of the 5 drugs (indinavir , efavirenz , zidovudine , lamivudine , abacavir and their appropriate combination) inhibited reproduction of the parasites (unpublished data). Therefore we should explore the possibility of eradicating HIV *in vivo* through such a

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