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Expression of monocyte markers in HIV-1 infected individuals with or without HIV associated dementia and normal controls in Bangkok Thailand*

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Abstract

HIV Associated Dementia (HAD) is a complication of HIV infection in developed countries and is still poorly defined in resource-limited settings. In this study we investigated the expression of the monocyte phenotype CD14CD16HLADR and the inflammatory profiles in monocytes supernatants by surface-enhanced laser desorption/ionization-time of flight (SELDI-TOF) mass spectrometry in a cohort of HAD and non-HAD Thai volunteers prior to the initiation of ARV. The CD14CD16HLADR phenotype was significantly increased in monocytes from HAD and non-HAD versus negative controls, but there was no difference in phenotype and in the secretion protein profiles between the two seropositive groups. In addition, monocytes supernatants from HAD and non-HAD did not induced apoptosis or cell death in brain aggregate culture. In conclusion it appears that HAD in Thai individuals has a different immunological profile then in North America cohorts.

Keywords

HIV-1; Thailand; Monocyte markers; HAD

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1. Introduction

Neurological dysfunction is a common complication of HIV-1 infection, ranging from early acute neurological disease, such as aseptic meningitis, to mild neurocognitive dysfunction and frank dementia (Gendelman et al., 1998; Price, 1996). An estimated 20–40% of infected individuals will develop significant cognitive dysfunction (Gendelman et al., 1998; Kolson and Gonzalez-Scarano, 2000); however the incidence of HAD has decreased with the advent of highly active antiretroviral therapy (HAART). HIV-1 in the brain is associated with macrophages and microglia and likely enters the brain via infected monocytes/macrophages (M/M) early after infection (Davis et al., 1992; Williams and Hickey, 2002). The role that HIV-infected M/M in the periphery and central nervous system (CNS) play in the development and pathogenesis of CNS disease is suggested by studies that have detected increased CD14CD16 and CD14CD69 blood monocyte sub-populations in HIV-1-infected patients, SIV-infected monkeys (Fingerle et al., 1993; Otani et al., 1998; Thieblemont et al., 1995; Ziegler-Heitbrock et al., 1993) and in persons with HAD (Amirayan-Chevillard et al., 2000; Pulliam et al., 1997; Thieblemont et al., 1995). In HAD, macrophages appear to be activated, with increased expression of MHC antigens, TNF- α , TGF- β , IL-1 α , IFN- γ , and nitric oxide synthase (Orandle et al., 2001; Roitt et al., 2001; Sippy et al., 1995; Tyor et al., 1992; Wesselingh et al., 1993). A novel technology, surface-enhanced laser desorption/ionization time of flight (SELDI-TOF) Protein Chip, that allows the study of M/M protein secretion profiles has revealed different inflammatory profiles between HAD and non-HAD individuals in subtype B virus (Everall and Grant, 2004; Luo et al., 2003; Sun et al., 2004). These observations, taken together, suggest that M/M are infected in the periphery, acquire a proinflammatory phenotype, and transit to the CNS likely transporting HIV in the process (Ohagen et al., 2003; Shiramizu et al., 2005; Simmonds et al., 1991).

Based on HIV-1 prevalence among Royal Thai Army conscripts and antenatal clinics, there may be 75,000–94,000 persons between the ages of 20–30 years who are infected with HIV-1 in Bangkok. Early published data from Bangkok (multicenter WHO Neuropsychiatric AIDS study, 1992) identified cognitive impairment but did not identify HIV-associated dementia (HAD) among HIV-1 patients (Maj et al., 1991), although other HIV-1 neurological complications have been reported in Thailand, including neurocognitive impairment (Folstein et al., 1975; Hemachudha et al., 1994; Piyasirisilp et al., 1994). The predominant HIV-1 strain in Thailand is CRF01_AE with a minor component of subtype B viruses (McCutchan, 2000). While there are limited data on HAD in Thailand, reports from India (predominantly subtype C) indicate that HAD exists in non-subtype B infections (Ranga et al., 2004; Satishchandra et al., 2000). The Southeast Asia Research Collaboration with Hawaii (SEARCH), a newly formed international collaboration that includes the University of Hawaii, Phramongkutklao Hospital, the Thai Red Cross AIDS Research Center and the Department of Retrovirology, Armed Forces Research Institute of Medical Sciences (AFRIMS) has recently established a neuroAIDS cohort in Bangkok. The principal finding of the study was that there are neurological abnormalities in Thai HIV-1 CRF01_AE infected individuals consistent with HAD and that the pattern of abnormalities is different from that observed in North American individuals (Valcour et al., 2007).

All participants in the cohort undergo formal neurological testing using the WHO International HIV Neuropsychological battery and provide blood samples for flow cytometric analyses. The immunological assays were aimed at confirming whether monocyte markers were increased in HIV-infected Thais with HAD compared to non-HAD and to determine if ARV therapy had any effect on the expression of these markers. In addition the study of monocyte supernatants by SELDI-TOF mass spectrometry would help to further characterize HAD in Thais.

Data showed that CD14CD16 monocyte markers are increased in HIV disease and decreased with the initiation of ARV but do not discriminate HAD from non-HAD. Profiles of secreted proteins from M/M supernatants do not differ between HAD and non-HAD individuals. In addition, Thai HIV-negative individuals have higher background levels of CD14CD16 expression than controls in United States.

2. Materials and methods

2.1. Thai volunteers

60 volunteers were enrolled at Phramongkutklo Hospital in Bangkok, Thailand. This cohort has been extensively described elsewhere (Valcour et al., 2007). Briefly, thirty volunteers were HIV-1 infected individuals (15 HAD and 15 non-HAD), and 30 matched HIV-negative controls. HAD volunteers were eligible for the study if they had: 1) no previous exposure to antiretroviral medications (one patient had limited past exposure to AZT) and 2) cognitive complaints with confirmed impairment by the study neurologist (P.S.) using proxy and patient histories, a complete neurological examination, simple cognitive tests, and the International HIV Dementia Scale (Sacktor et al., 2005). Exclusion criteria included: possible causes of cognitive abnormalities other than HIV-1 infection (e.g. head injury, learning disability, any active opportunistic infection or illness, past or current CNS opportunistic infection, major depression, and drug use). Individuals were tested for antibody to hepatitis C infection and excluded if positive (Forton et al., 2005). Individuals with remote or minimal past drug use were allowed to enroll, but all individuals were required to have a negative urine drug test at the screening and enrollment visits. All HAD individuals underwent gadolinium-enhanced brain MRI and lumbar puncture, if indicated, to exclude CNS opportunistic infection. Non-HAD volunteers were matched to HAD by age (within a decade), education (<12 years, 12–15 years, >15 years), gender, and CD4 (<200 cells/mm³, 200–350 cells/mm³, >350 cells/mm³). The thirty age-, gender-, and education-matched (1:1) HIV-negative controls were enrolled using the same exclusion criteria.

In addition to the neurological evaluation, all individuals enrolled in the study underwent neuropsychological testing. Only individuals with sufficient language skills to complete the NP testing were enrolled. We assessed depressive symptoms with the Thai Depression Inventory (TDI) which has been validated in Thailand (Lotrakul and Sukanich, 1999). After enrollment 24 patients started ARV therapy with GPOvirTM, the combination antiretroviral regimen (containing stavudine, lamivudine and nevirapine) produced by the Thai Government Pharmaceutical Organization (Anekthananon et al., 2004), 2 patients started with EFV+3TC+d4T, 1 patient started with EFV+3TC+AZT, 1 patient started with an unknown ARV (the subject is a participant in an ARV therapy research study) and 1 patient started with IDV+RTV+3TC+AZT. One individual in the dementia group refused to start ARV therapy.

Volunteers return to the clinic every 6 months to undergo repeat neuropsychological testing, viral load and CD4+ measurements. At one year volunteers received a full neurological exam, neuropsychological testing, viral load and CD4+ T-cell measurements. For this study we report data collected at entry prior to ARV therapy and at 6 months and 1 year after initiation of ARV.

2.2. Hawaii volunteers

Twenty HIV-negative individuals were enrolled to obtain data on monocyte markers in the general population living in Honolulu, Hawaii. Age, gender and education were recorded.

2.3. Human subject protection

The protocol and consents were approved by the Ethical Review Committee of the Royal Thai Army Medical Department, the University of Hawaii Committee on Human Subjects and the Human Use Review Committee of the Walter Reed Army Institute of Research.. Signed, informed consent (in Thai) was obtained for all participants. When appropriate, authorized representatives (usually family members) co-signed consents, in accordance with Thai IRB policy. All volunteers from Honolulu, Hawaii signed a consent form that was approved by the University of Hawaii Committee on Human Subjects.

2.4. HIV viral load, viral subtype and CD4+ count

Lymphocyte immunotyping and plasma HIV viral load were performed at the Department of Retrovirology Laboratory, AFRIMS. HIV viral load at visit 1 (V1) was determined with the Roche Amplicor v1.5 standard assay, and subsequent samples visits were tested using the UltraSensitive method. CD4 counts were obtained using dual platform. CD4 percent was obtained with BD TriTEST™ reagents (Becton Dickinson, San Jose, CA), and white blood cell counts were obtained by using cell counter (Coulter Max-M™ Burlington, Ontario, Canada).

Viral subtype was determined by the AFRIMS Retrovirology laboratory using V3 loop peptide ELISA serotyping, as previously described (Gaywee et al., 1996) The peptides used were V3-CM237 (Thai subtype B) and V3 CM242 (CRF01_AE). This approach has previously been shown to distinguish HIV-1 subtype B and CRF01-AE infection in Thai individuals (Mason et al., 1998; Polonis et al., 2001). Only one patient showed dual reactivity and could not be resolved using this technique.

2.5. Flow cytometry

Each volunteer provided 10 ml of heparinized blood. Leukocytes were separated on a Ficoll-Hypaque gradient, washed, counted and stained with a battery of monoclonal antibodies (mAb) specific for monocyte markers within 2 h of collection. Blood from entry (V1, pre-ARV), 6 months post ARV (V2) and 1 year post ARV (V3) was collected and stained with the CD14CD16HLADR marker combination. The following mAb were used: CD14 FITC, CD16 PE, and HLADR-PerCP (Becton Dickinson, San Jose, CA). IgG1 FITC, IgG2a-PE, IgG2a-PerCP were used as negative controls. The PBMC and antibody mixture was incubated for 20 min in the dark at room temperature. Cells were washed with 2 ml wash buffer (2% FBS in D-PBS) and resuspended in 500 µl of 1% paraformaldehyde. Stained cells were analyzed on a FACScalibur (Becton Dickinson, San Jose, CA) within 24 h. At least 75,000 total events were acquired. Monocyte gating was performed on a SSC/FSC scatter plot. Data analyses were performed with FlowJo software (Tree Star Inc., Ashland, OR). Absolute monocyte counts were calculated by multiplying the percent monocytes by the number of white blood cells in the complete blood count (CBC). Assay reproducibility was established by evaluation of intra-assay variability by performing the assay in duplicate for 10 controls, 5 HAD, and 5 non-HAD subjects. Only one laboratory technician performed the isolation of the PBMC, staining, acquisition, and analysis. The coefficient of reliability was <1% variation for both subsets of combined markers demonstrating small variability between replicates. Technicians from Hawaii and Bangkok were supervised by the same investigator (SRK), who provided direct oversight at each location. In addition, the technician from Hawaii worked in the laboratory in Thailand for one month to ensure consistency in procedures between sites.

2.6. Monocyte/macrophage (M/M) supernatants

CD14+ monocyte conditioned media were generated as previously described (Pulliam et al., 1997; Sun et al., 2004). Briefly, PBMCs were separated from 10 ml whole blood using Percoll gradient separation. CD14+ cells were positively selected with MACS anti-CD14 ferrous microbeads by magnetic retention in a lymphocyte separation column (Miltenyi Biotech, Auburn, California, USA). Purity of CD14+ cells was assessed by flow cytometry using anti-CD14-FITC and was generally within 80–90%. CD14+ monocytes were cultured in RPMI 1640 with 10% FBS at a density of 1×10^6 in 6-well plates. Conditioned media were collected on day 7, frozen to -80°C and shipped to the US for SELDI-TOF analyses.

2.7. SELDI-TOF mass spectrometry

Mass spectrometry analysis was modified from a previously described protocol (Sun et al., 2004). Conditioned media were subjected to ultracentrifugation at $90000 \times g$ at 4°C for 16 h with a Beckman LH-8 ultracentrifuge (Beckman Coulter Inc., Fullerton, CA), for clearance of HIV. M/M supernatants were fractionated using HPLC (Waters Milford Massachusetts, USA) with a Phenomenex $5 \mu\text{m}$ C18 column (Torrance, California, USA). The mobile phase was acetonitrile/water gradient. Fractions were collected every 2 min for 30 min. Each fraction was analyzed on Ciphergen gold chips using the SELDI-TOF ProteinChip system (Ciphergen Biosystems, Fremont, California, USA). Data were analyzed as previously described (Sun et al., 2004). All SELDI-TOF-generated spectra were normalized to total ion current intensity prior to analysis using Biomarker Wizard Software version 3.1 (Ciphergen). The intensities of the m/z values (mass-to-charge ratios) were subjected to statistical analysis.

2.8. Toxicity assay on brain aggregates

To further evaluate neurotoxicity of the M/M supernatants, human brain aggregates were prepared as previously reported (Pulliam et al., 1991) from human brain tissue. Conditioned supernatants (20% v/v with DMEM) were incubated with 2 different human brain cell aggregate cultures for 3 days. After incubation, supernatants from the brain aggregate cultures were used in a lactate dehydrogenase (LDH) assay (Roche) for cell death and the brain aggregates used in a programmed cell death (PCD) assay (Roche).

2.9. Statistical analyses

Descriptive analysis was done to assess means, medians, and frequencies, including tests of normality. Bivariate analyses utilizing contingency tables were used to assess associations between groups, as well as potentially confounding variables. Fisher's exact test was used for statistical inference for dichotomous variables. Analysis of continuous variables was done primarily with non-parametric tests of inference based on rank procedures due to the non-normal distribution of monocyte marker measurements. Peak intensities of SELDI-TOF spectra were compared using Student's *t*-test. All analyses were performed using Stata Statistical Software, release 9.2 (StataCorp, College Station, Texas).

3. Results

3.1. Monocyte marker expression in HIV-infected Thai individuals

At entry study volunteers were divided into three groups: HAD, non-HAD and seronegative controls; volunteers were well-matched for most demographic characteristics (Table 1). Individuals in the HAD and non-HAD groups were followed at 6 and 12 months after their initial visit. Twenty nine HIV-infected individuals started an ARV regimen. Monocytes expressing CD14CD16HLADR were significantly increased in HIV infection ($p < 0.01$ for both HAD and non-HAD volunteers versus controls), but there was no significant difference

between the two seropositive groups (HAD versus non-HAD) at V1 (Table 2), suggesting that the up-regulation of CD16 on monocytes does not distinguish those with HAD in this cohort. At V2 (6 months post-initiation of ARV) all HIV-infected volunteers had substantial and statistically significant decreases in viral load and attendant increases in CD4 counts (Fig. 1). Nine of 14 HAD subjects and 11/15 non-HAD subjects had undetectable viral load (<50 copies/mL, data not shown); the volunteer who did not start ARV therapy was excluded from the follow up analyses. There was no statistically significant difference in the reduction in viral load or CD4+ T cell gain between the HAD and non-HAD groups (Fig. 1). At the 6-month visit (V2), a statistically significant reduction in the percentage of monocytes carrying the markers CD14CD16HLADR was observed in both HAD and non-HAD groups when compared to V1. At V3 the percentage and number of monocytes carrying the CD14CD16HLADR markers increased to similar levels of pre ARV treatment, despite continued ARV therapy.

3.2. Comparison of HIV-negative Thai control and North American controls

Interestingly, the median value and range for CD14-CD16HLADR markers in the HIV-negative control group from Bangkok was much higher than expected based on published reports in seronegative control groups from North America (Pulliam et al., 1997). To evaluate this observation, we collected data from HIV-negative volunteers in Honolulu, Hawaii. Twenty HIV-negative individuals were enrolled, and their blood cells studied with the same protocol used for the Thai cohort (Table 1, for demographic of Hawaii cohort). Seronegative Thai individuals had a higher percentage of CD14HLADRCD16 staining cells compared to respective controls from Hawaii. These differences were statistically significant ($p < 0.001$) whether assessed as a percentage or absolute number, (Fig. 2, absolute number data not shown).

3.3. Proteomic profile and neurotoxicity of M/M supernatants

Supernatants harvested from cultured M/M obtained from controls ($n=13$), non-HAD subjects ($n=13$) and HAD subjects ($n=13$) were analyzed by SELDI-TOF mass spectrometry using a previously described protocol with modifications (Sun et al., 2004). When spectra generated from M/M supernatant samples were compared, no consistent differential peaks were identified between HAD and non-HAD subjects suggesting that the profile of M/M secreted proteins from HAD and non-HAD subjects were similar. However, when comparing M/M supernatant spectra from HIV-negative and HIV-positive individuals, 73 proteins peaks out of 441 detected exhibited a significant difference in peak intensity with 8 proteins (2.6 kDa–5.1 kDa) in HIV-positive M/M supernatants that were virtually undetectable in controls (Fig. 3). The M/M supernatants were also evaluated to determine if secreted factors were neurotoxic. When brain aggregate cultures that were treated with M/M supernatants (20%) and cultured for 3 days were analyzed for apoptosis and evidence of cell death, no neurotoxicity was associated with M/M supernatants from any of the 3 groups (results not shown).

4. Discussion

In this study, we sought to confirm the association of HAD and CD14CD16HLADR described in subtype B HIV infection, M/M supernatant neurotoxicity and whether the initiation of ARV has any effect on the expression of these markers in peripheral blood monocytes. These monocyte markers have been reported in association with HAD (Pulliam et al., 1997) but have not been evaluated longitudinally to monitor the effect of ARV on expression.

Using a three-color flow cytometry assay we did not identify differences in the expression of these markers and cognitive status; however, we identified different levels of expression in association with HIV infection for monocytes stained for CD14CD16HLADR when compared to seronegative controls, confirming previous data collected from a North American cohort (Pulliam et al., 1997). We conclude that these markers do not appear to be discriminatory for HIV-associated dementia in this cohort of CRF01_AE HIV infected subjects. To ensure that factors associated with the diagnosis of HAD did not influence this finding, we evaluated the relationship between these markers and raw neuropsychological testing scores among all participants, independent of HAD diagnosis. We were not able to identify any relationship between CD14CD16HLADR and neuropsychological performance (data not shown).

We also demonstrated an effect of ARV therapy on the expression of these monocyte markers albeit transient. These observations taken together may be attributed to a decreased viral replication and resulting effect on the numbers of these monocytes in circulation. It is quite intriguing that the CD14CD16 monocytes decrease early after ARV initiation but rebound somewhat after 1 year of treatment. The rebound is not statistically significant, and may be the spurious result of a small sample size combined with the variability in CD14CD16 monocytes. If the rebound in CD14CD16 monocytes is real, however, one possible explanation is that the CD14CD16 monocyte subpopulation is comprised of a mixture of infected monocytes with actively replicating and non-replicating virus. Low level HIV replication persists after ARV (Havlir et al., 2005) and similarly, CNS inflammation in HIV infection persists despite >48 months of HAART (Eden et al., 2007). Whether the activation of monocytes is due to an effect of latent virus or of local (CNS) lymphokine/monokine production is unknown. The role of these monocytes in chronic disease has not been clearly defined but their increased presence has been described in many chronic inflammatory diseases (Ziegler-Heitbrock, 2000). In HAD it has been shown that these monocytes may carry HIV to the brain (Williams and Hickey, 2002) and CD14CD16HLADR monocytes have been reported to be more susceptible to HIV infection (Crowe et al., 2003; Ellery et al., 2007). What mechanism induces these cells to migrate to the brain is not yet understood, but under ARV it has been shown that neurological symptoms improve possibly because of a decrease in CNS trafficking of actively infected M/M. To further characterize this population, M/M supernatants collected at baseline were analyzed by SELDI-TOF mass-spectrometry. Comparison of spectra between HAD and non-HAD M/M supernatants did not detect any proteins unique to either group. There were 73 elevated distinct protein peaks in the supernatants from the HIV infected individuals compared to non-HIV infected suggesting an altered secretion pattern in monocytes from Thais. In addition we found no evidence of neurotoxicity in brain aggregate cultures when cultured with the M/M supernatants. It is important to note that although SELDI-TOF mass spectrometry identified altered protein secretion products, these may not be neurotoxic. The brain aggregate assay measures cell death and apoptosis and it is possible that the secreted proteins were of a different nature and not involved in neurotoxic events. This is in contrast to North American data where clearly only the HAD individuals had altered inflammatory profile in M/M supernatants compared to non-HAD (Everall and Grant, 2004; Luo et al., 2003; Sun et al., 2004). A potential explanation is that different HIV subtypes may induce different immunological responses. While neuropsychological testing differences were identified between HAD and non-HAD groups in this cohort (Valcour et al., 2007) there is a suggestion that the cognitive testing profiles may differ from that classically described in North American HIV dementia cohorts, which may also suggest subtype-specific processes.

It is also possible that previous co-morbid infections, environmental, or host genetic factors may influence monocyte phenotypes and neuropsychological testing. We have observed that the percentage of CD14CD16HLADR in normal, non-infected control Thai individuals

living in Bangkok is significantly higher than similar populations in Honolulu, Hawaii. Differences in the percentage of NK cells and absolute CD4 T cell counts have been reported in Thais compared with North American controls (de Souza et al., 2000; Webster et al., 1996). It is therefore possible that the differences observed in this cohort are due to factors that are independent of HIV infection or disease status. These findings highlight the importance of testing baseline immunological markers in international cohorts. While speculative, it is possible that differences in baseline immunological phenotype prior to HIV infection can influence the pathogenesis of HIV infection.

In conclusion we have demonstrated that CD14CD16-HLADR monocytes are increased in HIV infection but do not appear to discriminate for HAD in this Thai cohort. In addition protein secretion profiles in M/M supernatant are altered in HIV infected individuals but are similar between HAD and non-HAD. Cultured M/M supernatants do not appear neurotoxic. We further demonstrated that the expression of these monocyte markers is influenced by ARV therapy. In addition, CD14CD16HLADR monocyte phenotype is significantly higher in healthy HIV-negative Thai individuals compared to HIV negative controls from Hawaii, and we conclude that it is possible that these monocyte phenotypes are influenced by other factors other than HIV infection.

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Fig. 1. CD4⁺ lymphocyte counts and plasma RNA viral load in the two groups at baseline (V1) and after initiation of ARV therapy. V2=6 months post ARV initiation, V3=12 months post ARV initiation.

Fig. 2.

Box plot representation of the percentage of monocytes carrying the markers CD14CD16HLADR in the two normal control cohorts. There was a statistical significant difference between the expressions of the two markers in the two cohorts ($p < 0.001$).

Fig. 3. SELDI-TOF intensities of selected peaks (each line represents peak intensity at the indicated m/z value (mass-to-charge ratio) of representative samples (HIV $n=3$, HAD $n=4$, Control $n=4$) showing a similar profile between HIV and HAD groups at 20 min HPLC fraction using Ciphergen gold chips.

Table 1

Demographic information

Baseline demographics				
Characteristics	C group	ND group	HAD group	Hawaii group
Sample size, <i>n</i>	30	15	15	20
Mean age $\pm$ SD	34.1 $\pm$ 9.6	33.7 $\pm$ 8.0	33.1 $\pm$ 8.6	34.1 $\pm$ 10.1
% Female	62.1%	71.4%	60.0%	55.0%
Education, mean years $\pm$ SD	7.6 $\pm$ 1.8	6.6 $\pm$ 1.7	6.9 $\pm$ 2.3	18.1 $\pm$ 3.3 *
Risk category				
Heterosexual only (%)	N/A	11 (73)	11 (73)	N/A
Homosexual only (%)	N/A	1 (7)	3 (20)	N/A
Medical parameters				
Current CD4 count $\pm$ SD	797.6 $\pm$ 245.2	53 $\pm$ 57.2 *	64.5 $\pm$ 90.8 *	N/A
Log ₁₀ viral load $\pm$ SD	N/A	5.15 $\pm$ 0.7	5.26 $\pm$ 0.5	N/A
Hemoglobin $\pm$ SD	14.0 $\pm$ 1.8	11.9 $\pm$ 0.9 *	11.2 $\pm$ 1.5 *	14.6 $\pm$ 1.3
TDI $\pm$ SD	7.2 $\pm$ 6.8	15.7 $\pm$ 6.6 *	22.9 $\pm$ 9.2 *	N/A

Statistical tests compare the two seropositive groups to controls using student *t*-test or chi square analysis, unless otherwise stated.

* Indicates a significant difference at a *p*=0.05 level versus the control group.

TDI = Thai depression inventory.

Table 2

Median and range of percentage of monocytes carrying the CD14CD16HLADR combination markers

Percentage of CD14CD16HLADR monocytes			
	Controls	Non-HAD	HAD
Visit 1	9.08 [12.65–21.97]	17.32 [10.63–34.37] ^{a, b}	17.55 [8.82–36.85] ^{a, b}
Patient number	N=25	N=12	N=13
Visit 2	ND	7.67 [2.35–48.18] ^b	13.92 [4.84–26.3] ^b
Patient number		N=15	N=14
Visit 3	ND	13.75 [1.45–21.62]	15.19 [7.58–35.93]
Patient number		N=12	N=12

The number of individuals tested differs between time points due to insufficient number of cell recovered for staining. Only 1 sample (HAD group) was excluded from analysis at V2 and V3 because the volunteer did not start ARV therapy.

^aKruskal–Wallis $p < 0.01$ controls versus Non-HAD and controls versus HAD.

^bWilcoxon matched-pairs sign-rank test, $p < 0.05$, for comparison of subjects between visits 1 and 2 ($n=12$), and 2 and 3 within each group. The Wilcoxon test was used for paired observations within each group for those who have paired data (12 for each group) between visits 1 and 2 and visits 2 and 3.