Immune profile of HIV–1 discordant couples attending AMPATH clinics and MTRH in Western Kenya.

Ndede I, Mining S.K, Were E.O, Ng’wena A.G.M, Emonyi W.I

1Department of Immunology, School of Medicine, Moi University, P. O. Box 4606 - 30100 Eldoret, Kenya.
2Dept. of Reproductive Health, School of Medicine, Moi University, P. O. Box 4606 - 30100 Eldoret, Kenya.
3Dept. of Medical Physiology, School of Medicine, Moi University, P. O. Box 4606 - 30100 Eldoret, Kenya.
4AMPATH Reference Laboratory, MTRH, P. O. Box 4606 - 30100 Eldoret, Kenya.

Corresponding Author: Isaac Ndede, Department of Immunology, School of Medicine, Moi University, P. O. Box 4606 - 30100 Eldoret, Kenya; Tel.: +254723247938 Email: iandede@yahoo.com

SUMMARY

Background: The basis of apparent resistance to HIV-1 infection by some HIV-1 exposed heterosexual couples is unclear. The nature and functional characteristics of their immune cells could offer insight.

Objective: To characterise immune profile in terms of CD8+, CD4+, CD56+/16+ and CD19+ cell number and Th1/Th2 cytokine expression in heterosexual HIV–1 discordant couples.

Methods: Thirty-three (33) untreated HIV-1 discordant (index with CD4 ≥ 250 cells/µL), concordant positive and negative control couples were recruited at MTRH/USAID-AMPATH partnership clinics in Western Kenya. Lymphocyte phenotypes, complete blood count, viral load (VL) and Th1/Th2 cytokine levels were determined and compared between the study groups.

Results: Differences were observed between: HIV-1 discordant index females and concordant positive females CD8+ 711.0 cells/ml vs 1070.8 cells/ml (p = 0.005); %CD8+ 43.0% vs 56.3%; (p = 0.017); %CD4+ 28.0% vs 18.3% (p = 0.02); CD56+/16+ 208.0 cells/ml vs 447.5 cells/ml (p = 0.011); discordant index males and concordant negative males CD4+ 700.7 cells/ml vs 1000.4 cells/ml (p = 0.003); CD8+ 554.7 cells/ml vs 1062.4 cells/ml (p = 0.002); CD56+/16+ 290.5 cells/ml vs 450.7 cells/ml (p = 0.027). Uninfected female partners had higher mean cells/µL and percentage CD19+ than index females. In discordant index, log10 viral load was negatively correlated with CD4+ (rho = -0.274) and CD56+/16+ (rho = -0.319). No Th1 to Th2 cytokine shift occurred in both index and partners of discordant couples.

Conclusion: Lymphocyte subsets counts and cytokine levels correlate with HIV-1 resistance among the studied heterosexual HIV-1 discordant couples. Studies using HIV-1 specific antigens and lymphocytes from similar cohorts are necessary to improve understanding the role of immune response in HIV-1 discordance.

Key words: HIV-1 discordant; partner; index


Introduction

Various studies have reported HIV exposed but uninfected individuals who appear to resist HIV-1 infection even after repeated exposure [1]. This phenomenon has been observed among people having unprotected sexual intercourse with HIV seropositive partners or prostitutes, intravenous drug users (IDU) with history of needle sharing, newborn infants of HIV positive mothers, recipients of blood and blood products contaminated with HIV, occupationally exposed health care workers, men having sex with men (MSM), and heterosexual discordant couples. These people often lack both serologic and virologic evidence for active infection. In 2001 Kaul et al. [2], reported a group of commercial sex workers (CSW) in Nairobi who remained free of HIV-1 infection for up to three years. Similar cohorts have been recorded in North America and elsewhere in the world.
The course of HIV exposure varies widely among individuals. A small proportion of exposed people (5% - 10%) appears to be resistant to HIV-1 or are long term non-progressors [3]. A study by the University of Washington in 2005, Phase III Randomised Placebo-Controlled Trial of HSV-2 Suppression to Prevent HIV-1 Transmission/infection between HIV-1 discordant Couples, estimated that in high HIV prevalent countries, as many as 18–31% of couples may be serodiscordant [4]. In Kenya, more than half of all new infections take place within the family and an estimated 7.5% of all married couples are HIV-1 discordant [5].

The precise nature and functional characteristics of T and other immune cells that can respond to the virus in HIV-1 exposed but uninfected individuals are unclear. Limited information is available on the properties of CD4+ T, CD8+ T, CD19+ B and CD56+/16+ NK cells in the protection against HIV infection and transmission. HIV-1 specific CTL cells have been shown to control viraemia and may play a role in protecting against overt HIV-1 infection in heterosexual partners of HIV infected people [6, 26]. However, some studies have reported low frequency and transient CTL in HIV exposed but uninfected individuals to provide effective protection in absence of persistent infection [7]. High concentrations of mucosal HIV-1 specific IgA have also been detected in HIV infection and exposure [25]. Mucosal epithelial chemokine (CCL28) modulate immune response at mucosal level by binding to CCR3 and CCR10 and recruiting IgA secreting plasma cells (IgA-ASC). Despite seemingly high prevalence of HIV-1 discordance among heterosexual Africans, most studies on HEPU individuals have been largely on well known high-risk groups such as commercial sex workers, intravenous drug users, homosexual men having sex with men (MSM) and health workers but not in heterosexual couple setting which is the predominant mode of HIV transmission is heterosexual intercourse in Kenya.

The present study characterised immune responses profile against HIV–1 in discordant couples to locally circulating strains of HIV-1 in terms of CD4+, CD8+, CD19+ and CD56+/16+ cell number and their representative Th1/Th2 cytokines (IL–2, IL–4, IL–10, and IFN–γ) in HIV-1 infected (index) and uninfected (partners) in married HIV-1 discordant relationships and compared the values with concordant HIV-1 negative and positive control couples.

Materials and Methods

Study area: The study was conducted at USAID – Academic Model for Prevention and Treatment of HIV/AIDS (AMPATH) Partnership’s clinics and Moi Teaching and Referral Hospital (MTRH) couples’ HIV voluntary counselling and testing (CHVCT) and voluntary counselling and testing (VCT) in Eldoret between June 2006 and July 2007. These institutions cater for clients from northern Rift Valley, Nyanza and Western provinces in Kenya.

Study population: The study cohort consisted of 33 married heterosexual pairs, of whom 11 were HIV-1 discordant, 11 concordant HIV-1 negative and 11 concordant HIV-1 positive couples. Trained HIV counsellors and interviewers assessed prospective participants for inclusion using set eligibility criteria. Laboratory staff carried out screening by HIV-1/2 rapid ELISA tests. To be eligible, couples had to be sexually active, in married relationship for ≥6 months, index participants with CD4 ≥250 cells/µL and must not have met national guidelines for antiretroviral therapy (ART). Sexual inactivity, use of protective sex barriers, current use of ART and pregnant subjects were excluded from the study. Only those of legal age, willing to participate and provided independent informed consent were enrolled. At all stages, every effort was made to protect the participant’s privacy and confidentiality. Institutional Research and Ethics Committee (IREC) of MTHR and Moi University approved the study.

Laboratory methods: Anti-HIV–1/2 antibody ELISA were done in parallel using Determine™ (Abbott, Tokyo, Japan) and Uni-Gold™ (Trinity Biotech, Ireland) to screen participants. Lymphocyte subsets were quantitated in whole blood (in EDTA) by a standardised method using a 4–colour Facscalibur™ (BD immunocytometry system BDIS, San Jose, CA) with CD45/CD3/CD4/CD8 and CD45/CD3/CD19/CD56/16 antibody panels. Acquired data were analysed by MultiSET™ software. Complete blood cell count (CBC) was determined using Beckmann™ coulter haematology analyser.

Peripheral blood mononuclear cells (PBMC) were separated on Ficoll–hypaque (Pharmacia, Uppsala, Sweden) by density gradient centrifugation at 500 x g for 20 minutes, and then washed in balanced phosphate saline. The separated PBMC’s were re suspended in
RPMI 1640 (Gibco BRL Grand Island, NY), containing 10% fetal calf serum, 100 µg/ml penicillin, 100 µg/ml streptomycin and 2 mMol L–glutamine/L. Finally, the cell concentration was adjusted to 1 x 106 cells/ml and preserved in 10% DMSO and frozen at -80 oC until use. Cryopreserved PBMC were thawed, allowed to rest overnight at 37°C in 5% CO2 in about 1 x106 cells/ml in 100 µL of complete medium before incubating in 96 well “U” plate previously coated with 50 µL of 10 µL/ml IFN-γ monoclonal antibody (mAb) for 18-24 hours at 4°C, with 1 µg phytohaemagglutinin (PHA; Sigma) to make a final concentration of 2 µg/ml to detect low levels of cytokines. Cell culture supernatants were then harvested frozen at -80 oC until use. Becton Dickinson (BD) Cytometric bead array (CBA) assay was used to detect and quantitate Th1/Th2 cytokines IL–2, IL–4, IL–10, and IFN–γ following manufacturer’s instructions. Acquired data were analysed using BD cytometric bead array (CBA) software.

Plasma HIV-1 RNA viral load (VL) were quantitated using standardized reverse transcriptase polymerase chain reaction (RT–PCR Amplicor HIV-1 monitor; test version 1.5 kit, Roche, Diagnostics, Branchburg, NJ, USA and COBAS TaqMan 48), each according to the manufacturer’s instructions. Detection limit was set at <40 HIV-1 RNA copies/ml. HIV viral loads were then transformed to log10 before statistical analyses. Differences between mean values were compared using Mann-Whitney and Wilcoxon rank sum test.

We assumed that resistance attributed to by CCR5 coreceptor mutation b y 32 base pairs deletion was non-existent in the studied population. Recruitment of participants relied on self-reported sexual activity, histories and symptoms. No other medical and/or laboratory examinations were done to evaluate the subjects’ general state of health. Some study subjects may have been within the window period of HIV infection at screening and recruitment. The impact of circumcision status of male partners on HIV-1 transmission was not assessed as part of eligibility evaluation. The protective nature of this practice only became apparent later on in the course of this study [8].

Results

Study population: The study population consisted of total of 33 couples aged between 18 – 60 years, of whom 11 were HIV-1 discordant, consisting of 5 (22.7%) male partners (mean age ±SD, 36±8 years), 6 (27.3%) male index (mean age ±SD 44±10 years), 6 (27.3%) female partners (mean age ±SD 38±8 years) and 5(22.7%) female index (mean age ±SD, 33±11 years).

Table 1. shows CD4/CD8 ratio, mean cells/µL and percent (%) of lymphocyte subsets

<table>
<thead>
<tr>
<th></th>
<th>Discordant (n =11)</th>
<th>Concordant (n = 11)</th>
<th>Concordant (n =11)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>HIV-1 Negative</td>
<td>HIV-1 Positive</td>
<td>HIV-1 Positive</td>
</tr>
<tr>
<td></td>
<td>Males Females</td>
<td>Males Females</td>
<td>Males Females</td>
</tr>
<tr>
<td>CD4/CD8</td>
<td>1.3 1.4</td>
<td>.8 .7</td>
<td>1.1 1.6</td>
</tr>
<tr>
<td>CD8(%)</td>
<td>554.7(30)</td>
<td>943.3(43)</td>
<td>711.0(43)</td>
</tr>
<tr>
<td></td>
<td>710.6(32)</td>
<td>1062.4(37)</td>
<td>925.4(53)</td>
</tr>
<tr>
<td>CD4(%)</td>
<td>700.7(38)</td>
<td>602.3(31)</td>
<td>922.9(42)</td>
</tr>
<tr>
<td></td>
<td>727.1(37)</td>
<td>496.6(28)</td>
<td>392.6(22)</td>
</tr>
<tr>
<td>CD5616(%)</td>
<td>290.5(13)</td>
<td>308.0(12)</td>
<td>450.7(14)</td>
</tr>
<tr>
<td></td>
<td>295.1(13)</td>
<td>493.0(19)</td>
<td>493.0(19)</td>
</tr>
<tr>
<td>CD19(%)</td>
<td>231.0(11)</td>
<td>192.9(9)</td>
<td>422.0(9)</td>
</tr>
<tr>
<td></td>
<td>289.8(14)</td>
<td>190.4(10)</td>
<td>351.5(9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>198.3(8)</td>
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<td>297.1(15)</td>
</tr>
</tbody>
</table>
Both the mean absolute cells/µL CD56+/16+ and CD19+ were higher in male partners compared to male index participants (290.5 cells/ml vs. 140.9 cells/ml; p = 0.005); 231.0 cells/ml vs. 192.9 cells/ml) respectively, but lower in concordant positive males (251.5 vs 198.3).

Between discordant female index and concordant HIV-1 positive females, differences were; CD8+ 711.0 cells/ml vs 1070.8 cells/ml; (p = 0.005), %CD8+ 43% vs 56%; (p = 0.017), %CD4+ 28% vs 18%; (p = 0.021), CD4+/CD8+ 0.7 vs 0.4; (p = 0.014), CD56+/16+ 208.0 vs 447.5; (p = 0.011). Between discordant male partners and concordant HIV-1 negative males, the differences were: CD4+ 700.7 cells/ml vs 1000. cells/ml 4; (p = 0.003), CD8+ 554.7 cells/ml vs 1062.4 cells/ml; (p = 0.002), CD56+/16+ 290.5 cells/ml vs 450.7 cells/ml; (p = 0.027). Higher mean absolute cells/µL CD4+, CD56+/16+ and CD19+ were also observed in concordant HIV-1 negative females compared to both discordant and concordant HIV-1 positive couples. The mean percentage CD19+ cells for discordant partner females were higher (14%) compared with 9% for discordant HIV-1 negative females. The ratios and percentage of lymphocyte subsets for discordant index were lower but the general profile for males and females resembled those of concordant negative control couples (Figure 1).

**Figure 1.** Mean %/ratio of lymphocyte subsets in study couples

A significant negative correlation (Spearman) was observed between CD4+/CD8+ ratios and HIV status between partners and index participants (p=0.038) but a significant positive correlation existed between HIV status and %CD8+ (p = 0.041) in HIV-1 discordant couples.

Human immunodeficiency virus plasma RNA levels were determined in eleven discordant index participants and equal number of concordant HIV-1 positive males and females participants. The log10 mean±SD of RNA copies/ml for concordant HIV-1 positive females were 3.87±1.4, males 4.03±1.4 and in discordant index females 3.04±2.1 and index males 4.05±0.7. Plasma RNA (log10 copies/ml) was negatively correlated with mean absolute CD4+ and CD56+/16+ counts (rho = -0.274, rho = -0.319) respectively in index discordant
participants, though not significantly. The mean absolute CD4+ counts were positively correlated with WBC (p = 0.023) in discordant index females and percentage CD56+/16+ were negatively correlated (p = 0.039) with white blood cells (WBC) in both male and female concordant HIV-1 negative participants. Table 2. shows mean ±SD cytokine levels (pg/ml) of discordant index, partners and concordant positive couples

<table>
<thead>
<tr>
<th></th>
<th>IL – 2</th>
<th>IFN – γ</th>
<th>IL – 4</th>
<th>IL – 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Partners</td>
<td>6.4± 1.2</td>
<td>13.2± 1.1</td>
<td>28.5± 6.0</td>
<td>194.8 ±0.1</td>
</tr>
<tr>
<td>Index</td>
<td>6.6± 1.2</td>
<td>13.0± 1.4</td>
<td>26.4± 7.1</td>
<td>194.1± 12.5</td>
</tr>
<tr>
<td>Concordant HIV +ve</td>
<td>4.6± .56</td>
<td>12.0± .28</td>
<td>20.5±0.7</td>
<td>181.5 ±0.7</td>
</tr>
</tbody>
</table>

The mean ±SD cytokine levels (pg/ml) of IL–2, IL–4, IL–10, and IFN–γ were 6.4±1.2, 28.5±6.0, 194.8±10.1, and 132.2±1.1 respectively for partners compared to index (IL–2 6.6±1.2, IL–4 26.4±7.1, IL–10 194.1±12.5 and IFN–γ 130.0±1.4). The ratios of IFN–γ/IL–10 in index was (0.618) and in partners (0.667), meaning there no Th1 to Th2 cytokine shift in both index and partners in the studied HIV–1 discordant couples. The CD4+/CD8+ ratios were negatively correlated (spearman) with IFN–γ among discordant partners (rho = -.316) and index (rho = -.607). Percentage of CD8+ cells was strongly positively correlated with IL–2 (rho = 0.800) in both index and partners, while negatively correlated among HIV concordant positive participants.

White blood cell (WBC) differential counts and RBC indices showed no statistical differences between discordant and concordant negative couples except mean absolute neutrophils (p = 0.042), atypical lymphocytes (p = 0.019), percentage (%) atypical lymphocyte (p = 0.000) and percentage (%) eosinophil (p = 0.025). The mean absolute monocytes counts were higher in HIV-1 infected participants compared to uninfected partners.

**Discussion**

Both the mean absolute lymphocyte and other haematological values for HIV-1 uninfected partners in discordant relationship and concordant HIV–1 negative participants were generally lower than published reference ranges from North America but similar to other studied African population in conformity with Tsegaye et al. [9]. The difference may be due to environmental, age, sex, ethnic origin, dietary pattern, seasonal rhythms, physical and psychological stresses, drugs and prevailing infections [7]. However, lower mean absolute CD4+ T cells/µL in index partners compared to uninfected partners in discordant couples, consistent with the fact that HIV-1 infection leads to a progressive and generalized haematological suppression [10].

Higher mean absolute CD4+ T cells/µL in index males than a median 395 cells/µL in concordant HIV positive males may be suggestive of the cell subset role in controlling HIV infection or transmission within HIV-1 discordant couples [11]. The differences in mean absolute CD8+ T cells/µL between index males and their uninfected counterparts in discordant relationship
is similar to those determined by Denny et al. [12], who demonstrated increased mean absolute CD3+CD8+ cells/µL among index partners compared to their HIV-1 negative partners. Increased mean absolute CD3+CD8+ cells/µL was accompanied by concomitant decrease in the amount of viral activities measured by HIV culture end point and quantitative plasma RNA. Other studies have since regularly demonstrated nef specific CD8+ CTL in heterosexual partners of HIV-1 infected individuals [13, 26]. Thus, the relatively higher CD8+ cell number in index compared to concordant HIV-1 positive participants observed in this study may be indicative of immune mediated suppression of replication and transmission of HIV-1 between index and partners in a discordant relationship. The fact that HIV infection elicits both cytotoxic and non-cytotoxic CD8+ T cell responses is documented and increased non-cytotoxic CD8+ subset which suppress HIV-1 have been found to be positively correlated and of good clinical prognosis in HIV-1 infected individuals [14, 15].

The higher CD4+/CD8+ ratios in partners than index and concordant HIV-1 positive participants can be interpreted to mean that both quality (function) and cell number (quantity) of CD4+ or CD8+ T cells subsets may be important in controlling infection and transmission of HIV-1 among the studied discordant couples. Percentage values and ratios are proportions and therefore less subjected to analytical fluctuation and may be more predictive of CD4+ and CD8+ lymphocyte subset values and thus more useful surrogates for determining immune response to infection of HIV-1 [16].

There was a significant lower mean absolute CD56+/16+ cells/µL in index females in discordant couples compared to HIV-1 positive females in concordant couples. Scott–Algara et al. [17] and Jiang et al. [18], also found lower mean absolute CD56+/16+ cells/µL in HIV-1 infected individuals compared to normal controls. A reduction in the number and quantity of NK cells may be accompanied by enhanced cytolytic and secretory activity of NK in HIV-1 infected individuals [19]. Immune activation associated with HIV-1 infection leads to increased apoptosis and reduction in NK cells number. Scott – Algara et al. [17] have demonstrated increased lysis of infected NK cells and production of IFN–γ, TNF–α and β cytokines in exposed but uninfected Vietnamese intravascular drug users (IDU) than seroconverters. Reduced NK cells/µL observed here, may due to their increased activity and prevention of HIV-1 infection among the discordant couples through Major Histocompatibility Complex unrestricted cytotoxic activities, secretion of immunoregulatory cytokines and antibody-dependent cell cytotoxicity (ADCC), before development of adaptive immune response.

The reason for the observed differences in mean absolute CD19+ cells/µL between HIV-1 negative females in discordant couples and HIV-1 negative females in concordant couples observed here, is unclear. Higher mean absolute CD19+ B cells/µL in female partners than index counterparts in discordant relationships probably imply a protective role by B cells in HIV-1 discordant female partners. Some studies have indicated that HEPU generate HIV-1 specific IgA in genital tracts and plasma [20, 25]. Some authors have reported high concentrations of mucosal HIV specific IgA in Saliva, cervico-vaginal secretions, breast milk in HIV infected and exposed and may characterise HIV exposed but uninfected individuals. It has also been proposed that relative resistance to HIV-1 infection may be mediated by alloimmune antibody responses directed against MHC molecules such as HLA DRB3, B5701, A2, and DR13 of incoming infected cells from partner [21]. In this study, plasma HIV RNA log10 copies/ml were negatively correlated with CD4+, CD56+/16+, but positively so with CD8+ (rho = 0.26) and CD19+ (rho = 0.257) in infected discordant participants. This implies underlying control of viral replication and transmission between discordant pairs.

The cytokines IFN–γ (Th1) and IL–10 (Th2) observed are in nearly equal proportions in both index and partners participants, meaning that there is no apparent Th1 to Th2 cytokines shift in HIV discordant couples. Th1 (IFN–γ) cytokines have antiviral activity and are also essential for inducing HIV-1 specific CD8+ cytotoxic T lymphocyte effector responses [22]. This observation is in agreement with studies carried out by Douglas et al. [23] who found no shift in Th1 to Th2 cytokine pattern between HIV–1 discordant couples. A positive correlation between %CD8 and IL–2 and IL–10 among discordant uninfected partners compared with a negative correlation among discordant positive couples further implicate immune response and their possible protection against HIV-1 infection among the discordant couples.
Thus, the apparent resistance to HIV-1 infection by HIV exposed but uninfected individuals may be due to their immune profiles and responses. Non-productive infections from one partner to the other or repeated exposures to the virus probably trigger CD4+, CD8+, CD19+, and CD56+/16+ immune cells, without overt infection like a natural vaccine in some of these discordant couples. A few early reports also indicated that HIV-2 positive prostitutes were partially protected via unknown mechanisms, possibly by immune responses [24].

Conclusions and Recommendations
In conclusion, lymphocytes CD4+, CD8+, CD19+ and CD56+/16 cell numbers and their cytokines can be the basis of resistance to HIV-1 transmission and/or infection among HIV-1 discordant couples. Chronic exposure at finite viral load may trigger HIV-1 specific and non-specific immune responses, which then suppress overt HIV-1 infection among discordant couples.

A larger sample size longitudinal study using HIV-specific antigens to stimulate cells from similar cohorts are necessary to improve our understanding of correlates of immune protection in an African context, using data presented here as baseline are needed. Genetic factors contributing resistance to HIV-1 infection by uninfected partners should also be characterised among this study cohort.

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