Journal of
Clinical & Cellular Immunology

Nakanjako et al., J Clin Cell Immunol 2014, 5:1
DOI: 10.4172/2155-9899.1000186

Research Article Open Access

CD4 T-cell Activation and Reduced Regulatory T-cell Populations are Associated with Early Development of Cataracts among HIV-infected Adults in Uganda

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Abstract

Background: Cataracts contribute 12% of visual loss among HIV-infected adults (HIV-positive) in Uganda. Immuno-pathogenesis of cataracts may differ among HIV-negative and HIV-positive individuals; thus the need for innovative therapeutic interventions for cataracts among HIV-positive adults. We compared the regulatory T-cell (Treg) dysfunction among HIV-positive-with-cataracts, HIV-negative-with-cataracts and respectively age-matched HIV-healthy-volunteers.

Methods: In a laboratory based case-control study, nested within a clinical/surgical community outreach camp, within the Rakai Health Sciences Program (RHSP) rural cohort, 50 adults with cataracts eligible for surgery were selected consecutively. Routine provider-initiated HIV testing was done for individuals with unknown HIV sero-status. Peripheral Blood Mononuclear Cells (PBMC) were collected from all HIV-positive adults with cataracts (cases) and HIV-negative adults with cataracts (comparative group) and age-matched HIV-negative and HIV-positive-adults-without-cataracts (comparative group). Treg were measured as $CD3^+CD4^+FoxP3^+CD25^{+bright}$ and immune activation as $CD3^+CD4^+CD38^+HALDR^+$ using a Facs Canto II flowcytometer. Mann Whitney test was used to compare expression among the four groups.

Results: Of 50 adults operated for cataracts, 24 (48%) were female, 25 (50%) were HIV-positive. HIV-positive individuals had cataracts earlier [median; Inter-quartile Range (IQR); 49 (44-53) years] than HIV-negative [70 (IQR 59-75) years]; p=0.0005. Treg were lower among individuals with cataracts irrespective of HIV status; p=0.001; but comparable among younger HIV-positive and elderly HIV-negative with cataracts; p=0.301. Immune activation levels were comparable among HIV-positive and HIV-negative individuals with cataracts. However, HIV-positive individuals with cataracts expressed higher levels of immune activation than HIV-positive individuals without cataracts; p=0.012 and HIV-negative individuals with cataracts expressed higher levels of immune activation that HIV-negative-without-cataracts; p<0.0001.

Conclusion: CD4 T-cell activation and reduced regulatory T-cell populations were associated with cataracts among adults aging with HIV. We recommend studies on clinical relevance of immune modulation in the prevention of early development of cataracts among adults aging with HIV in Africa.

Keywords: HIV/AIDS; Regulatory T-cells; Immune activation; Aging; Cataracts; Blinding; Cataract surgery; Aging with HIV; Sub-Saharan Africa

Background

Cataract is the world's largest single cause of blindness, accounting for 50% to 80% of blindness in developing countries [1]. Cataracts range from minor lens opacities not interfering with vision, to total opacity causing blindness [2]. In a survey among all residents aged 40 years and older in Kongwa, Tanzania, the prevalence of age-related blinding cataracts was 1.3% [3]. However, among HIV-infected individuals in Uganda, cataracts contributed to up to 12% of visual loss [4-5]. A combination of demographic, systemic, environmental and nutritional factors have been associated with cataract development [6], in addition to common biochemical pathways through which these factors may interact [7]. The HIV-1 virus has also been attributed to nonspecific intraocular inflammation which could serve as a contributing factor in the pathogenesis of cataracts [8]. Most studies to understand cataract development among HIV-infected adults have described various aetiological pathogens including CMV retinitis, herpes simplex and toxoplasmosis among the immune suppressed individuals [4-5,9]. Similarly, many therapeutic interventions have concentrated on treatment options to eliminate or prevent the implicated pathogens [10]. There is limited data on specific immunological pathways that increase risk of cataracts during HIV infection and subsequently little has been reported about immune modulation in the management of cataracts.

With increasing populations of HIV-infected adults receiving antiretroviral therapy, aging with HIV/AIDS and associated co-

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Received October 28, 2013; **Accepted** January 07, 2014; **Published** January 17, 2014

Citation: Nakanjako D, Otiti-Sengeri J, Ssewanyana I, Nabatanzi R, Bayigga L, et al. (2014) CD4 T-cell Activation and Reduced Regulatory T-cell Populations are Associated with Early Development of Cataracts among HIV-infected Adults in Uganda. J Clin Cell Immunol 5: 186. doi:10.4172/2155-9899.1000186

morbidities are emerging challenges in sub-Saharan Africa [11]. In Uganda, over 2 million adults are living with HIV at an HIV prevalence of 7.3%, with peaks at age 35-39 years for women (12%) and at age 40-44 years for men (11%) [12]. Human aging exhibits significant changes in innate and adaptive immune responses [13]. As described in the White House Meeting on HIV and Aging [14], there is increasing emphasis on aging with HIV due to the rapidly increasing numbers of HIV-infected individuals above 50 years [14,15]. Evidence suggests that chronic HIV-infection accelerates the immune changes that otherwise occur during normal aging. The synergistic effects of aging and HIV precipitate immune decline and associated complex pathologies [13]. Thus the need to understand mechanisms and triggers of diseases associated with aging, such as cataracts, among the adults aging with HIV.

This paper describes the immune dysfunction associated with blinding cataracts among individuals aging with HIV/AIDS. We compared expression of markers of immune activation among HIV-infected and HIV-negative individuals that were operated for blinding cataracts in a Ugandan community. To understand the role of regulatory T-cells (Treg) in the development of cataracts, we compared Treg populations among HIV-infected and HIV-negative individuals with cataracts and their age-matched HIV-infected and HIV-negative counterparts without cataracts, respectively. Results from this study give insight on the similar immune dysfunction associated with cataracts in the elderly HIV-negative individuals and the relatively younger HIV-infected individuals.

Methods

Study design and setting

This was a laboratory based case-control study, nested within a clinical/surgical community outreach camp where adults with cataracts, within the Rakai Health Sciences Program (RHSP) rural HIV treatment cohort, are routinely operated by a visiting ophthalmologist. RHSP is a collaborative program between the Uganda Virus Research Institute (UVRI), scientists at Makerere and Johns Hopkins Universities (MU and JHU) and intramural National Institutes of Health (NIH) via an International Center of Excellence in Research (ICER) award. RHSP in rural south-western Uganda represents one of the largest and longest-running population-based research programs in sub-Saharan Africa. HIV–positive controls without cataracts were selected from a rural HIV-treatment cohort in mid-western Uganda (Kiboga), supported through an outreach program of the Infectious Diseases Institute (IDI). All HIV-positive individuals were receiving first-line Highly Active Antiretroviral therapy (HAART) for at least six months (initiated at CD4<250 cells/UL according to the 2012 national guidelines for HAART initiation) at RHSP and IDI rural HIV treatment programs respectively. Similarly, HIV-negative controls without cataracts were selected from the Kiboga HIV-negative cohort that was initiated by IDI within the Kiboga rural community.

Study participants

Cases were consecutive HIV-infected adults>18 years of age, that presented at RHSP with visually significant cataracts (blinding cataracts) and were eligible for cataract surgery during the surgical outreach camps in 2012. HIV-negative adults>18 years of age presenting with blinding cataracts and eligible for cataract surgery at RHSP were consecutively included as controls. We excluded patients with traumatic cataracts and patients that were severely ill or showed any other contraindications for cataract surgery, such as suppurative conjunctival infections. Routine provider-initiated HIV testing was done for the patients who were unaware of their HIV sero-status according to the national Ministry of Health guidelines for routine HIV

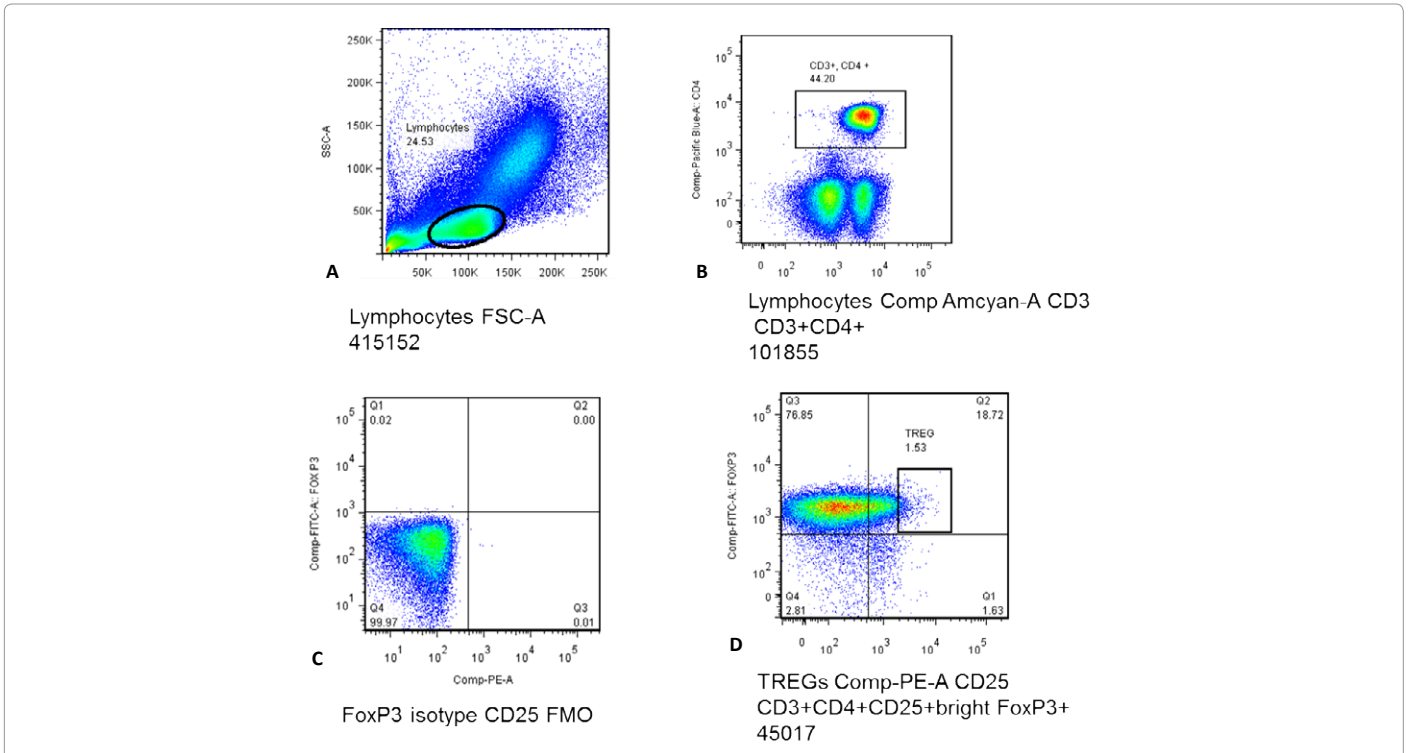

Figure 1: Gating strategy of regulatory T-cells ($CD3^{+}CD4^{+}CD25^{Bright}$ $FoxP3^{+}$). (A) Shows the live lymphocyte gate, (B) Shows the CD3+CD4+lymphocytes, (C) Shows FoxP3 isotype and the Fluorescence minus one (FMO) for CD25, and (D) Shows the Treg ($CD3+CD4+CD25^{Bright}$ FoxP3+ T cells).

testing within health care settings. HIV-negative comparative group included 57 age-matched HIV-negative adults that were consecutively selected from the patients' register of HAART-treated HIV-infected individuals in the Kiboga rural HIV-treatment cohort. Similarly, 21 age-matched HIV-negative adults were consecutively selected from the Kiboga HIV-negative patient register. All participants gave written informed consent to participate in the study. The study was approved by the Uganda National Council for Science and Technology and it was conducted according to the principles of the declaration of Helsinki and the international guidelines of biomedical research involving human subjects.

Laboratory procedures

Peripheral blood mononuclear cells (PBMC) were collected using the Cell Preparation Tube with Sodium Heparin protocol (BD Vacutainer® CPT™). PBMC were washed and re-suspended in RPMI-1640 medium (Sigma-Aldrich, R0883) containing heat inactivated fetal bovine serum (FBS), Sigma-Aldrich, F2442. PBMC were frozen and stored in FBS with 10% dimethyl sulfoxide (DMSO), in liquid nitrogen until assay time.

Cell surface and intracellular staining

PBMC were thawed and batch analyzed using the Becton Dickson Facs Canto II flow cytometer (BD Biosciences) at the Infectious Diseases Institute and housed in the Immunology laboratory at Makerere University College of Health Sciences. Cell surface and intracellular staining were performed to measure expression of markers of immune activation and regulatory T-cells using antibodies CD3, CD4, CD38, HLADR, CD25 and FoxP3 (BD Biosciences) and samples were acquired using a Facs Canto II flow cytometer. In general, at least 300,000 events were collected. Gating was standardized and set using fluorescence minus one controls (FMOs) for HLADR, CD38, CD25 and FoxP3 isotype (Figure 1). Regulatory T-cells were defined as $CD3^{+}CD4^{+}FoxP3^{+}CD25^{+bright}$ cells, and immune activation was defined as $CD3^{+}CD4^{+}CD38^{+}HLA\ DR^{+}$ cells.

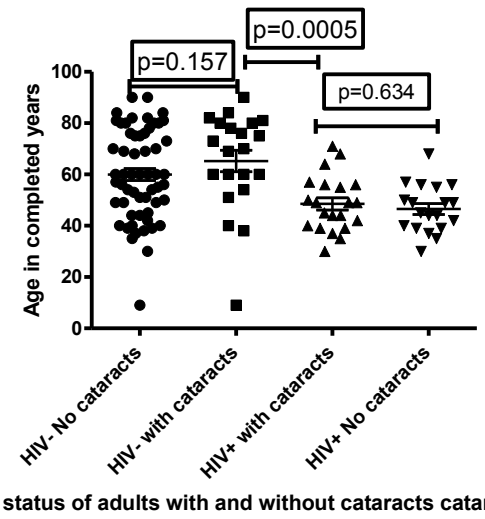

Figure 2a: Age of HIV-infected and HIV-negative adults with cataracts and their HIV-negative counterparts. Data is shown for 25 HIV+ adults with cataracts and 25 HIV-negative adults with cataracts. HIV+ adults with cataracts were age-matched with 21 HIV+ adults without cataracts; p=0.634 and HIV-adults with cataracts were age-matched with 21 HIV-without cataracts, p=0.157 for comparison.

Data analysis

Flowcytometry data was analyzed using Flowjo software for PC, Version X, 64 bit (Tree Star Inc). Graphs and comparisons were done using Graphpad prism version 5.0. Comparisons of medians of parameters between HIV-negative and HIV-positive individuals with cataracts and their age-matched HIV-negative adults without cataracts were made using the Mann Whitney test for non-parametric tests. Associations with p-value ≤ 0.05 were considered statistically significant.

Results

Demographic characteristics of study participants

Overall, 50 adults were operated for blinding cataracts; of whom 25 (50%) were HIV-positive [median age; Inter-quartile Range (IQR); 49 (44-53) years] within the RHSP HIV treatment program, with the other half as HIV-negative adults [median age; 70 (IQR 59-75) years] from Rakai community in south-western Uganda. In addition, we investigated 57 age-matched HIV-negative adults without cataracts; 37% female and 21 age-matched HIV-positive adults without cataracts from the IDI HIV treatment cohort; 48% female, in mid-western Uganda. All HIV-positive adults had received NNTI-based first-line HAART for at least 6 months. HIV-infected adults developed cataracts at a younger age [(IQR); 49 (44-53)] than HIV-negative individuals [70 (IQR 59-75) years]; p=0.0005 (Figure 2a).

Regulatory T-cell dysfunction and blinding cataracts

PBMC were analysed for 23 HIV-positive and 21 HIV-negative with cataracts; excluding 2 HIV-positive and 3 HIV-negative that had less than 1 million PBMC collected. Ten HIV-negative age-matched and 16 HIV-positive age-matched adults without cataracts were consecutively selected and analysed as comparative groups. Treg populations ($CD3^{+}CD4^{+}CD25^{Bright+}FoxP3^{+}$ cells) were comparable among HIV-negative and HIV-positive adults with cataracts; p=0.301. However, Treg were significantly lower among HIV-positive individuals with cataracts relative to HIV-positive without cataracts (P=0.0028); despite the higher Treg among HIV-positive without cataracts relative to HIV-negative cataracts, p-0.025 (Figure 2b).

Immune activation among HIV-Infected adults with cataracts

Immune activation, measured as co-expression of CD38 and HLADR by CD^{+} T-lymphocytes, was measured among a representative sample of 37 HIV-negative without cataracts, 21 HIV^{-} negative with cataracts, 23 HIV-positive with cataracts, and 22 HIV^{+} without cataracts. In general, immune activation ($CD3^{+}CD4^{+}HLADR^{+}CD38^{+}$) was higher among HIV-positive individuals with cataracts than age-matched HIV-positive individuals without cataracts (p=0.012). Similarly, immune activation was higher among HIV-negative individuals with cataracts than HIV-negative individuals without cataracts; p<0.0001. Furthermore, immune activation was higher among HIV-positive individuals with cataracts than HIV-negative individuals with cataracts; p=0.007. Specifically, immune activation was higher among the elderly HIV-negative individuals with cataracts than age-matched HIV-negative individuals without cataracts; p=0.0001 (Figure 3).

Discussion

Regulatory T-cell dysfunction and blinding cataracts

We found reduced regulatory T-cells among individuals with cataracts (HIV-negative and HIV-infected), when compared with age-matched HIV-negative and HIV-positive individuals without cataracts.

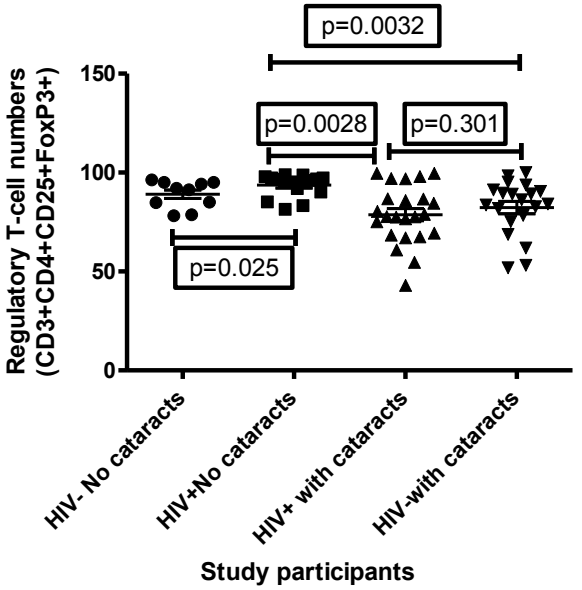

Figure 2b: Regulatory T-cells among individuals with cataracts and their age-matched HIV-negative counterparts without cataracts. This Figure shows regulatory T-cell numbers ($CD3^{+}CD4^{+}CD25^{Bright+}FoxP3^{+}$) among 10 HIV-negative without cataracts, 16 HIV-positive without cataracts, 23 HIV-positive with cataracts and 21 HIV-negative with cataracts respectively.

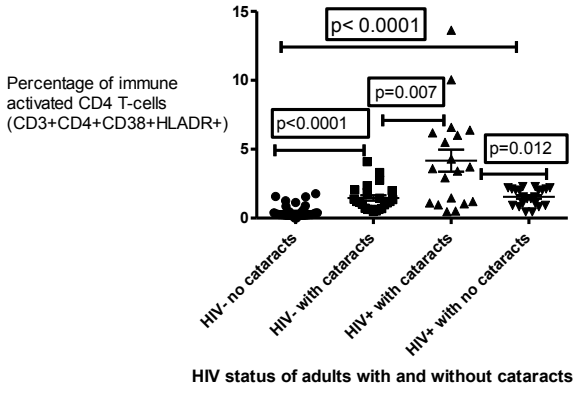

Figure 3: T-cell immune activation among individuals with cataracts when compared with their age-matched HIV-negative counterparts. Percentages of activated CD4 T-cells among 37 HIV-negative without cataracts, 21 HIV-negative with cataracts, 23 HIV-positive with cataracts, and 22 HIV+ without cataracts are shown respectively.

Indeed, our data is consistent with previous findings of expansion of Treg during HIV infection as shown by the significantly higher levels of Treg among HIV-positive without cataracts relative to HIV-negative without cataracts [16,17]. Despite persistent expansion of Treg among HAART-treated HIV-infected adults in this study, Treg populations were reduced to comparable levels among relatively younger-HIV-infected-adults-with-cataracts and elderly-HIV-negative-adults-with-cataracts. To our knowledge, this is the first paper to describe regulatory T-cell dysfunction among HIV-infected adults with cataracts. The authors postulate that persistent immune activation and exhaustion previously described after long-term HAART [18,19] could be associated with accelerated aging and early occurrence of non-AIDS diseases of aging including cataracts among adults aging with HIV. Given the regulatory T-cell dysfunction demonstrated by our results, there is need to understand the role of immune modulation interventions as strategies to delay development of cataracts. More so among 'at-risk' HIV-infected adults aging with HIV where the prevalence of blinding cataracts is 12% [5], relative to 1.3% among the general population [3].

Majority of previous studies on cataracts among HIV-infected adults, in Africa and USA, described aetiological pathogens in the setting of immune suppression such as Cytomegalovirus retinitis, toxoplasmosis and HIV vasculopathy [4,10]. In fact many interventions revolved around different therapeutic options to eliminate or reduce incidence of the offending pathogens such as choice of drug, time at which drug is given and duration of therapy for CMV retinitis in the immune compromised patient [10]. It is important to note that individuals that underwent cataract surgery during this study did not have a prior history of symptomatic ocular infections. Hence the need for more comprehensive assessment of subclinical ocular infections among HIV-infected individuals in HIV treatment programs to identify preventable causes of cataracts.

Aging and cataracts among HIV-infected adults

Human aging is associated with changes in innate and adaptive immune responses at phenotypic, functional and molecular levels [13]. We demonstrated that HIV-infected adults developed blinding cataracts at a significantly younger age than HIV-negative adults. Our data implies that the immune system aging associated with cataracts in the elderly occurs about two decades earlier in the HIV-infected individuals. This finding is consistent with previous reports that HIV infection affects the aging and development of illnesses typically associated with advanced age and chronic inflammation [13]. However, there is yet no consensus on whether HIV-infected adults develop HIV-associated cataracts because chronic HIV disease accelerates aging or because HIV infection is an additive risk factor just like age, smoking and use of glucocorticoids. Immune dysfunction and inflammation concomitant with viral infections and multi-morbidity have been associated with premature functional decline, susceptibility to additional illnesses and mortality [14,20]. Similarly, emerging data suggests that immunologic aging processes among HIV-infected individuals include accumulation of terminal stage CD8 T-cells and limited T-cell proliferation potential, which have been associated with clinical problems and frailty among the individuals aging with HIV [13].

Immune activation and immune exhaustion among HIV-Infected adults with cataracts

Overall, we found that immune activation was higher among individuals with cataracts relative to age-matched HIV-negative individuals without cataracts. Among the individuals with cataracts, immune activation was higher in the HIV-positive than HIV-negative subgroups. The trend was similar with cell exhaustion as shown by expression of programmed death-1 cell surface marker (data not shown). These data imply that immune activation was associated with blinding cataracts in both the younger HIV-infected and elderly HIV-negative individuals. Given that immune activation was even higher among the relatively younger HIV-infected individuals with cataracts and the previously described persistence of immune activation during chronic HIV disease [17,19,21], we hypothesise that chronic immune activation and inflammation during HIV-infection drive immune senescence and contribute to the unifying mechanisms leading to greater incidence of blinding cataracts among individuals aging with HIV disease [13,21]. This, together with the social isolation of older HIV-infected adults and care delivery issues, impair the quality of life

of individuals aging with HIV infection [14]. Therefore, there is need to integrate clinical measures with emerging biomarkers to inform innovative interventions to delay or treat clinical syndromes that complicate aging with HIV. In view of our findings, we hypothesise that HIV treatment interventions to reduce immune activation such as HAART and anti-immune activation agents could potentially modify the risk of cataracts and other age-related HIV-associated non-AIDS conditions (HANA). Although not evaluated in this study, it is important to note that HAART could contribute to the T-cell immune dysfunction among adults aging with HIV [22,23]. With increasing numbers of individuals receiving HAART, carefully designed studies are required to understand the effect of HAART on HANA including cataracts, in sub-Saharan Africa.

Limitations

We did not have elderly HIV-infected adults without cataracts to compare with elderly HIV-negative adults with cataracts. This was due to the fact that majority of HIV-infected adults in Uganda are below 70 years [12]; which was the mean age for the HIV-negative individuals with cataracts. However, we were able to get age-matched HIV-negative adults that were consecutively selected from the HIV-negative rural cohort in mid-western Uganda. Similarly, because of the multifactorial aetiology of cataracts ranging from low birth weight at first birth day to nutrition, chronic inflammation, HIV infection and associated ocular opportunistic infections, steroid therapy as well as co-morbidities such as diabetes mellitus [6], further studies are required to determine the cause of cataracts among adults aging with HIV in addition to understanding the triggers of immune activation in the elderly adults with cataracts. However, we raise important issues about the overall immune dysfunction that might inform interventional immune-modulation studies to delay non-AIDS diseases of aging among high-risk populations of HIV-infected adults above 40 years. With more individuals receiving HAART in Uganda, the population of aging HIV-infected individuals is likely to increase and direct comparisons between the aging HIV-infected and HIV-uninfected will become more feasible in resource limited settings [13].

Conclusion

CD4 T-cell activation and reduced regulatory T-cell populations were associated with cataracts among adults aging with HIV. We recommend studies on clinical relevance of immune modulation in the prevention of early development of cataracts among adults aging with HIV infection in Africa.

Acknowledgement

The authors acknowledge the study participants from Rakai Health Science Project and the Infectious Diseases Institute (IDI) rural HIV treatment cohorts. In addition, the authors acknowledge the Gladstone Institute of Vaccinology and Immunology (GIVI) at UCSF and the Vaccine and Gene Therapy Institute (VGTI), Florida where Damalie Nakanjako received training in multicolour flow cytometry during her post-doctoral training under the Wellcome Trust-Funded Makerere University Infection and Immunity program at Makerere University and Uganda Virus Research Institute, Grant # 084344.

Authors' Contributions

DN and JO made substantial contribution to the conception, design, data collection, analysis and drafting of the manuscript. IS, RN, LB and SK contributed to the immune assays, data analysis and interpretation. MJ, YCM, and AK contributed to the conception, design, data interpretation and revision of the manuscript. BC and HMK made substantial contribution to the conception, design, immune assays, data analysis, interpretation and revision of the manuscript. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare no conflict of interest.

Funding

This study was funded by the Belgian Government grant through the Belgium Technical Cooperation's "Own Initiatives 2007" Vlamse Interuniversitaire Raad (VLIR) Interuniversity Cooperation award to Institute of Tropical Medicine (ITM), University of Antwerp/Makerere University titled "Capacity Building in Clinical Research in Infectious Diseases" (VLIR Reference ZEIN2007PR335) and a Wellcome Trust Uganda post-doctoral Fellowship in Infection and Immunity held by Damalie Nakanjako, funded by a Wellcome Trust Strategic Award, grant number 084344.

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