

HIV infection induces age-related changes to monocytes and innate immune activation in young males which persist despite cART

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Objectives: To compare the impact of HIV infection and healthy ageing on monocyte phenotype and function and determine whether age-related changes induced by HIV are reversed in antiretroviral treated individuals.

Design: A cross sectional study of monocyte ageing markers in viremic and virologically suppressed HIV+ males aged ≤ 45 years and age-matched and elderly (≥ 65 years) HIV-uninfected individuals.

Methods: Age-related changes to monocyte phenotype and function were measured in whole blood assays *ex vivo* on both CD14⁺⁺CD16⁻ (CD14⁺) and CD14^{variable}CD16⁺(CD16⁺) subsets. Plasma markers relevant to innate immune activation were measured by ELISA.

Results: Monocytes from young viremic HIV+ males resemble those from elderly controls and show increased expression of CD11b ($p < 0.0001$ on CD14⁺ and CD16⁺ subsets), and decreased expression of CD62L and CD115 ($p = 0.04$ and 0.001 respectively on CD14⁺ monocytes) when compared to young uninfected controls. These changes were also present in young virologically suppressed HIV+ males. Innate immune activation markers neopterin, sCD163 and CXCL10 were elevated in both young viremic ($p < 0.0001$ for all) and virologically suppressed ($p = 0.0005$, 0.003 and 0.002 respectively) HIV+ males with levels in suppressed individuals resembling those observed in elderly controls. Like the elderly, CD14⁺ monocytes from young HIV+ males exhibited impaired phagocytic function ($p = 0.007$) and telomere shortening ($p = 0.03$) as compared to young uninfected controls.

Conclusions: HIV infection induces changes to monocyte phenotype and function in young HIV+ males that mimic those observed in elderly uninfected individuals, suggesting HIV may accelerate age-related changes to monocytes. Importantly, these defects persist in virologically suppressed HIV+ individuals.

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Introduction

The increased life-expectancy of HIV+ individuals due to combination antiretroviral therapy (cART) has revealed an increased incidence of non-AIDS comorbidities more commonly seen in the elderly, such as cardiovascular disease [1], frailty [2], bone and renal disease [3] and neurocognitive decline [4]. While the pathogenesis of age-related diseases is complex and multifactorial, these conditions are associated with an ageing and dysregulated immune system. The increased prevalence of these diseases and their premature onset in HIV+ individuals suggest HIV may induce premature immune ageing.

Immune ageing results from a lifetime of pathogen stimulation and is characterized by an increase in senescent CD8⁺CD28⁻T cells [5] and a state of chronic inflammation which drives age-related immune dysfunction and disease [6,7]. Elevated plasma levels of inflammatory markers (e.g. IL-6) predict age-related diseases including cardiovascular disease, frailty and mortality [8–12]. HIV+ individuals share many immunological characteristics with the elderly including chronic inflammation (reviewed in [13]), an increased proportion of senescent T cells with shortened telomeres [14,15] and increased risk of age-related disease. Similar factors may drive chronic immune activation and inflammation in HIV+ individuals and the elderly and include a) increased pathogen burden due to impaired immunity, b) chronic viral replication by viruses such as cytomegalovirus, a well recognized driver of ageing [16,17] and HIV and c) microbial translocation of bacterial products across damaged mucosal surfaces e.g. the gut [18,19].

In untreated HIV+ individuals, the level of T cell activation is the strongest predictor of mortality [20], but the predictive value of T cell activation markers in the post-cART era is unclear. T cell activation in HIV+ individuals is primarily driven by viral replication, which is significantly suppressed by cART, although residual activation persists [21]. In contrast, cART does not normalize levels of innate immune stimulants such as LPS [22], and innate immune activation persists in cART-treated individuals [22,23]. A recent study of HIV+ individuals (the majority being virologically suppressed, VS) found levels of the monocyte/macrophage activation marker soluble CD14 (sCD14) independently predict all cause mortality [24]. In cART treated subjects, sCD14 levels correlate with inflammatory markers such as IL-6, hsCRP and d-dimer [24] that predict cardiovascular disease while elevated sCD163 levels are associated with increased formation of non-calcified plaques [25]. Thus, the persistence of innate immune stimuli in VS individuals may contribute to HIV-related morbidity and mortality in the post-cART era.

LPS levels remain elevated in cART-treated individuals with a decay half life of up to 13 years [22], yet the impact of this on the phenotype and function of innate immune cells remains unknown. Monocytes/macrophages are the major responders to LPS due to their high expression of the LPS-receptor TLR4 and its related signalling molecule CD14 [26] and are primary producers of pro-inflammatory cytokines like TNF and IL-6. Monocytes have specific roles in the pathogenesis of age-related diseases; e.g. in foam cell formation during the development of atherosclerotic plaques [27] and in HIV-associated dementia [28]. Given that the stimuli to which monocytes respond remain elevated in cART-treated individuals, monocyte activation may be an independent predictor of age-related co-morbidities in VS HIV+ individuals.

We sought to determine whether HIV infection prematurely induces age-related changes to monocytes by directly comparing the phenotype and function of monocytes from young HIV+ individuals to both age-matched and elderly HIV-uninfected controls. Our findings confirm that HIV infection is associated with a premature induction of age-related changes to monocytes and support the hypothesis that HIV may accelerate immune ageing and thus hasten the development of age-related diseases.

Methods

Participant recruitment and blood separation

Viremic (median viral load 41,600 RNA copies/ml, range 80–>100,000; median CD4 T cells 434/μL, range 11–1092) and VS (viral load <50 RNA copies/ml, median CD4 T cells 695/μL, range 209–1199, median time of viral suppression 2.8 years, range 0.7–7.6 years) HIV+ individuals ≤45 years were recruited from the Infectious Diseases Unit at The Alfred hospital, Melbourne. Young (≤45 years) and aged (≥65 years) HIV- controls were recruited from the community. Ethical approval was obtained from The Alfred hospital Research and Ethics Committee. Male participants were exclusively recruited because HIV+ individuals in Australia are predominantly male. Exclusion criteria included current use of anti-inflammatory medication and recent (≤3 weeks) vaccination or self-reported illness/injury. Plasma separation and peripheral blood mononuclear cell (PBMC) preparation (via Ficoll gradient centrifugation) was performed within 2 hours of blood collection.

Monocyte phenotyping

Blood collected into EDTA anticoagulant was mixed with a 20x volume of 1x FACS lysing solution (BD Biosciences) to lyse erythrocytes (shown not to significantly alter expression of relevant surface markers),

incubated on ice for 10 min, then washed twice with FACS wash (1% heat-inactivated cosmic calf serum, 2 mM EDTA in calcium- and magnesium-free phosphate-buffered saline (Invitrogen)). Cells were stained on ice for 30 minutes using pre-titrated volumes of the following antibodies: CD14-APC, CD16-PE.Cy7, CD38-PE, HLA-DR-FITC, CD11b-PE, CD62L-FITC (BD Biosciences), TLR4-FITC (R&D Systems) and CD115-PE (eBiosciences), or appropriate isotype control antibodies. Cells were washed once in FACS wash and fixed in 1% formaldehyde. Samples were analysed on a dual-laser BD FACSCalibur flow cytometer.

Assessment of monocyte phagocytosis

Phagocytic capacity of monocytes was determined using heat-killed *E.coli* labelled with the pH-dependent dye pHRODO (Invitrogen) as per manufacturer's instructions. 2×10^8 labelled *E.coli* were added to 100 μ l of whole blood collected into heparin anticoagulant and incubated either at 37°C or on ice for 10 minutes. Red blood cells were lysed and monocytes labelled with CD14-APC and analysed as described above.

Analysis of telomere length

Telomere length in monocyte subsets was determined via immunophenotyping and FISH-Flow using modification of a previously described protocol [29]. PBMCs were labelled with anti-CD14-Qdot 800, anti-CD3-Alexa-Fluor 405 (both from Invitrogen) and anti-CD16-AlexaFluor 647 (Biolegend) and cross-linked with 4 mM bis(sulfosuccinimidyl)suberate. Telomeres were stained with the FITC FISH-Flow telomere labelling kit (Dako) as per manufacturer's instructions using a 7AAD DNA stain (0.1 μ g/ml). Samples were analysed on a LSR II flow cytometer and relative telomere length of monocyte subsets determined as a percentage of the internal control cell line 1301 [30].

Measurement of soluble markers of innate immune activation

Plasma protein and endotoxin measurements were performed using frozen EDTA plasma (subjected to only one freeze-thaw), that was clarified via centrifugation at 10,000 \times g for 10 minutes prior to analysis. LPS levels were determined in plasma diluted 1:10 and heat inactivated (80°C for 10 min) using the chromogenic Lonza LAL kit (Cat # 50–647U). Commercial ELISA kits were used to determine levels of sCD163 (IQ products, Cat. # IQP-383), neopterin (Screening EIA, Brahms, Cat. # 99R.096), sCD14, CXCL10/IP-10 and M-CSF (Cat. #DC140, DIP100 and DMC00B respectively, all from Quantikine, R&D Systems) as per manufacturer's instructions.

Data and statistical analysis

Analysis of flow cytometric data was performed using GateLogic software (Inivai). Graphing and statistical analysis was performed using GraphPad Prism version

5.0. Mann-Whitney U test was used to detect significant differences between sample groups (for non-parametric data) and significant correlations detected via Spearman's correlation analysis.

Results

Monocyte subsets

Peripheral blood monocytes were gated as CD14^{variable}CD16⁺ (referred to as CD16⁺) and CD14⁺⁺CD16⁻ (referred to as CD14⁺) subsets (Fig. 1a). There was a significant increase in the proportion of CD16⁺ monocytes in elderly individuals ($p = 0.003$, Fig. 1b). An expansion of this population was also observed in young viremic ($p = 0.003$) but not in VS ($p = 0.47$) HIV+ males (Fig. 1b). In addition to the expansion of total CD16⁺ monocytes, the CD14⁺ monocyte subset (typically negative for CD16) in both viremic and VS HIV+ males exhibited an increased expression of CD16 (exemplified in Fig. 1c; $p = 0.001$ for both, Fig. 1d).

Changes to monocyte phenotype induced by age and HIV

A panel of monocyte phenotypic markers that reflect immune activation and monocyte function was initially screened using blood from healthy young and elderly individuals to identify biomarkers of monocyte ageing (see Supplementary data). We used *ex vivo* whole blood analyses in this study, since we and others have shown that PBMC preparation can alter surface expression of certain monocyte receptors [31,32]. Monocytes from elderly individuals had significantly altered expression of the adhesion molecules CD62L (CD14⁺ monocytes, $p = 0.02$) and CD11b (CD16⁺ and CD14⁺ monocytes, $p = 0.002$ and 0.0005, respectively), the LPS-receptor TLR4 (CD16⁺ monocytes, $p = 0.007$) and the M-CSF receptor CD115 (CD14⁺ monocytes, $p = 0.03$) as compared to young men. Expression of HLA-DR and CD38 was high on all monocytes, but was not significantly altered by age in our cohort (data not shown) thus was not further analysed.

Age-related changes to monocyte phenotype were then analysed in young HIV+ individuals. The same pattern of phenotypic change was found in young viremic HIV+ males as in the elderly, but the magnitude of these changes was greater. Compared to young controls, monocytes from viremic HIV+ subjects had significantly decreased expression of CD62L (CD14⁺ monocytes, $p = 0.04$, Fig. 2a), decreased expression of CD115 (CD14⁺ monocytes, $p = 0.001$, Fig. 2b) and increased expression of CD11b ($p = <0.0001$ for both monocyte subsets, Fig. 2c and d). Surface expression of CD11b on monocytes from young viremic HIV+ subjects was significantly higher than seen in the elderly ($p = 0.003$

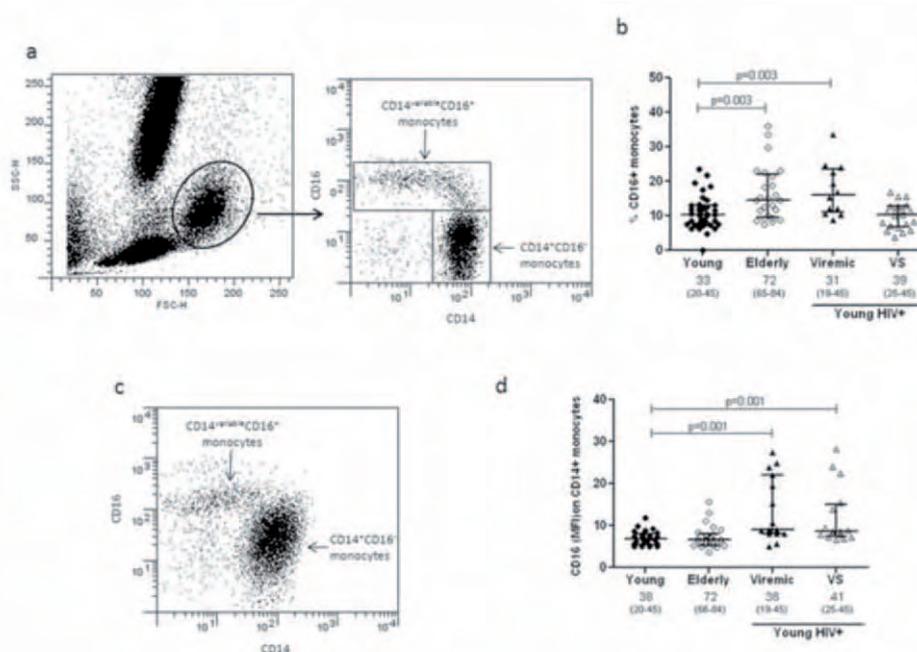


Fig. 1. Proportions of monocyte subsets and CD16 expression levels. a) Peripheral blood monocytes were gated initially via forward and side scatter then separated into subsets based on expression of CD14 and CD16. Monocyte subsets were analysed as either CD14^{variable}CD16⁺ (CD16⁺) or CD14⁺CD16⁻ (CD14⁺). b) The proportion of monocytes expressing CD16 calculated as [CD16⁺ monocytes]/[CD16⁺ + CD14⁺ monocytes] in young healthy controls (closed circles), elderly healthy controls (open circles), young viremic HIV+ (closed triangles) and young cART-treated virologically suppressed HIV+ males (open triangles). Median ages and ranges for each group are shown below the relevant group label on the x axis. Median values and interquartile ranges are shown. P values were calculated by Mann-Whitney analysis. c) Expression of CD14 and CD16 on monocytes from a representative HIV+ male. d) Expression of CD16 (MFI, geometric mean fluorescence intensity) on the CD14⁺ monocyte population in young and elderly healthy controls and young HIV+ males (viremic and virologically suppressed) as detailed in (b).

and 0.0003 for CD14⁺ and CD16⁺ monocytes respectively) whilst expression of CD62L and CD115 was not significantly different between young HIV+ individuals and the elderly. Monocytes from young HIV+ individuals did not show significantly altered expression of TLR4 (data not shown).

Similar age-related changes in monocyte phenotype were also observed in young VS HIV+ individuals (Fig. 2a-d). Their monocytes showed significantly increased expression of CD11b ($p = 0.002$ for both CD14⁺ and CD16⁺ monocytes) and decreased expression of CD62L (CD14⁺ monocytes, $p = 0.03$) and CD115 expression (CD14⁺ monocytes, $p = 0.03$) as compared to age-matched controls; expression levels were not significantly different to the elderly. VS HIV+ individuals showed reduced CD11b expression as compared to viremic patients, however changes in expression of CD62L or CD115 with treatment were not observed. These data indicate that monocytes from young HIV+ individuals exhibit a phenotype that mimics changes seen in elderly healthy HIV- individuals and that these defects persist in cART-treated individuals.

Soluble mediators and markers of innate immune activation

To compare the impact of ageing and HIV on innate immune activation, we measured plasma levels of candidate innate immune activation markers. Neopterin ($p = 0.0001$), CXCL10 ($p = 0.001$) and sCD163 ($p = 0.004$) were significantly elevated in the elderly compared to young individuals (Fig. 3a-c) whilst levels of sCD14 and M-CSF were not significantly altered (data not shown). In young viremic HIV+ individuals, plasma levels of neopterin, CXCL10 and sCD163 were significantly elevated when compared to both young ($p < 0.0001$ for all) and elderly ($p < 0.0001$ for neopterin and CXCL10 and 0.005 for sCD163) controls (Fig. 3a-c). Plasma levels of these markers were significantly reduced in VS as compared to viremic HIV+ individuals, but remained elevated above those of age-matched HIV-uninfected controls ($p = 0.0005$, 0.004 and 0.003 for neopterin, CXCL10 and sCD163 respectively) and were not significantly different to elderly controls. We also measured plasma levels of the innate immune stimulant LPS and found significantly elevated levels in elderly controls ($p = 0.006$), viremic ($p = 0.005$) and VS

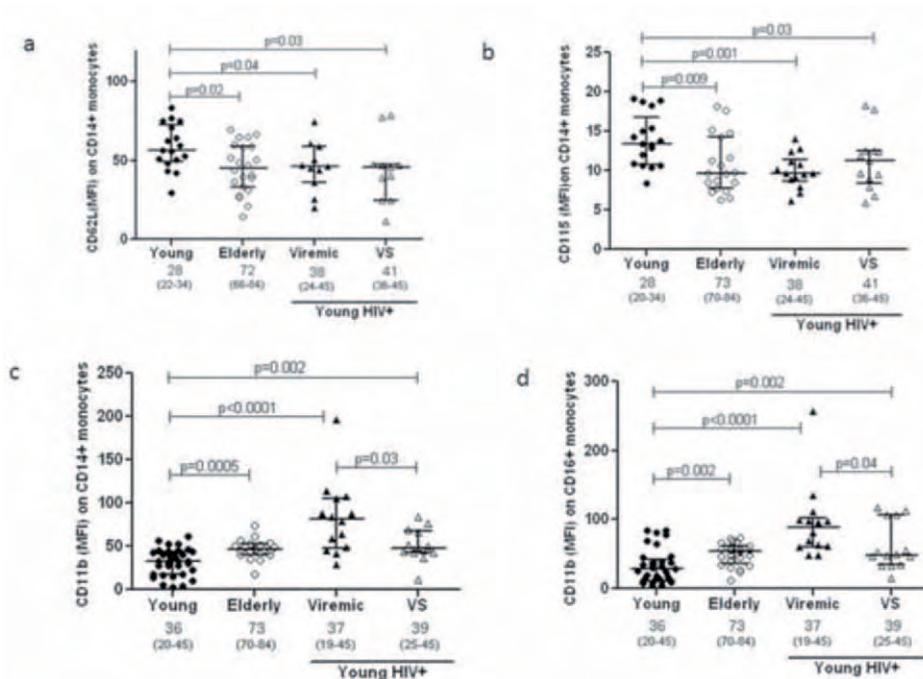


Fig. 2. Expression of CD62L, CD11b, CD115 on monocytes. Phenotyping of surface receptors was performed on peripheral monocytes in whole blood from young healthy controls (closed circles), elderly healthy controls (open circles), young viremic HIV+ (closed triangles) and young cART-treated virologically suppressed HIV+ males (open triangles). Median ages and ranges for each group are shown below the relevant group label on the x axis. Expression of (a) CD62L and (b) CD115 on CD14⁺ monocytes and CD11b on (c) CD14⁺ and (d) CD16⁺ monocytes measured as Δ MFI (geometric mean fluorescence intensity of stain minus fluorescence of isotype control) is shown. Median values and interquartile ranges are shown. P values were calculated by Mann-Whitney analysis and are shown for values <0.05.

($p=0.02$) HIV+ individuals as compared to young controls (Fig. 3d). These findings support the use of these markers as biomarkers of innate immune ageing and show that they are elevated in young HIV+ individuals irrespective of viral load.

LPS binding to TLR4 receptors on monocytes leads to production of pro-inflammatory cytokines like TNF; thus we hypothesised that elevated LPS levels may be associated with increased production of TNF by monocytes. Basal intracellular levels of TNF in both CD14⁺ and CD16⁺ whole blood monocytes from elderly and HIV+ individuals were significantly higher than young controls (see Supplementary data), suggesting increased plasma LPS is associated with increased basal production of pro-inflammatory cytokines by monocytes.

We performed regression analyses between soluble innate immune activation markers and phenotypic markers of monocyte ageing and found a significant correlation between CXCL10 and all identified phenotypic markers (Table 1). Plasma levels of neopterin were significantly associated with all phenotypic markers except the proportion of CD16⁺ monocytes; sCD163 levels correlated with expression of CD11b on CD16⁺

monocytes and plasma LPS levels correlated with the proportion of CD16⁺ monocytes.

Monocyte function

To determine whether monocyte function is altered by ageing or HIV infection, we examined the phagocytic ability of peripheral blood monocytes (Fig. 4a). The percentage of phagocytic monocytes was significantly reduced in elderly ($p=0.04$) and young viremic HIV+ males ($p=0.007$) as compared to young controls with the proportion of phagocytic cells not differing significantly between elderly controls and HIV+ individuals ($p=0.6$).

Monocyte telomere length

HIV infection is associated with accelerated telomere shortening in CD8⁺ T cells [33,34] and in B cells [35]. The impact of HIV infection on telomere length in innate immune cells is unknown. Using a multicolour FISH-Flow protocol, we demonstrated that both CD14⁺ and CD16⁺ monocytes from young HIV+ individuals contain significantly shorter telomeres than young controls ($p=0.03$ and 0.02 for CD14⁺ and CD16⁺ respectively, Fig. 4b and c) which were not significantly different to elderly controls ($p=0.3$ and 0.4 for CD14⁺ and CD16⁺ monocytes respectively). In contrast, whilst age was associated with a significant decrease in telomere

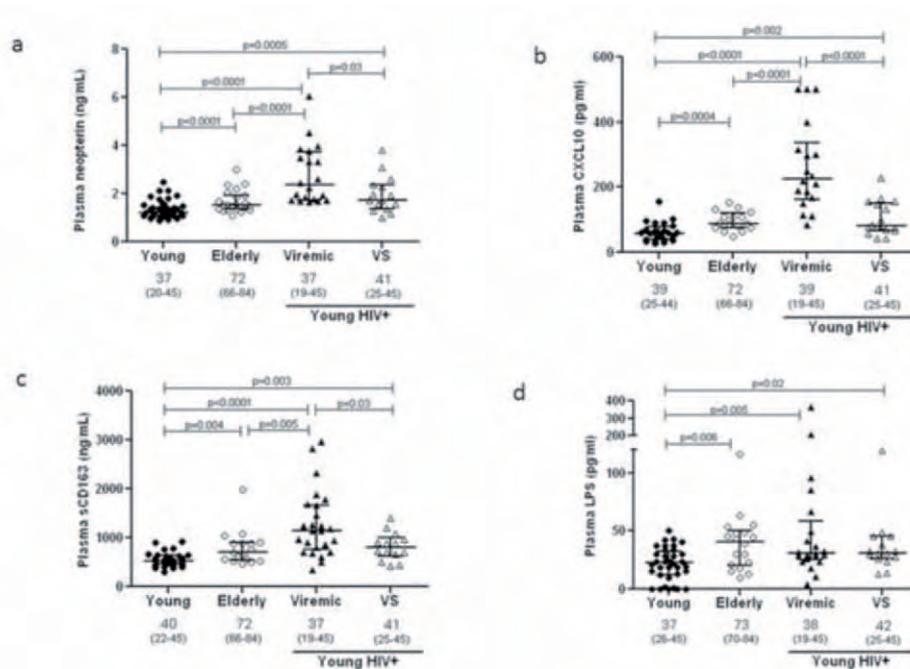


Fig. 3. Plasma levels of innate immune activation markers. Plasma levels of (a) neopterin, (b) CXCL10 and (c) sCD163 were determined via ELISA and (d) LPS determined by the Limulus Amoebocyte Lysate assay. Values are shown for young healthy controls (closed circles), elderly healthy controls (open circles), young viremic HIV+ (closed triangles) and young cART-treated HIV+ individuals (open triangles). Median ages and ranges for each group are shown below the relevant group label on the x axis. Median values and interquartile ranges are shown. P values were calculated via Mann-Whitney analysis and are shown for values <0.05 .

length in CD3⁺ T cells, telomere length in T cells from young HIV+ individuals was not different to young controls ($p=0.5$, Fig. 4d). These data indicate that HIV infection is associated with telomere shortening in monocytes.

Discussion

Increasing evidence suggests HIV infection induces premature ageing of the adaptive immune system. Ageing also hastens the development of age-related diseases with inflammatory aetiologies, yet the impact of HIV on age-related changes to innate cells critical for regulating

inflammation (eg. monocytes) is unknown. Here we directly compared monocytes from young HIV+ males with young and elderly healthy controls and found that young HIV+ individuals show changes to monocyte phenotype, function and telomere length that closely resemble those observed in elderly controls aged approximately 30 years older. Furthermore, our data suggest these immune defects are not fully restored by cART.

We found that similar to elderly controls, the proportion of CD16⁺ monocytes (considered inflammatory due to their high production of TNF [36]) was increased in young viremic HIV+ individuals. An increase in this subset has been reported in other inflammatory

Table 1. Correlation of soluble and monocyte phenotypic markers of ageing.

	Neopterin		CXCL10		sCD163		LPS	
	rho	p value	rho	p value	rho	p value	rho	p value
% CD16 ⁺ monocytes	0.09	NS (0.53)	0.405	0.009	0.098	NS (0.55)	0.317	0.032
CD11b on CD14 ⁺ monocytes	0.300	0.03	0.675	<0.0001	0.151	NS (0.36)	0.177	NS (0.24)
CD11b on CD16 ⁺ monocytes	0.418	0.002	0.653	<0.0001	0.318	0.05	0.024	NS (0.88)
CD62L on CD14 ⁺ monocytes	-0.36	0.01	-0.584	<0.0001	-0.084	NS (0.63)	-0.177	NS (0.26)
CD115 on CD14 ⁺ monocytes	-0.291	0.03	-0.37	0.02	0.011	NS (0.95)	-0.115	NS (0.45)

Plasma levels of soluble markers of innate immune activation were correlated with age-related changes to monocyte phenotypic markers for all samples tested using Spearman's rank correlation coefficient (rho, shown in Table). NS = not statistically significant.

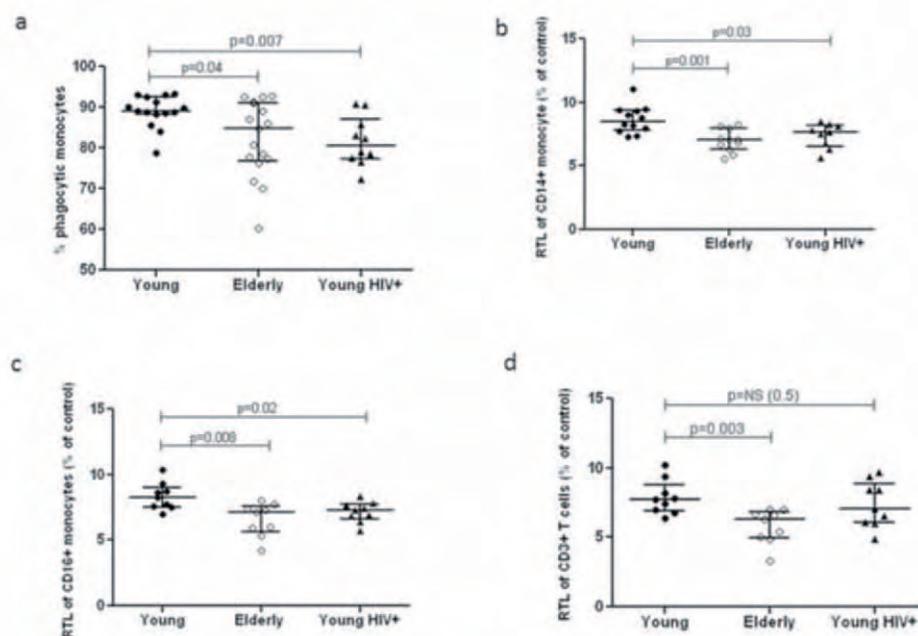


Fig. 4. Monocyte function and telomere length. a) The phagocytic function of monocytes was determined by whole blood flow cytometry using pHRODO labeled *E.coli* phagocytic target. Values shown indicate % CD14⁺ monocytes that had phagocytosed the target for young healthy controls (closed circles), elderly healthy controls (open circles), young viremic HIV+ (closed triangles) individuals. Median ages and ranges for each group are shown below the relevant group label on the x axis. Median values and interquartile ranges are shown. P values were calculated via Mann-Whitney analysis. b-d) Telomere length of CD14⁺ (b) and CD16⁺ (c) monocyte subsets and CD3⁺ T cells (d) was determined via FISH-Flow and immunophenotyping. Values represent Relative Telomere Length (RTL) expressed as a percentage of the internal control cell line 1301 for young healthy controls (closed circles; median age 28, range 20–32 years), elderly healthy controls (open circles; median age 72, range 70–82 years), young viremic HIV+ (closed triangles; median age 39, range 27–45 years). Median values and interquartile ranges are shown. P values were calculated via Mann-Whitney analysis.

conditions [37–39], in association with ageing [40] and in patients with HIV-related dementia [28]. Our finding of increased CD16⁺ monocytes in viremic but not cART-treated individuals is consistent with data from ourselves and others [41,42]. Distinct from the increased proportion of CD16⁺ monocytes, we also observed an increase in CD16 expression on the CD14⁺ monocyte population seen specifically in HIV+ individuals. Whilst the functional implications of this novel finding remain to be defined, CD14⁺ monocytes are thought to be precursors for the mature CD16⁺ subsets and an increase in double positive (CD14⁺CD16⁺) monocytes is a preliminary step in M-CSF-induced expansion of CD16⁺ monocytes *in vivo* [43]. This is consistent with our finding of significantly increased plasma M-CSF levels in viremic (median 177 pg/ml, $p = 0.0002$) and VS (median 141, $p = 0.04$) HIV+ individuals but not elderly individuals as compared to young controls (median 115 pg/ml, data not shown), suggesting increased CD16 expression on CD14⁺ monocytes may reflect an increased rate of monocyte maturation induced by cytokines such as M-CSF in HIV+ individuals. The fact that increased expression of CD16 on CD14⁺

monocytes persists in VS individuals while the proportion of CD16⁺ monocytes is normalised suggests that different factors may drive these two processes, but the net effect of these changes on the inflammatory potential of monocyte subsets remains to be determined.

Monocytes from young HIV+ individuals exhibited other age-related changes including increased surface expression of CD11b (a component of the β 2-integrin MAC-1) and decreased expression of the adhesion molecule CD62L. CD11b is a marker of monocyte activation; MAC-1 is involved in migration of monocytes into atherosclerotic plaques [44] and increased CD11b expression has been associated with atherosclerosis in a mouse model [45]. Exposure of monocytes from healthy donors to either TNF α , LPS or immune complexes reduces CD62L expression and increases CD11b expression [46,47], suggesting that changes in monocyte phenotype observed here may be in part due to a pro-inflammatory state present in both the elderly and HIV+ individuals. CD62L and CD11b are required for endothelial attachment and migration of monocytes, and HIV infection of monocytic cell lines increases

CD11b expression and increases endothelial attachment [48]. Reduced expression of the M-CSF receptor CD115 was also found on monocytes from the elderly and viremic HIV+ individuals, which in HIV+ individuals may be a consequence of elevated M-CSF levels leading to increased CD115 internalization and degradation [49]. Alternatively, LPS has been shown to reduce M-CSF receptor expression on mouse macrophages [50]. The functional implications of reduced CD115 expression are not known, but may alter the ratio of M-CSF-stimulated 'anti-inflammatory' M2 monocytes and GM-CSF-stimulated 'inflammatory' M1 macrophages [51].

We have confirmed and extended previous findings of elevated plasma levels of neopterin, sCD163 and CXCL10 in HIV+ individuals [23,52,53] by identifying that levels of these innate immune activation markers in VS HIV+ individuals were similar to those in elderly controls and are linked with age-related changes to monocyte phenotype. Elevation of these markers in VS HIV+ individuals suggests factors other than HIV viremia may drive persistent innate immune activation, although the contribution of residual viral replication cannot be excluded. Neopterin and CXCL10 are implicated in frailty, atherogenesis and neurological defects in elderly individuals [54–56] whilst in HIV+ individuals, elevated neopterin is associated with impaired cognitive function and AIDS-related dementia [57] and elevated sCD163 with non-calcified coronary plaques [25]. The neurotoxic chemokine CXCL10 is also up-regulated in patients with HIV-associated neurocognitive disorders [58,59] and was the only plasma marker that correlated with all the observed age-related changes to monocyte phenotype, supporting its use as a robust biomarker of monocyte ageing. CXCL10 is produced by monocytes, endothelial cells and fibroblasts in response to IFN γ [60] and our finding of elevated CXCL10 in elderly and HIV+ individuals suggests elevated IFN γ levels in these individuals. LPS directly stimulates monocytes and is elevated in both the elderly and HIV+ individuals irrespective of viral load. Our finding of increased basal levels of the pro-inflammatory cytokine TNF in monocytes from both elderly and HIV+ individuals suggests that persistent innate immune activation by factors such as LPS may contribute to chronic inflammation in both groups. The significant association of innate immune activation markers with age-related HIV co-morbidities warrants further work to elucidate the drivers of persistent innate immune activation in VS individuals.

Our data showing telomere shortening in monocytes from both young HIV+ individuals and the elderly was surprising. Human monocytes do not undergo significant cell division, thus shortened telomeres are unlikely to reflect enhanced peripheral cell division. These data more

likely reflect telomere shortening in bone marrow precursor cells, suggesting HIV infection drives increased precursor cell turnover. This hypothesis is consistent with the findings of Burdo *et al* who show increased monocyte turnover in SIV-infected macaques which correlated with increased levels of sCD163 and severity of encephalitis [61]. Increased monocyte turnover during HIV infection may be due to persistent immune activation (e.g. secondary to microbial translocation) causing increased monocyte mobilisation from the bone marrow. Reduced activity of the telomere repair enzyme telomerase in CD34⁺ haematopoietic progenitor cells from HIV⁺ individuals [62] may also contribute. Despite the small sample size, telomere length of CD14⁺ monocytes correlated with plasma levels of CXCL10 in our study ($p=0.03$, Spearman's ρ -0.475 , data not shown), supporting a link between monocyte activation and telomere shortening. The functional implications of shortened telomeres in monocytes/macrophages are not known but telomere shortening of leukocytes is associated with age-related diseases including cancer and cardiovascular disease [63]. HIV infection is associated with telomere shortening in CD8⁺ but not CD4⁺ T cells [33,34], which may explain why significant telomere shortening was not detected in total CD3⁺ T cells in this study. Our data showing HIV infection also induces age-related changes to monocyte phagocytosis is consistent with our previous findings [64,65]. Given the observed similarities in monocyte activation and phenotype, it would be of interest to compare the gene expression profiles of monocytes from young and elderly HIV-uninfected and young HIV+ individuals, which to date has not been done.

In summary, we have shown that young HIV+ men exhibit age-related changes to monocyte phenotype and levels of innate immune activation that resemble those observed in elderly HIV-uninfected individuals. Importantly, age-related changes to plasma and phenotypic markers of monocyte activation (eg. neopterin, CXCL10, sCD163 and CD11b expression) are not normalised by cART, which may have implications for the development of co-morbidities involving activated monocytes (e.g. atherosclerosis) in VS HIV+ individuals. Given the strong links between chronic inflammation and age-related co-morbidities, our findings have important clinical implications. Future longitudinal studies are required to investigate the impact of time of viral suppression on innate ageing biomarkers and determine whether the rate of ageing is increased by HIV. Elucidating the mechanisms driving age-related changes in the absence of overt viremia is required to prevent co-morbidities in this population. This study has identified biomarkers which may be useful for monitoring immune activation and disease risk in HIV+ individuals and may also represent new targets for interventional strategies.

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Conflicts of interest

None declared.

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