HIV infection induces age-related changes to monocytes and innate immune activation in young men that persist despite combination antiretroviral therapy

Anna C. Hearps[a,b], Anna Maisa[a], Wan-Jung Cheng[a], Thomas A. Angelovich[a,c], Gregor F. Lichtfuss[a,b], Clovis S. Palmer[a,d], Alan L. Landay[e], Anthony Jaworowski[a,b,f,*] and Suzanne M. Crowe[a,b,g,*]

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-positive males aged 45 years or less 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-positive 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 with young uninfected controls. These changes were also present in young virologically suppressed HIV-positive males. Innate immune activation markers neopterin, soluble CD163 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-positive males with levels in suppressed individuals resembling those observed in elderly controls. Like the elderly, $CD14^{+}$ monocytes from young HIV-positive males exhibited impaired phagocytic function ($P = 0.007$) and telomere-shortening ($P = 0.03$) as compared with young uninfected controls.

Conclusion: HIV infection induces changes to monocyte phenotype and function in young HIV-positive 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-positive individuals.

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[a]Centre for Virology, Burnet Institute, [b]Department of Medicine, Monash University, [c]School of Applied Sciences, RMIT University, Melbourne, Victoria, [d]School of Medical Sciences, University of New South Wales, Sydney, New South Wales, Australia, [e]Rush University Medical Center, Chicago, Illinois, USA, [f]Department of Immunology, Monash University, and [g]Infectious Diseases Unit, Alfred Hospital, Melbourne, Victoria, Australia.

Correspondence to Dr Anna C. Hearps, Centre for Virology, Burnet Institute, GPO Box 2284, Melbourne, VIC 3001, Australia.
Tel: +61 03 9282 2150; fax: +61 03 9282 2142; e-mail: annah@burnet.edu.au

[*] Anthony Jaworowski and Suzanne M. Crowe contributed equally to the writing of this article.

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Introduction

The increased life expectancy of HIV-positive 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]. Although 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-positive 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 that drives age-related immune dysfunction and disease [6,7]. Elevated plasma levels of inflammatory markers [e.g. interleukin (IL)-6] predict age-related diseases including cardiovascular disease, frailty and mortality [8–12]. HIV-positive 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-positive individuals and the elderly and include the following: first, increased pathogen burden due to impaired immunity; second, chronic viral replication by viruses such as cytomegalovirus, a well recognized driver of ageing [16,17] and HIV; and, third, microbial translocation of bacterial products across damaged mucosal surfaces, for example the gut [18,19].

In untreated HIV-positive 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-positive 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 lipopolysaccharide (LPS) [22], and innate immune activation persists in cART-treated individuals [22,23]. A recent study of HIV-positive individuals (the majority being virologically suppressed) found levels of the monocyte/macrophage activation marker, soluble CD14 (sCD14), independently predict all-cause mortality [24]. In cART-treated individuals, sCD14 levels correlate with inflammatory markers such as IL-6, high-sensitivity C-reactive protein and D-dimer [24] that predict cardiovascular disease, whereas elevated sCD163 levels are associated with increased formation of noncalcified plaques [25]. Thus, the persistence of innate immune stimuli in virologically suppressed 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 Toll-like receptor (TLR)4 and its related signaling molecule CD14 [26] and are primary producers of pro-inflammatory cytokines such as tumor necrosis factor (TNF) and IL-6. Monocytes have specific roles in the pathogenesis of age-related diseases, for example 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 comorbidities in virologically suppressed HIV-positive 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-positive 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 RNA viral load 41 600 copies/ml, range 80–>100 000; median CD4 T cells 434 cells/μl, range 11–1092) and virologically suppressed (RNA viral load <50 copies/ml; median CD4 T cells 695 cells/μl, range 209–1199; median time of viral suppression 2.8 years, range 0.7–7.6) HIV-positive individuals 45 years or less were recruited from the Infectious Diseases Unit at The Alfred Hospital, Melbourne, Victoria, Australia. Young (≤45 years) and aged (≥65 years) HIV-negative 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-positive 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 h of blood collection.

Monocyte phenotyping

Blood collected into EDTA anticoagulant was mixed with a 20 : 1 FACS (fluorescence-activated cell sorting)

lysing solution (BD Biosciences, Franklin Lakes, New Jersey, USA) to lyse erythrocytes (shown not to significantly alter expression of relevant surface markers), incubated on ice for 10 min and then washed twice with FACS wash [1% heat-inactivated cosmic calf serum, 2 mmol/l EDTA in calcium-free and magnesium-free PBS (Invitrogen, Carlsbad, California, USA)]. Cells were stained on ice for 30 min using pretitrated volumes of the following antibodies: CD14-APC, CD16-PE.Cy7, CD38-PE, HLA(human leukocyte antigen)-DR-FITC, CD11b-PE, CD62L-FITC (BD Biosciences), TLR4-FITC (R&D Systems, Minneapolis, Minnesota, USA) and CD115-PE (eBiosciences, San Diego, California, USA) or appropriate isotype control antibodies. Cells were washed once in FACS wash and fixed in 1% formaldehyde. Samples were analyzed on a dual-laser BD FACSCalibur flow cytometer.

Assessment of monocyte phagocytosis

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

Analysis of telomere length

Telomere length in monocyte subsets was determined via immunophenotyping and fluorescence *in situ* hybridization (FISH)-Flow using modification of a previously described protocol [29]. PBMCs were labeled with anti-CD14-Qdot 800, anti-CD3-AlexaFluor 405 (both from Invitrogen) and anti-CD16-AlexaFluor 647 (Biolegend, San Diego, California, USA) and cross-linked with 4 mmol/l bis(sulfosuccinimidyl)suberate. Telomeres were stained with the FITC FISH-Flow telomere labelling kit (Dako, Glostrup, Denmark) as per manufacturer's instructions using a 7-Aminoactinomycin D DNA stain (0.1 μg/ml). Samples were analyzed on a LSR II flow cytometer (BD Biosciences) 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), which was clarified via centrifugation at 10 000*g* for 10 min prior to analysis. LPS levels were determined in plasma diluted 1 : 10 and heat inactivated (80°C for 10 min) using the chromogenic Limulus Amebocyte Lysate kit (Lonza, Basel, Switzerland, catalogue number 50–647U). Commercial ELISA kits were used to determine levels of sCD163 (IQ products, Groningen, The Netherlands, catalogue number IQP-383), neopterin (Screening EIA, Brahms, Berlin, Germany, catalogue number 99R.096), sCD14, CXCL10 and macrophage-colony stimulating factor (M-CSF; catalogue number 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, Melbourne, Australia). Graphing and statistical analysis was performed using Prism version 5.0 (GraphPad Software, La Jolla, California, USA). Mann–Whitney *U*-test was used to detect significant differences between sample groups (for nonparametric 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 virologically suppressed ($P = 0.47$) HIV-positive males (Fig. 1b). In addition to the expansion of total $CD16^+$ monocytes, the $CD14^+$ monocyte subset (typically negative for CD16) in both viremic and virologically suppressed HIV-positive 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, http://links.lww.com/QAD/A207). We used ex-vivo whole blood analyses in this study, as 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 with 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 analyzed.

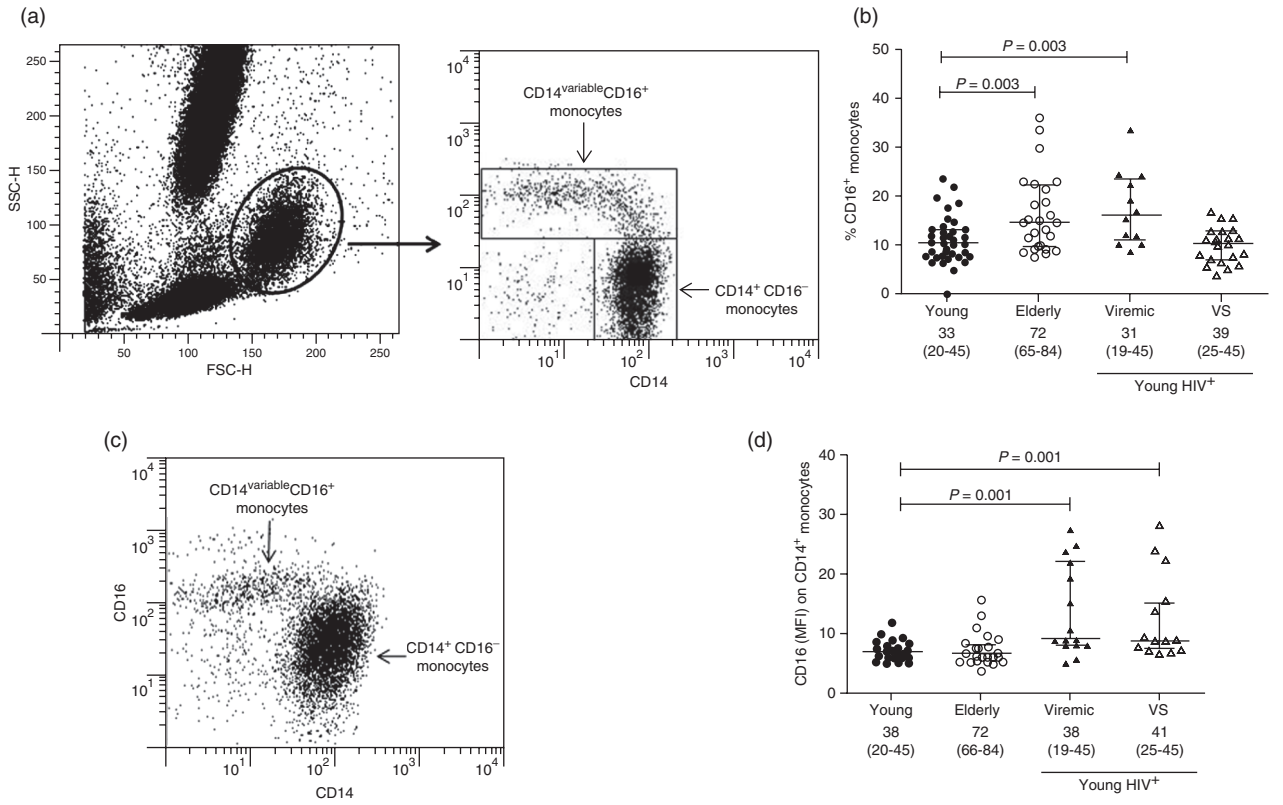

Fig. 1. Proportions of monocyte subsets and CD16 expression levels. (a) Peripheral blood monocytes were gated initially via forward and side scatter then separated into subsets based on expression of CD14 and CD16. Monocyte subsets were analyzed as either $CD14^{variable}CD16^{+}$ ($CD16^{+}$) or $CD14^{+}CD16^{-}$ ($CD14^{+}$). (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-positive (closed triangles) and young combination antiretroviral therapy (cART)-treated virologically suppressed HIV-positive 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. The *P* values were calculated by Mann–Whitney analysis. (c) Expression of CD14 and CD16 on monocytes from a representative HIV-positive male. (d) Expression of CD16 (MFI, geometric mean fluorescence intensity) on the $CD14^{+}$ monocyte population in young and elderly healthy controls and young HIV-positive males (viremic and virologically suppressed) as detailed in (b).

Age-related changes to monocyte phenotype were then analyzed in young HIV-positive individuals. The same pattern of phenotypic change was found in young viremic HIV-positive males as in the elderly, but the magnitude of these changes was greater. Compared with young controls, monocytes from viremic HIV-positive individuals 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-positive individuals was significantly higher than that seen in the elderly ($P = 0.003$ and 0.0003 for $CD14^{+}$ and $CD16^{+}$ monocytes, respectively), whereas expression of CD62L and CD115 was not significantly different between young HIV-positive individuals and the elderly. Monocytes from young HIV-positive individuals did not show significantly altered expression of TLR4 (data not shown).

Similar age-related changes in monocyte phenotype were also observed in young virologically suppressed HIV-positive 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 with age-matched controls; expression levels were not significantly different to the elderly. Virologically suppressed HIV-positive individuals showed reduced CD11b expression as compared with viremic patients; however, changes in expression of CD62L or CD115 with treatment were not observed. These data indicate that monocytes from young HIV-positive individuals exhibit a phenotype that mimics changes seen in elderly

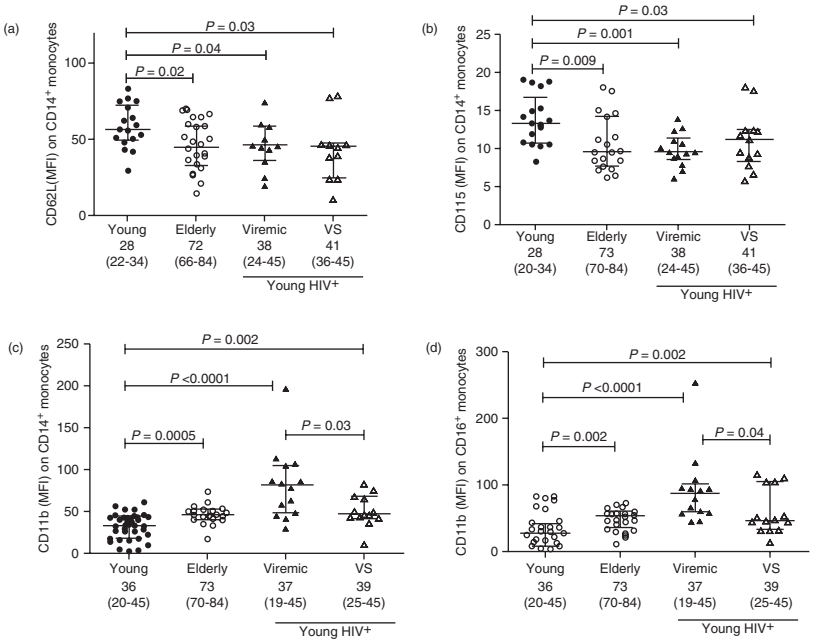

Fig. 2. Expression of CD62L, CD11b and CD115 on monocytes. Phenotyping of surface receptors was performed on peripheral monocytes in whole blood from young healthy controls (closed circles), elderly healthy controls (open circles), young viremic HIV-positive (closed triangles) and young combination antiretroviral therapy (cART)-treated virologically suppressed HIV-positive 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 ΔMFI (geometric mean fluorescence intensity of stain minus fluorescence of isotype control) is shown. Median values and interquartile ranges are shown. The *P* values were calculated by Mann–Whitney analysis and are shown for values less than 0.05.

healthy HIV-negative 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 ($P = 0.004$) and sCD163 ($P = 0.004$) were significantly elevated in the elderly compared with young individuals (Fig. 3a–c), whereas levels of sCD14 and M-CSF were not significantly altered (data not shown). In young viremic HIV-positive individuals, plasma levels of neopterin, CXCL10 and sCD163 were significantly elevated when compared with 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 virologically suppressed as compared with viremic HIV-positive individuals, but remained elevated above those of age-matched HIV-uninfected controls ($P = 0.0005$, 0.002 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 virologically suppressed ($P = 0.02$) HIV-positive individuals as compared with 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-positive individuals irrespective of viral load.

LPS binding to TLR4 receptors on monocytes leads to production of pro-inflammatory cytokines such as TNF; thus, we hypothesized 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-positive individuals were significantly higher than young controls (see supplementary data, http://links.lww.com/QAD/A207), 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.

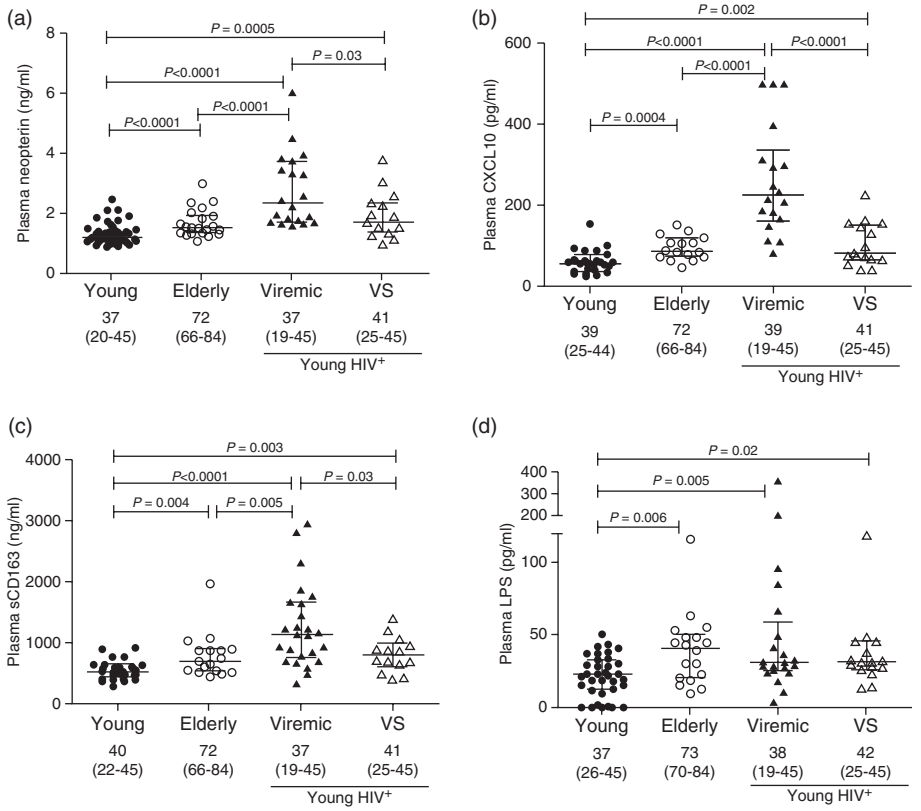

Fig. 3. Plasma levels of innate immune activation markers. Plasma levels of (a) neopterin, (b) CXCL10 and (c) soluble CD163 (sCD163) were determined via ELISA, and (d) lipopolysaccharide (LPS) determined by the Limulus Amoebocyte Lysate assay. Values are shown for young healthy controls (closed circles), elderly healthy controls (open circles), young viremic HIV-positive (closed triangles) and young combination antiretroviral therapy (cART)-treated HIV-positive 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. The *P* values were calculated via Mann–Whitney analysis and are shown for values less than 0.05.

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-positive males ($P = 0.007$) as compared with young controls with the proportion of phagocytic cells not differing significantly between elderly controls and HIV-positive 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 multicolor FISH-Flow protocol, we demonstrated that both $CD14^+$ and $CD16^+$ monocytes from young HIV-positive 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

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

	Neopterin		CXCL10		Soluble CD163		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*). LPS, lipopolysaccharide.

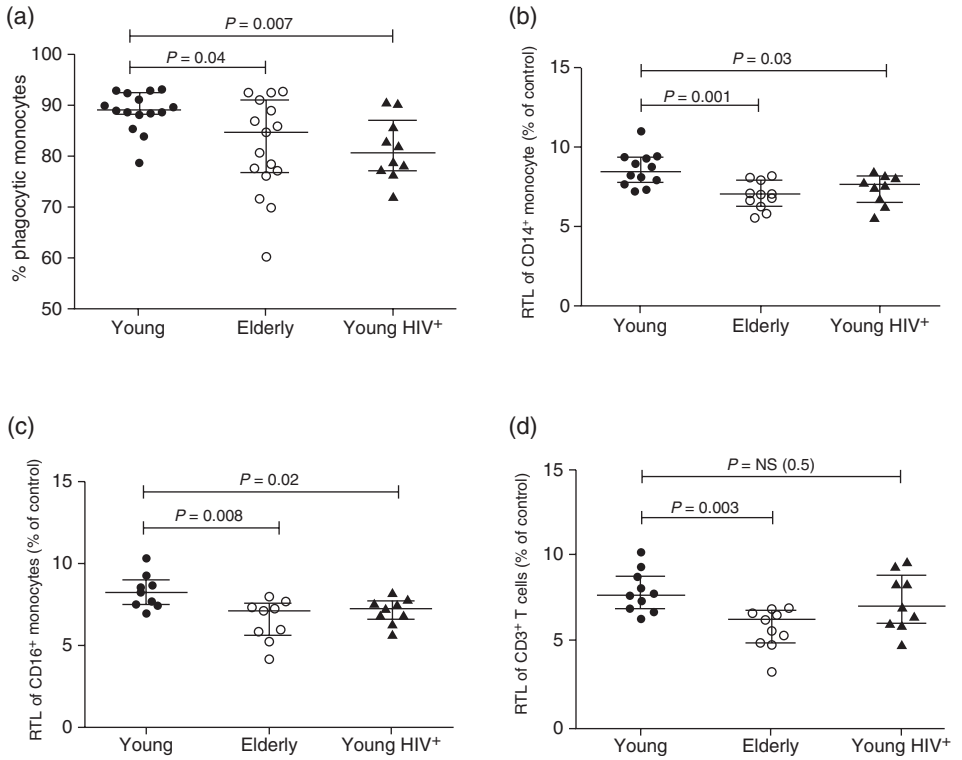

Fig. 4. Monocyte function and telomere length. (a) The phagocytic function of monocytes was determined by whole blood flow cytometry using pHRODO-labeled *Escherichia coli* phagocytic target. Values shown indicate percentage $CD14^+$ monocytes that had phagocytosed the target for young healthy controls (closed circles), elderly healthy controls (open circles) and young viremic HIV-positive (closed triangles) individuals. Median values and interquartile ranges are shown. The *P* values were calculated via Mann–Whitney analysis. 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 years, range 20–32), elderly healthy controls (open circles; median age 72 years, range 70–82), young viremic HIV-positive (closed triangles; median age 39 years, range 27–45). Median values and interquartile ranges are shown. The *P* values were calculated via Mann–Whitney analysis.

elderly controls ($P = 0.3$ and 0.4 for $CD14^+$ and $CD16^+$ monocytes, respectively). In contrast, although age was associated with a significant decrease in telomere length in $CD3^+$ T cells, telomere length in T cells from young HIV-positive 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 causes, yet the impact of HIV on age-related changes to innate cells critical for regulating inflammation (e.g. monocytes) is unknown. Here we directly compared monocytes from young HIV-positive males with young and elderly healthy controls and found that young HIV-positive 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-positive individuals. An increase in this subset has been reported in other inflammatory 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-positive individuals. Although 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 virologically suppressed (median 141, $P = 0.04$) HIV-positive individuals, but not elderly individuals as compared with 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-positive individuals. The fact that increased expression of CD16 on $CD14^+$ monocytes persists in virologically suppressed individuals while the proportion of $CD16^+$ monocytes is normalized 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-positive individuals exhibited other age-related changes including increased surface expression of CD11b (a component of the β2-integrin macrophage-1 antigen) and decreased expression of the adhesion molecule CD62L. CD11b is a marker of monocyte activation; macrophage-1 antigen 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 TNFα, 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-positive 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-positive individuals, which in HIV-positive 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 granulocyte-macrophage (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-positive individuals [23,52,53] by identifying that levels of these innate immune activation markers in virologically suppressed HIV-positive individuals were similar to those in elderly controls and are linked with age-related changes to monocyte phenotype. Elevation of these markers in virologically suppressed HIV-positive 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], whereas in HIV-positive individuals elevated neopterin is associated with impaired cognitive function and AIDS-related dementia [57] and elevated sCD163 with noncalcified coronary plaques [25]. The neurotoxic chemokine CXCL10 is also upregulated 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 interferon (IFN)γ [60] and our finding of elevated CXCL10 in elderly and HIV-positive individuals suggests elevated IFNγ levels in these individuals. LPS directly stimulates monocytes and is elevated in both the elderly and HIV-positive individuals irrespective of viral load. Our finding of increased basal levels of the pro-inflammatory cytokine TNF in monocytes from both elderly and HIV-positive 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 comorbidities warrants further work to elucidate the drivers of persistent innate immune activation in virologically suppressed individuals.

Our data showing telomere-shortening in monocytes from both young HIV-positive 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 morrow precursor cells, suggesting HIV infection drives increased precursor cell turnover. This hypothesis is consistent with the findings of Burdo *et al.* [61] that show increased monocyte turnover in simian immunodeficiency virus-infected macaques that correlated with increased levels of sCD163 and severity of encephalitis. Increased monocyte turnover during HIV infection may be due to persistent immune activation (e.g. secondary to microbial translocation), causing increased monocyte mobilization from the bone marrow. Reduced activity of the telomere repair enzyme telomerase in $CD34^+$ hematopoietic progenitor cells from HIV-positive 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 *rho* −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 that 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-positive individuals, which to date has not been done.

In summary, we have shown that young HIV-positive 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 (e.g. neopterin, CXCL10, sCD163 and CD11b expression) are not normalized by cART, which may have implications for the development of comorbidities involving activated monocytes (e.g. atherosclerosis) in virologically suppressed HIV-positive individuals. Given the strong links between chronic inflammation and age-related comorbidities, 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 comorbidities in this population. This study has identified biomarkers that may be useful for monitoring immune activation and disease risk in HIV-positive individuals and may also represent new targets for interventional strategies.

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Conflicts of interest

There are no conflicts of interest.

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