OPEN ACCESS Freely available online

PLOS ONE

Phenotypic Alteration of Neutrophils in the Blood of HIV Seropositive Patients

Tom Cloke[1], Markus Munder[2], Philip Bergin[3], Shanthi Herath[1], Manuel Modolell[4], Graham Taylor[5], Ingrid Müller[1], Pascale Kropf[1]*

1 Department of Immunology, Faculty of Medicine, Imperial College London, London, United Kingdom, **2** Third Department of Medicine (Hematology, Oncology, and Pneumology), University Medical Center Mainz, Mainz, Germany, **3** International AIDS Vaccine Initiative Human Immunology Laboratory, Faculty of Medicine, Imperial College London, London, United Kingdom, **4** Department of Cellular Immunology, Max-Planck-Institute for Immunobiology and Epigenetics, Freiburg, Germany, **5** Section of Infectious Diseases, Imperial College London, London, United Kingdom

Abstract

We have recently identified a novel population of activated low-density granulocytes (LDGs) in peripheral blood mononuclear cells of HIV seropositive patients. LDGs have a similar morphology to normal density granulocytes (NDGs), but are phenotypically different. Here we measured the expression levels of different phenotypic markers of granulocytes in the blood of HIV seropositive patients at different stages of HIV infection to determine whether the phenotype of NDGs and LDGs are affected by disease severity. Our results reveal that the phenotype of NDGs, but not that of LDGs, varies according to the severity of the disease.

Citation: Cloke T, Munder M, Bergin P, Herath S, Modolell M, et al. (2013) Phenotypic Alteration of Neutrophils in the Blood of HIV Seropositive Patients. PLoS ONE 8(9): e72034. doi:10.1371/journal.pone.0072034

Editor: Adriano Boasso, Imperial College London, United Kingdom

Received March 27, 2013; **Accepted** July 4, 2013; **Published** September 9, 2013

Funding: This work was supported by a grant from The Wellcome Trust (07664/Z/05/Z, PK) and TC is a recipient of a Imperial College London MB/PhD fellowship. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: p.kropf@imperial.ac.uk

Introduction

Neutrophils play a central role in the elimination of pathogens by using several strategies such as the production of reactive oxygen species, the release of antimicrobial peptides and neutrophil extracellular traps (NETs) (summarised in [1]). In HIV infections, both the number and the functions of neutrophils are impaired (summarised in [2]). Functional abnormalities of neutrophils include impaired phagocytosis and production of toxic oxygen species [2]. Furthermore, it has been recently shown that whereas NETs formation can capture and eliminate HIV, HIV can counteract this by inducing the production of IL-10 by dendritic cells and therefore inhibiting NETs formation [3].

Human neutrophils constitutively express arginase [4], an enzyme that catalyses the conversion of L-arginine into ornithine and urea [5,6]. Recently, the metabolism of L-arginine by arginase has emerged as a crucial mechanism for the regulation of immune responses: increased catabolism of L-arginine by arginase results in the depletion of L-arginine from the microenvironment; since L-arginine is essential for efficient T cell activation, this decrease in L-arginine results in impaired T cell responses [7,8,9,10]. Increased arginase activity has been described in malaria [11], tuberculosis [12], leishmaniasis [13,14,15] and HIV [16,17].

We have recently shown that PBMCs from HIV seropositive patients with low $CD4^+$ T cell counts expressed significantly more arginase activity as compared to patients with high $CD4^+$ T cell counts or uninfected controls [16]. Higher arginase expression in PBMCs from HIV seropositive patients was associated with decreased levels of CD3ζ expression, a marker of T cell dysregulation [16]. The phenotype of arginase-expressing cells was identified as low-density granulocytes (LDGs) as these cells co-purify with PBMCs following density gradient centrifugation. This difference in density distinguishes this population from the remaining granulocytes that co-purify with the erythocyte fraction following density gradient centrifugation and thus have been named normal-density granulocytes (NDGs). LDGs purified from HIV+ patients display a similar morphology as NDGs, but have major phenotypic differences suggesting that LDGs were activated neutrophils that had degranulated and released arginase [17]. In the present study, we aim to determine whether the phenotype of NDGs and LDGs differs in different stages of HIV infection.

Materials and Methods

Subjects and samples

Twenty-one HIV seropositive (HIV+) treatment-naïve individuals were recruited from St Mary's Hospital (Table 1). Plasma HIV-1 viral RNA was quantified by real-time PCR (Bayer Quantiplex assay (bDNA) PCR test; lower detection level of 50 copies/mL). The standard T lymphocyte markers CD3, CD4, CD8 were determined by flow cytometry. The study was approved by the National Research Ethics Service (05/Q0410/93) and all individuals gave written, informed consent before participation.

Twenty ml of peripheral blood was collected in EDTA tubes and PBMCs were isolated by density gradient centrifugation on Histopaque®-1077 (Sigma). Neutrophils were isolated from the erythrocyte fraction by dextran sulphate sedimentation [17]. All experiments were performed on fresh cell, immediately after processing.

Table 1. Clinical data.

Patients	Age	Sex (M/F)	CD4 count	Viral load
1	46	M	840	3.42
2	38	M	780	4.96
3	33	M	680	4.35
4	41	M	620	3.25
5	27	M	580	2.39
6	60	M	540	3.16
7	42	M	540	4.54
8	34	M	540	4.77
9	23	M	520	3.99
10	30	F	440	4.24
11	46	M	390	5.27
12	48	M	330	4.79
13	51	M	300	5.57
14	40	M	170	4.91
15	34	M	160	4.6
16	49	M	160	4.4
17	46	M	140	5.28
18	38	M	128	5.69
19	27	F	25	5.57
20	44	M	20	5.7
21	38	F	4	4.43

Age, sex, $CD4^+$ T cell counts and viral load were recorded for HIV+ patients (n = 21).

doi:10.1371/journal.pone.0072034.t001

Flow cytometry

The following antibodies were used: $CD14^{FITC}$, $CD15^{PE}$ (BD Pharmingen), $Arginase1^{Alexa\ Fluor®647}$ (Hycult Biotechnology), $CD11b^{PerCP\text{-}eFluro710}$, $CD16^{eFluro450}$, $CD33^{PE\text{-}Cy7}$ (eBioscience), $CD13^{APC\text{-}Cy7}$ (Biolegend), $CD66b^{FITC}$ and $CD63^{FITC}$ (Beckman Coulter). 1×10^6 PBMCs were incubated with FcR blocking reagent (BD Pharmingen) and the antibodies against extracellular markers were added directly to cells. Cells were washed after 20 min, fixed, permeabilised and anti-arginase $I^{Alexa\ FluorR\ 647}$ was added to the cells for 20 min as described in [18]. Analysis was performed on an FACS Canto II (BD Bioscience) and results were analyzed using FlowJo v8.7 (Tree Star, Ashland, OR).

Statistical analyses

Data were evaluated for statistical differences using a two-tailed Mann-Whitney test and a Spearman's rank test when appropriate (GraphPad Prism 5); differences were considered statistically significant at $p<0.05$. Results are expressed as median± SEM.

Results

We first analysed the phenotype of NDGs to determine whether it changes with increased disease severity, as measured by $CD4^+$ T cell counts and viral load. We measured the expression levels of CD11b, CD13, CD15, CD16, CD33, CD63, CD66b and arginase on NDGs from treatment naïve HIV+ patients with high (≥350 cells/μL, CD4high) and low (<350 cells/μL, CD4low) $CD4^+$ T cell counts. The division of patients based on a $CD4^+$ T cell count <350 cells/μL was chosen because 1) once the $CD4^+$ T cell count falls below 350 cells/μL differences in clinical outcome increasingly appear [19] and 2) the initiation of antiretroviral therapy is recommended once $CD4^+$ T cell count falls to <350 cells/μL [20]. Our results show that the MFIs of CD13 and arginase are statistically significantly lower ($p = 0.0008$ and $p = 0.0048$, respectively) and that of CD63 significantly higher ($p = 0.0346$) in the blood of CD4low HIV+ patients (Figure 1, Table 2) as compared to CD4high HIV+ patients. No significant difference was observed in the expression levels of CD11b ($p = 0.9159$), CD15 ($p = 0.3072$), CD16 ($p = 0.5495$), CD33 ($p = 0.3787$) and CD66b ($p = 0.6985$) between CD4high and CD4low HIV+ patients (Table 2). Of note, the expression levels of CD11b, CD13, CD15, CD16, CD33, CD63, CD66b and arginase 1 were homogenous (Figure S1).

To characterise further the association between these markers and disease severity, we plotted their MFI values against $CD4^+$ T cell counts. As shown in Figure 2, there are statistically significant positive correlations between $CD4^+$ T cell counts and the MFIs of CD13 ($p = 0.0007$), arginase ($p = 0.0196$) and CD16 ($p = 0.0400$); and a significant negative correlation between $CD4^+$ T cell counts and the MFI of CD63 ($p = 0.0097$) (Figure 2, Table 3). No significant correlation was observed between $CD4^+$ T cell counts and CD11b ($p = 0.5122$), CD15 ($p = 0.0580$), CD33 ($p = 0.0825$), and CD66b ($p = 0.6064$) (Table 3).

Next, we plotted expression levels of CD11b, CD13, CD15, CD16, CD33, CD63, CD66b and arginase against another marker of disease severity, viral load. A statistically significant negative correlation between VL and arginase ($p = 0.0063$, Figure 3) and a trend towards significance between VL and CD13 ($p = 0.0553$, Table 4) were observed, all other correlations were not statistically significant (Figure 3, Table 4).

These results suggest that in HIV+ patients, the phenotype of NDGs varies according to the severity of the disease.

Our previous results have identified a novel population of low-density granulocytes (LDGs) in the PBMCs of HIV+ patients that are morphologically similar, but phenotypically different from NDGs. Here, we determined whether the MFIs value differ in this population of LDGs and whether there was a correlation between markers of disease severity and the expression levels of CD11b, CD13, CD15, CD16, CD33, CD63, CD66b and arginase: MFIs values were similar between LDGs from CD4low and CD4high HIV+ patients (Table 5) and none of the correlations were statistically significant (Tables 6 and 7).

These results suggest that the phenotype of NDGs, but not that of LDGs varies according to the severity of the disease.

Phenotype of NDGs and LDGs in CD4low HIV+ patients

We have previously shown that LDGs are phenotypically different from NDGs, as they express increased levels of CD11b, CD15, CD33, CD66b, CD63 and decreased levels of CD16 and arginase 1 [17], suggesting that these cells are activated neutrophils that have degranulated and therefore change their density. Indeed, the results presented in Figure 2 suggest that NDGs get progressively more activated with increased disease severity, as measured by $CD4^+$ T cell counts. In the next step, we assessed how the phenotype of NDGs differs from that of LDGs in CD4high and CD4low HIV+ patients. As shown in Figure 4 and Tables 8 and 9, the expression levels of CD16, arginase and CD63 are significantly different between LDGs and NDGs from CD4high HIV+ patients, but are similar in CD4low patients. Differences between CD11b, CD13 and CD66b MFIs remain non-significant and differences between CD15 and CD33 remain significant in CD4low and CD4high HIV+ groups.

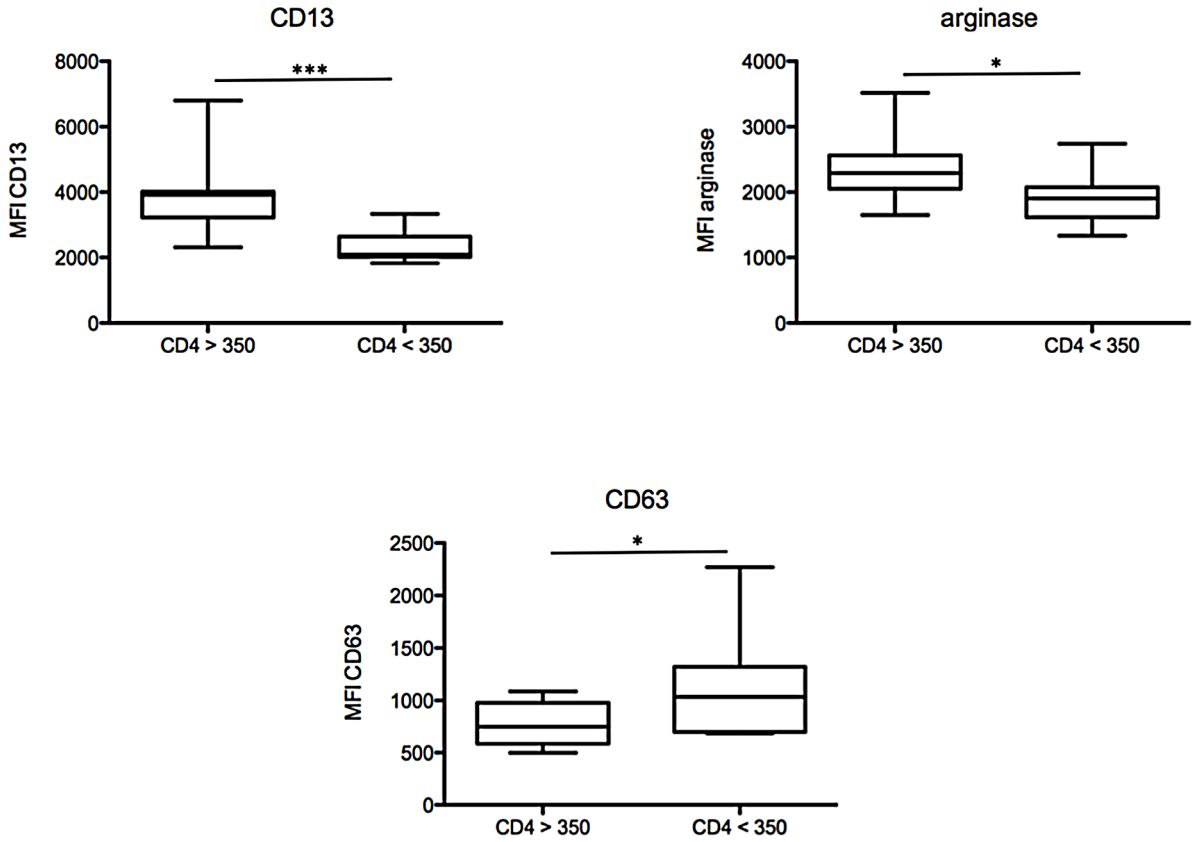

Figure 1. Phenotypic analysis of NDGs. NDGs were isolated from the blood of HIV+ patients with $CD4^+$ T cell counts >350 (n = 11) or <350 cells/μL (n = 10) as described in materials and methods and the expression levels of phenotypic markers were determined by flow cytometry. Isotype controls: <1%. Statistical significance was determined by a two-tailed Mann-Whitney test. Box = interquartile range and median; whiskers = range.
doi:10.1371/journal.pone.0072034.g001

Discussion

We have previously shown that arginase activity was significantly increased in the blood of CD4low HIV+ patients as compared to CD4high and healthy controls [16]. The phenotype of arginase-expressing cells in the PBMCs of HIV+ patients are a subset neutrophils, which were classified as low-density granulocytes (LDGs) [16,17]. These cells have a similar morphology as normal density granulocytes (NDGs) [17]. However, LDGs differ from NDGs as i) they co-localise with the PBMCs and not the erythrocytic fractions, suggesting that their density is lower; ii) they express different levels of phenotypic markers of neutrophils. In addition, our results show that the frequency of LDGs is significantly higher in HIV+ patients with low $CD4^+$ T cell counts and correlates with markers of disease severity in HIV+ patients [17]. We have already shown that the cells expressing arginase in PBMCs from HIV+ patients are LDGs, as they express CD15, but not CD14, and that the frequency of these cells increases with disease severity [16].

Table 2. Phenotype of NDGs.

	CD4high	CD4low	*p* value
	(median ± SEM)	(median ± SEM)	
CD11b	2684±767	3418±610	0.9159
CD13	3915±345	2091±152	**0.0008**
CD15	2270±693	3732±812	0.3072
CD16	10259±934	8731±743	0.5495
CD33	2114±196	1644±241	0.3787
CD63	751±57	1035±152	**0.0346**
CD66b	6326±771	6854±604	0.6985
arginase	2294±148	1904±124	**0.0448**

NDGs were isolated from the blood of HIV+ patients with $CD4^+$ T cell counts >350 cells/μL (n = 11) or <350 cells/μL (n = 10) as described in materials and methods and expression levels of phenotypic markers were determined by flow cytometry.
doi:10.1371/journal.pone.0072034.t002

Our results show that there is no difference between the phenotype of LDGs from CD4low and CD4high HIV+ patients; however, there are clear phenotypic differences in the expression levels of CD13, CD63 and arginase in NDGs from HIV+ patients with low $CD4^+$ T cell counts. Increased activation of neutrophils in HIV+ patients has already been described [21]. In this study, the expression levels of CD11b were shown to be increased on neutrophils from HIV+ patients as compared to HIV- controls. In our study we assessed whether the level of neutrophils' activation changes with disease severity, rather then comparing it to healthy controls. Our results are in agreement with the study by Elbim et al., as we did not find a change in CD11b expression on NDGs with lower $CD4^+$ T cell counts.

Our results suggest that during the course of HIV infection, there is no progressive activation of LDGs, as we found no correlation between $CD4^+$ T cell counts or viral load and the

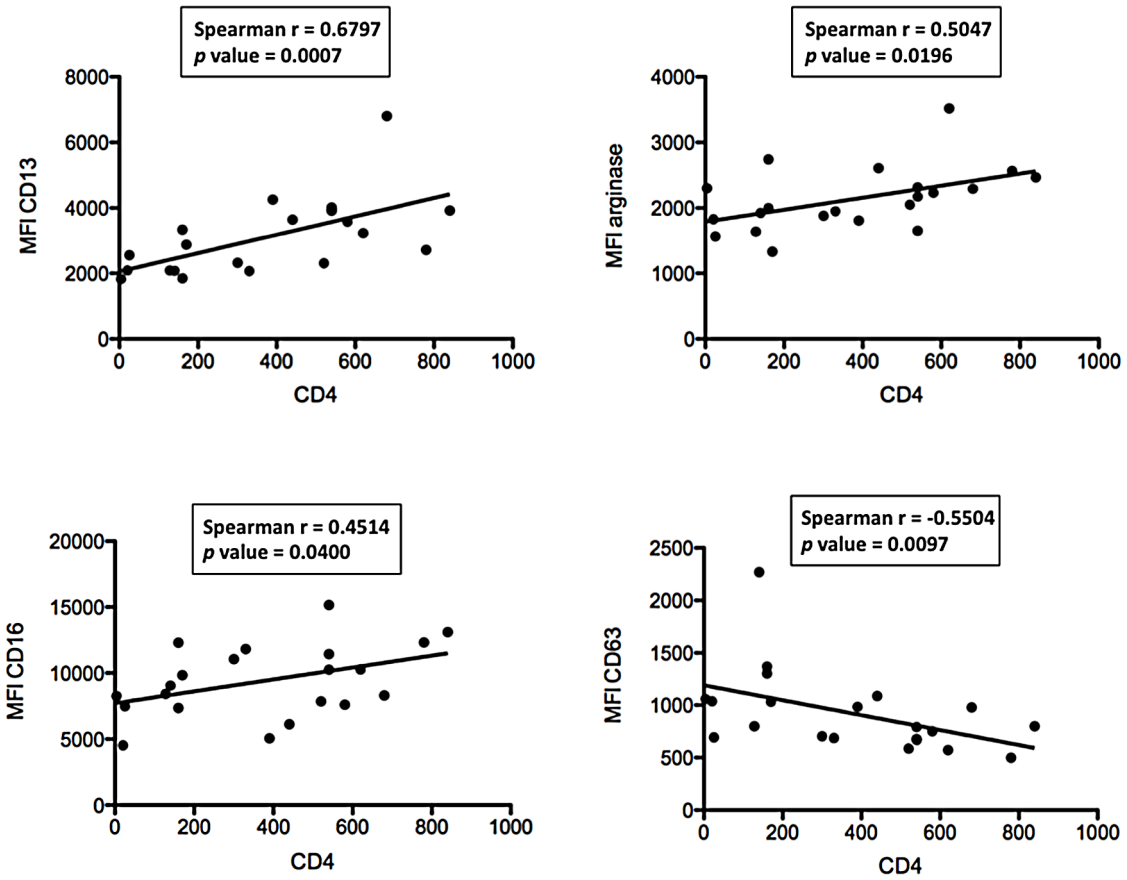

Figure 2. Correlation between CD4$^+$ T cells and phenotypic markers. NDGs were isolated from the blood of HIV+ patients (n = 21) as described in materials and methods and the expression levels of phenotypic markers were determined by flow cytometry. Correlation between CD4$^+$ T cell counts and phenotypic markers was determined by a Spearman's rank test.
doi:10.1371/journal.pone.0072034.g002

expression levels of CD11b, CD13, CD15, CD16, CD33, CD63, CD66b and arginase 1. However, our results suggest that NDGs become progressively and systemically more activated and more degranulated, since decreased expression levels of CD13, CD16 and arginase and increasing expression levels of CD63 on NDGs correlate with decreasing CD4$^+$ T cell counts. The degree of neutrophil activation is regulated by the intensity of the activating signal and occurs sequentially: 1) secretory granules; 2) gelatinous granules; 3) specific and 4) azurophilic granules. Arginase is found

Table 3. NDGs: Correlation between CD4$^+$ T cell counts and MFIs.

	Spearman r	*p* value
CD11b	−0.1522	0.5122
CD13	0.6797	**0.0007**
CD15	−0.3707	0.0980
CD16	0.4514	**0.0400**
CD33	0.3876	0.0825
CD63	−0.5504	**0.0097**
CD66b	−0.1213	0.6034
arginase	0.5047	**0.0196**

NDGs were isolated from the blood of HIV+ patients with CD4$^+$ T cell counts >350 cells/μL (n = 11) or <350 cells/μL (n = 10) as described in materials and methods and the correlations between CD4$^+$ T cell counts and phenotypic markers were determined by a Spearman's rank test.
doi:10.1371/journal.pone.0072034.t003

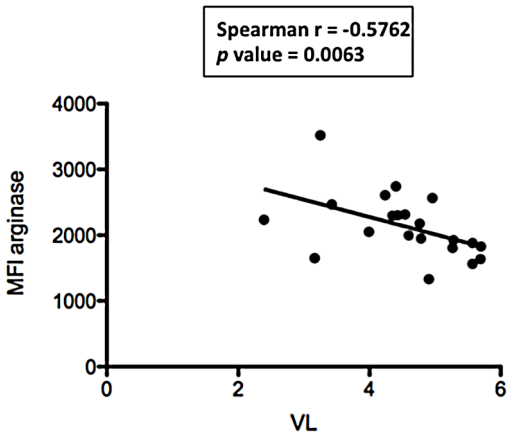

Figure 3. Correlation between viral load and phenotypic markers. NDGs were isolated from the blood of HIV+ patients (n = 21) as described in materials and methods and the expression levels of phenotypic markers were determined by flow cytometry. Correlation between viral load and phenotypic markers was determined by a Spearman's rank test.
doi:10.1371/journal.pone.0072034.g003

Table 4. NDGs: Correlation between viral loads and MFIs.

	Spearman r	*p* value
CD11b	0.1104	0.6337
CD13	−0.4242	0.0553
CD15	0.1241	0.5921
CD16	−0.2014	0.3814
CD33	−0.1845	0.4234
CD63	0.1358	0.5572
CD66b	0.1358	0.5574
arginase	−0.5762	**0.0063**

NDGs were isolated from the blood of HIV+ patients with $CD4^+$ T cell counts >350 cells/μL (n = 11) or <350 cells/μL (n = 10) as described in materials and methods and thes correlation between viral load and phenotypic markers were determined by a Spearman's rank test.
doi:10.1371/journal.pone.0072034.t004

Table 5. Phenotype of LDGs.

	CD4high	CD4low	*p* value
	(median ± SEM)	(median ± SEM)	
CD11b	4413±649	5148±890	0.2907
CD13	2137±564	2368±366	0.9159
CD15	10012±1277	8262±769	0.3072
CD16	1528±1483	6932±1112	0.5035
CD33	3575±285	2768±304	0.1300
CD63	1474±178	1355±246	0.9717
CD66b	8899±2404	10198±1775	0.6472
arginase	1700±141	1533±123	0.2453

LDGs were isolated from the blood of HIV+ patients with $CD4^+$ T cell counts >350 cells/μL (n = 11) or <350 cells/μL (n = 10) as described in materials and methods and the expression levels of phenotypic markers were determined by flow cytometry.
doi:10.1371/journal.pone.0072034.t005

Table 6. LDGs: Correlation between $CD4^+$ T cell counts and MFIs.

	Spearman r	*p* value
CD11b	−0.3507	0.1191
CD13	0.1028	0.6576
CD15	0.1502	0.5157
CD16	0.0072	0.9754
CD33	0.4039	0.0694
CD63	0.1158	0.6173
CD66b	−0.0134	0.9531
arginase	0.2738	0.7009

LDGs were isolated from the blood of HIV+ patients with $CD4^+$ T cell counts >350 cells/μL (n = 11) or <350 cells/μL (n = 10) as described in materials and methods and the correlations between $CD4^+$ T cell counts and phenotypic markers were determined by a Spearman's rank test.
doi:10.1371/journal.pone.0072034.t006

Table 7. Correlation between viral loads and MFIs.

	Spearman r	*p* value
CD11b	0.2242	0.3286
CD13	0.0071	0.9755
CD15	−0.2007	0.3830
CD16	−0.0084	0.9710
CD33	−0.3248	0.1509
CD63	−0.0364	0.8756
CD66b	0.0948	0.6826
arginase	−0.4722	0.2235

LDGs were isolated from the blood of HIV+ patients with $CD4^+$ T cell counts >350 cells/μL (n = 11) or <350 cells/μL (n = 10) as described in materials and methods and the correlations between viral load and phenotypic markers were determined by a Spearman's rank test.
doi:10.1371/journal.pone.0072034.t007

in gelatinous granules [22] and azurophilic granules [4] and upregulation of CD63 on neutrophils coincides with the release of azurophilic granule [23,24]. Therefore, our result showing that CD63 is expressed at increased levels and arginase at lower levels in NDGs from patients with low $CD4^+$ T cells suggest that NDGs get progressively more activated with increased disease severity. We hypothesise that as a result of degranulation, NDGs will change their density and become LDGs, and will be collected in the PBMC fraction following density gradient purification. Our results suggest that NDGs are activated and have already, at least partially, released azurophilic granules, as shown by increased CD63 and decreased arginase expression levels in CD4low HIV+ patients. Furthermore, our results show that the MFIs of CD16, CD63 and arginase are significantly different between LDGs and NDGs in CD4high HIV+ patients, but not any more in CD4low HIV patients; suggesting that there are less differences between the phenotype of LDGS and NDGs in CD4low as compared to CD4high HIV+ patients.

The origins of LDGs as well as the signals resulting in the degranulation of neutrophils remain unclear in HIV+ patients and we have not been able to activate NDGs to become LDGs. Stimulation of neutrophils with Phorbol 12-myristate 13-acetate

Table 8. LDGs and NDGs in HIV+ patients in CD4low HIV+ patients.

	LDGs	NDGs	*p* value
	(median ± SEM)	(median ± SEM)	
CD11b	5148±890	3418±610	0.2176
CD13	2368±366	2091±152	0.9705
CD15	8262±769	3732±812	**0.0015**
CD16	6932±1112	8731±743	0.0630
CD33	2768±304	1644±241	**0.0288**
CD63	1355±246	1035±152	0.1230
CD66b	10198±1775	7671±604	**0.0355**
arginase	1533±123	1904±124	0.0630

LDGs and NDGs were isolated from the blood of HIV+ patients with $CD4^+$ T cell counts <350 cells/μL (n = 10) as described in materials and methods. Expression levels of phenotypic markers were determined by flow cytometry.
doi:10.1371/journal.pone.0072034.t008

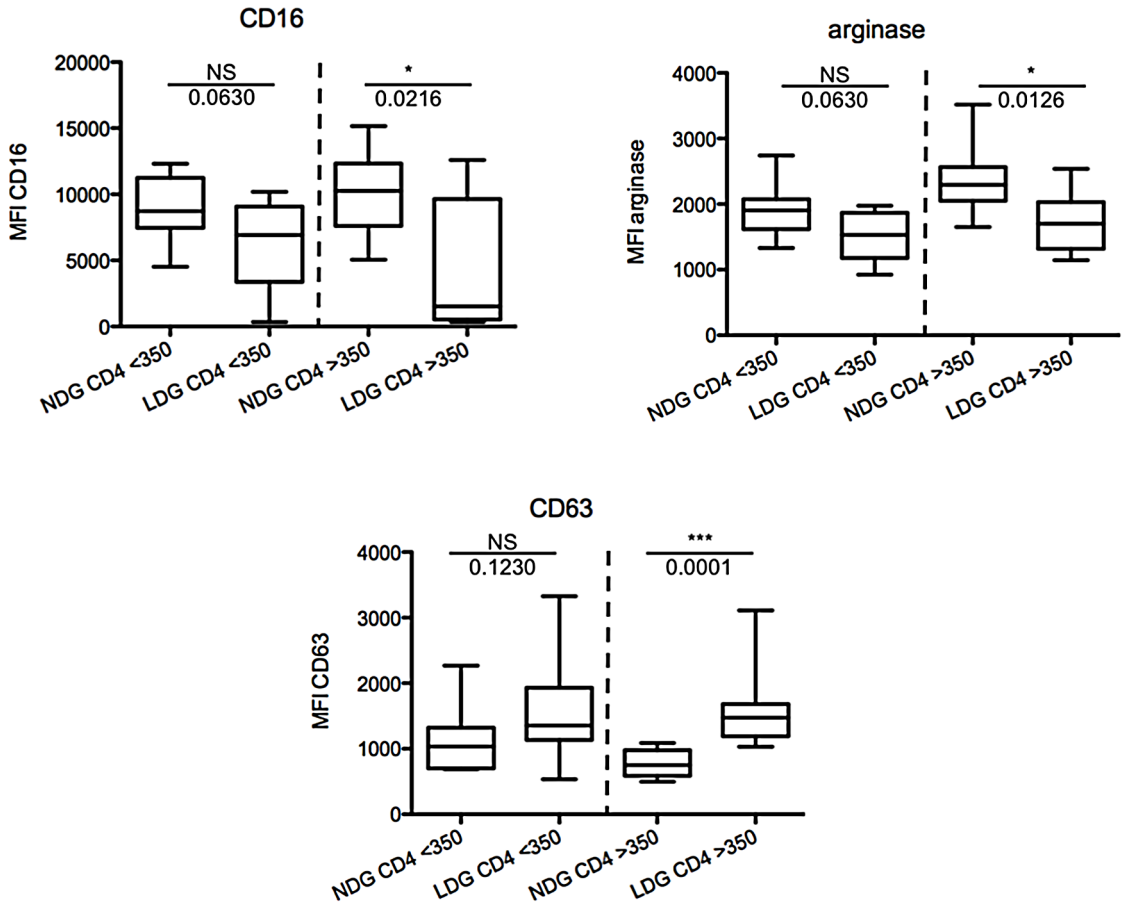

Figure 4. Phenotypes of NDGs and LDGs in CD4low and CD4high HIV+ patients. PBMCs and NDGs were isolated from the blood of HIV+ patients with $CD4^+$ T cell counts >350 (n = 11) or <350 cells/μL (n = 10) as described in materials and methods and the expression levels of phenotypic markers were determined by flow cytometry. Isotype controls: <1%. Statistical significance was determined by a two-tailed Mann-Whitney test. Box = interquartile range and median; whiskers = range.
doi:10.1371/journal.pone.0072034.g004

(PMA) and/or N-formyl-methionyl-leucyl-phenylalanine (fLMP) has been described previously ([23]: whereas these stimuli result in activation and degranulation of neutrophils, the phenotype of these activated neutrophils differs from that of LDGs we described [17], as CD13 and CD16 are both downregulated on activated NDGs.

Further work into novel markers of immune suppression, such as activation of granulocytic cells, is warranted as this may result in improvement in the clinical management of patients with HIV infection through: 1) better evaluation of disease severity (including the stage and rate of progression of the disease) and 2) informing the timing and choice of treatment initiation so as to minimise morbidity associated with opportunistic infections, drug resistance and medication side effects.

Table 9. LDGs and NDGs in HIV+ patients in CD4high HIV+ patients.

	LDGs	NDGs	*p* value
	(median ± SEM)	(median ± SEM)	
CD11b	4413±694	2684±767	0.5994
CD13	2137±564	3915±345	0.1007
CD15	10012±1277	2270±693	**0.0003**
CD16	1528±1483	10259±934	**0.0216**
CD33	3575±285	2114±196	**0.0016**
CD63	1474±178	751±57	**0.0001**
CD66b	8899±1775	7697±604	0.1679
arginase	1700±141	2294±150	**0.0126**

Ldgs And Ndgs Were Isolated From The Blood Of Hiv+ Patients With Cd4+ T Cell Counts >350 Cells/μL (N = 11) As Described In Materials And Methods. Expression Levels Of Phenotypic Markers Were Determined By Flow Cytometry.
doi:10.1371/journal.pone.0072034.t009

Supporting Information

Figure S1 Phenotypic analysis of NDGs. NDGs were isolated from the blood of HIV+ patients with $CD4^+$ T cell counts >350 (n = 11) or <350 cells/μL (n = 10) as described in materials and methods and the expression levels of phenotypic markers were determined by flow cytometry. Isotype controls: <1%. Statistical significance was determined by a two-tailed Mann-Whitney test. Box = interquartile range and median; whiskers = range.
(TIF)

Author Contributions

Conceived and designed the experiments: TC IM PK. Performed the experiments: TC PK. Analyzed the data: TC M. Munder PB SH M. Modolell GT IM PK. Wrote the paper: TC PK.

References

1. Drescher B, Bai F (2012) Neutrophil in viral infections, friend or foe? Virus Res.
2. Kuritzkes DR (2000) Neutropenia, neutrophil dysfunction, and bacterial infection in patients with human immunodeficiency virus disease: the role of granulocyte colony-stimulating factor. Clin Infect Dis 30: 256–260.
3. Saitoh T, Komano J, Saitoh Y, Misawa T, Takahama M, et al. (2012) Neutrophil extracellular traps mediate a host defense response to human immunodeficiency virus-1. Cell Host Microbe 12: 109–116.
4. Munder M, Mollinedo F, Calafat J, Canchado J, Gil-Lamaignere C, et al. (2005) Arginase I is constitutively expressed in human granulocytes and participates in fungicidal activity. Blood 105: 2549–2556.
5. Cederbaum SD, Yu H, Grody WW, Kern RM, Yoo P, et al. (2004) Arginases I and II: do their functions overlap? Mol Gen Metabol 81: S38–S44.
6. Munder M (2009) Arginase: an emerging key player in the mammalian immune system. Br J Pharmacol 158: 638–651.
7. Ochoa AC, Zea AH, Hernandez C, Rodriguez PC (2007) Arginase, prostaglandins, and myeloid-derived suppressor cells in renal cell carcinoma. Clin Cancer Res 13: 721s–726s.
8. Popovic PJ, Zeh HJ 3rd, Ochoa JB (2007) Arginine and immunity. J Nutr 137: 1681S–1686S.
9. Bronte V, Zanovello P (2005) Regulation of immune responses by L-arginine metabolism. Nat Rev Immunol 5: 641–654.
10. Munder M, Choi BS, Rogers M, Kropf P (2009) L-arginine deprivation impairs Leishmania major-specific T cell responses. European Journal of Immunology 39: 2161–2172.
11. Weinberg JB, Lopansri BK, Mwaikambo E, Granger DL (2008) Arginine, nitric oxide, carbon monoxide, and endothelial function in severe malaria. Curr Opin Infect Dis 21: 468–475.
12. Zea AH, Culotta KS, Ali J, Mason C, Park HJ, et al. (2006) Decreased Expression of CD3 zeta and Nuclear Transcription Factor kappa B in Patients with Pulmonary Tuberculosis: Potential Mechanisms and Reversibility with Treatment. J Infect Dis 194: 1385–1393.
13. Abebe T, Hailu A, Woldeyes M, Mekonene W, Bilch K, et al. (2012) Local increase of arginase activity in lesions of patients with cutaneous leishmaniasis in Ethiopia. PLoS NTD 6: e1684.
14. Takele Y, Abebe T, Weldegebreal T, Hailu A, Hailu W, et al. (2012) Arginase activity in the blood of patients with visceral leishmaniasis and HIV infection. PLoS NTD 7(1): e1977.
15. Abebe T, Takele T, Weldegebreal T, Cloke T, Closs E, et al. (2013) Arginase: a marker of disease status in patients with visceral leishmaniasis. PLoS NTD in press.
16. Cloke T, Garvery L, Choi BS, Abebe T, Hailu A, et al. (2010) Increased arginase activity correlates with disease severity in HIV seropositive patients. Journal of Infectious Diseases 202: 374–385.
17. Cloke T, Munder M, Taylor GP, Müller I, Kropf P (2012) Characterization of a novel population of low-density granulocytes associated with disease severity in HIV seropositive patients. PLOS ONE 7: e48939.
18. Kropf P, Baud D, Marshall SE, Munder M, Mosley A, et al. (2007) Arginase activity mediates reversible T cell hyporesponsiveness in human pregnancy. Eur J Immunol 37: 935–945.
19. Phillips A, Pezzotti P (2004) Short-term risk of AIDS according to current CD4 cell count and viral load in antiretroviral drug-naive individuals and those treated in the monotherapy era. AIDS 18: 51–58.
20. Gazzard BG (2008) British HIV Association Guidelines for the treatment of HIV-1-infected adults with antiretroviral therapy 2008. HIV Med 9: 563–608.
21. Elbim C, Prevot MH, Bouscarat F, Franzini E, Chollet-Martin S, et al. (1994) Polymorphonuclear neutrophils from human immunodeficiency virus-infected patients show enhanced activation, diminished fMLP-induced L-selectin shedding, and an impaired oxidative burst after cytokine priming. Blood 84: 2759–2766.
22. Rotondo R, Bertolotto M, Barisione G, Astigiano S, Mandruzzato S, et al. (2011) Exocytosis of azurophil and arginase 1-containing granules by activated polymorphonuclear neutrophils is required to inhibit T lymphocyte proliferation. J Leukoc Biol 89: 721–727.
23. Kuijpers TW, Tool AT, van der Schoot CE, Ginsel LA, Onderwater JJ, et al. (1991) Membrane surface antigen expression on neutrophils: a reappraisal of the use of surface markers for neutrophil activation. Blood 78: 1105–1111.
24. Martin-Martin B, Nabokina SM, Blasi J, Lazo PA, Mollinedo F (2000) Involvement of SNAP-23 and syntaxin 6 in human neutrophil exocytosis. Blood 96: 2574–2583.