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Differential expression of chemokine receptors on monocytes in TB and HIV S

Wegene Tamene [a,*], Vincent C. Marconi [b], Meseret Abebe [c], Liya Wassie [c], Yohannes Belay [a], Amha Kebede [a,1], Ulrich Sack [d], Rawleigh Howe [c]

[a] TB and HIV Directorate, Ethiopian Public Health Institute, Addis Ababa, Ethiopia
[b] Emory University School of Medicine and Rollins School of Public Health, Atlanta, Georgia
[c] Bacterial and Viral Diseases Research Directorate, Armauer Hansen Research Institute, Addis Ababa, Ethiopia
[d] Institute of Immunology, Leipzig University, Leipzig, Saxony, Germany

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ABSTRACT

In the present study, we defined multiple chemokine receptors expressed by classical, intermediate and non-classical monocyte subsets in TB, HIV and TB/HIV co-infection and associate it with the perturbation of monocyte subsets due to the diseases. Peripheral blood mononuclear cells from TB+ (n = 34), HIV+ (n = 35), TB + HIV+ (n = 12), as well as TB-HIV- healthy controls (n = 39), were tested for monocyte phenotyping by flow cytometry. Frequencies of intermediate and non-classical monocytes were significantly higher in TB and/or HIV disease relative to healthy controls. CCR2 and CX3CR1 were significantly higher on monocytes in TB disease, whereas CCR4 and CCR5 were present at higher levels in HIV disease. TB/HIV co-infected patients exhibited CCR2, CCR5 and CX3CR1 levels intermediate to TB and HIV subjects, while CCR4 was at a higher level than HIV. Despite the increase in the expression of chemokine receptors due to disease conditions, chemokine receptors maintained their original expression pattern on monocyte subsets. Our data provided new insight into the disease-specific but not monocyte subsets-specific modulation of chemokine receptors in TB and HIV.

1. Introduction

Tuberculosis (TB), HIV and TB/HIV co-infection continue to be major global health problems [1], with the continued need for a better understanding of immuno-pathogenesis for improved diagnosis, treatment and vaccines.

Monocytes or monocyte-derived cells represent the major host cells to restrain *M. tuberculosis* infection, and one of the two most important host cell types in HIV infection [2–5]. Studies in man and animal models indicate the importance of the IFNγ axis in the appropriate activation of macrophages as well as the immigration of CCR2-bearing monocytes in protection against high TB inoculums [6–8]. Along with CD4 T cells, monocytes represent a key infected cell in HIV disease and through tissue emigration likely contribute to viral dissemination, and may play a particularly prominent role in AIDS-related dementia complex [9].

Over the past several years, a new body of evidence has emerged delineating three major subsets of monocytes, the classical or CD14++, the non-classical or CD16++ and, intermediate monocytes expressing both CD14 and CD16 molecules [10]. While retaining

* Corresponding author.
E-mail addresses: wegeneta@yahoo.com, wegeneta7@gmail.com (W. Tamene).
[1] Present address: African Society of Laboratory Medicine, Addis Ababa, Ethiopia.

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some degree of plasticity, the monocytes have distinct homing patterns, with the classical monocyte (CM) subset migrating immediately into tissues, the non-classical monocytes (NCM) patrolling blood vasculature, and intermediate monocytes (IM) with varied function [11–14]. Remarkably, in most infectious diseases and inflammatory non-infectious diseases, the distribution of these subsets is skewed from mostly CM seen in healthy individuals to higher levels of IM and NCM [11]. The reason for the change in the frequency of monocyte subsets in TB and HIV necessitates exploration.

TB and HIV are not only implicated in changing monocyte subsets frequency but also in altering surface markers' expression including chemokine receptors [3,15,16]. The chemokine receptors such as CCR2 and CX3CR1 were used to classify monocytes into distinct subsets [17] as they are differentially expressed on the CM and NCM in healthy individuals. However, the role of these chemokine receptors is beyond simple subset classification as their expression alters in disease conditions and might impact disease pathogenesis. Change in the expression of chemokine receptors has either protective or deleterious outcomes. For example, CCR2 plays a vital role in restraining *M.TB* by recruiting monocytes from bone marrow to the circulation and then to the site of inflammation [18]. On the contrary, the same molecule is associated with disease severity [19,20]. Similarly, CCR5 expression has been implicated in enhanced recruitment of cells but also with immuno-regulatory roles [21]. A positive correlation between CCR5 expressing $CD4^{+}$ T cells frequency with disease progression and high viral load due to the continuous immune activation in HIV patients has been reported previously [22]. Most of the studies characterizing the expression and role of chemokine receptors have been from animal studies [7,8,20,18,23]. To our knowledge, there are no published studies on the expression of chemokine receptors on monocytes in TB/HIV co-infection. One recent study reported increased total monocyte counts and inflammatory monocytes in HIV with latent TB infection (LTBI) and prior history of TB infection compared to HIV patients with no TB infection [24]. The same group also reported an association of HIV with CX3CR1 expression in monocytes.

Furthermore, few studies have explored the levels of chemokine receptors on different subsets of monocytes and how these may be altered during disease. Therefore, this study explored the differential expression patterns of chemokine receptors in TB, HIV, and TB/HIV co-infection on the cell surface of monocytes subsets, the role of these differential expression patterns in the recruitment of monocyte subsets, and their association with disease pathogenesis.

2. Material and methods

2.1. Study participants

The study was ethically approved by the National Research and Ethics Review Board of Ethiopia (Ref. No. 3.10/786/07), University of Leipzig (Ref. No, 185-14-02,062,014) and Ethiopia Public Health Institute (Ref. No. SERO 152-1-2006) institutional review boards before the start of data collection. The study was conducted in agreement with the Declaration of Helsinki 1996. All participants were briefed about the study objective and gave written consent to be part of the study which involves the collection of both demographic data and biological specimens.

A total of 120 adult participants were recruited from health facilities in Addis Ababa, Ethiopia. These are i) TB ($n = 34$), ii) HIV ($n = 35$), iii) TB/HIV ($n = 12$) and iv) apparently healthy controls, HC, ($n = 39$). The presence of autoimmune disorders, chronic illness, pregnancy, corticosteroid medication, or previous TB history were exclusion criteria for all study participants. All participants were recruited before the start of both TB and HIV treatment except for TB/HIV patients. Five out of twelve participants were on ART in the TB/HIV group and all of them were not on *anti*-TB drugs.

2.2. Defining TB and HIV status

At the primary health facility, TB suspects with appropriate clinical presentation were screened with Ziehl-Neelsen sputum smear microscopy or GeneXpert molecular assay, and X-ray. Later TB status was confirmed by MIGT or LJ culture at the National TB Reference Laboratory, EPHI on sputum collected before the start of TB treatment. HIV infection was diagnosed using rapid HIV-1 diagnostic tests based on an established national HIV testing algorithm.

2.3. PBMC isolation and flow cytometry

Peripheral blood mononuclear cells (PBMCs) were isolated from 20 mL of heparinized venous blood according to our previous protocol [25]. Briefly centrifuged, plasma was separated from the whole blood, aliquot to multiple vials and stored at $-80\,^{\circ}C$ until use. PBMCs were isolated from the remaining packed cells layered on Ficoll-plaque plus (GE) in Leucosep tubes (Greiner). One million PBMCs each were allocated to test and control tubes followed by staining using anti-CD14 PE (BD Bioscience) and CD16 APC-H7 (Biolegend) in both test and control tubes while CCR2 PE-Cy7, CCR4 BV421, CCR5 PerCP, CX3CR1 FITC, (Biolegend) in test and IgG1 PE-Cy7, IgG1 BV421, IgG2a PerCP and IgG2b FITC, (Biolegend) in control tube. Phenotyping of monocytes was performed on FACSCanto II flow cytometer using FACSDiva software (BD Bioscience) then data were analyzed by using Flowjo 9.4.6 Software (FlowJo, USA).

Monocyte-enriched populations were gated by light scatter parameters and doublets were excluded (Supplementary figure 1). The three monocyte subpopulation, CM, IM and NCM, were defined by the expression of CD14 and CD16. Chemokine receptor expression was defined on monocyte subsets or the sum of all three. Specific fluorescence for a given chemokine receptor was calculated by subtracting the median fluorescence intensity (MFI) of isotype control from that of the marker of interest. Thus net MFI (nMFI) was calculated for both total monocytes and the three monocyte subsets. nMFI was used for statistical analysis. An average of three hundred

thousand, ranging from two to five hundred thousand, total events were acquired for each sample. The mean total monocytes gated was approximately 19,000. Samples with fewer than 1700 acquired monocytes and those with poor staining were excluded from further analysis.

2.4. $CD4^+$ T cell count and HIV-1 plasma RNA viral load determination

Venous blood collected using an EDTA tube was used for $CD4^+$ T cell count determination on FACSCalibur (BD Bioscience). The remaining blood was centrifuged, plasma separated and HIV-1 plasma RNA viral load measured on Cobas Amplipre/Taqman automated real-time PCR (Abbott Laboratories).

2.5. Statistical analysis

Data analysis was performed using Statistical Package for Social Science version 20.0 (SPSS, IBM, Armonk, USA) and GraphPad Prism 6 (GraphPad Software, La Jolla California USA). Group comparisons were made by the non-parametric Kruskal-Wallis test and p-values were adjusted according to Dunn's multiple comparisons. The middle horizontal line in dot plots represents the median value. The correlation was assessed with the non-parametric Spearman correlation test; results were considered statistically significant with p-values less than 0.05.

3. Results

3.1. Basic demographic and clinical characteristics of study participants

The median age of the participants was 30 years ranging from 18 to 72 years. While the age of HC, TB and HIV patients were in a similar range, TB/HIV patients were slightly older with a median age of 41 years. Male participants accounted for a slightly higher percentage, 55.1, than female participants. A significant proportion of HIV-positive subjects presented with WHO clinical stage I or II disease (83.9%) whereas all TB/HIV co-infected subjects presented with clinical stage III or IV. Both HIV and TB/HIV infected subjects had high HIV-1 plasma RNA viral load. The median HIV-1 plasma RNA viral load was 52,771 and 360,627 copies per milliliter for HIV and TB/HIV patients, respectively (Table 1).

3.2. Perturbations of monocyte subsets during TB, HIV and TB/HIV co-infection

Fig. 1 illustrates monocyte subsets frequency as well as expression intensity of CD14 and CD16 on monocytes in TB, HIV and TB/HIV co-infected patients. The frequency of IM and/or NCM in TB, HIV and TB/HIV patients was significantly higher than in the comparator HC (Fig. 1A). The highest frequency of IM subset was in TB patients ($p = 0.001$) followed by HIV ($p = 0.011$) and TB/HIV ($p = 0.070$) patients. On the other hand, the frequency of the NCM subset was the highest among HIV patients ($p < 0.0001$). Increased frequencies of both IM and NCM subsets in TB/HIV co-infected patients were apparent but the frequency of NCM was higher than that of IM (Supplementary table 1).

Panels B and C of Fig. 1 depict the density of CD14 and CD16, respectively, among total monocytes and among each subset, stratified by clinical cohort. We observed reduced CD14 density, particularly among HIV seropositive patients. On the other hand, the density of CD16 increased in all monocyte subsets, particularly in subjects with TB disease. Hence, each diseased cohort exhibited not only different frequencies of the three monocyte subsets but also different densities of CD14 and CD16 within each subset.

3.3. Chemokine receptors differentially increase in TB and HIV

Several chemokines and the level of their receptors altered in TB and HIV. Among those receptors, we focused on chemokine receptors more pertinent to monocytes/myeloid cells in TB and HIV [26–28]. The gating strategy for CCR2, CCR4, CCR5 and CX3CR1 on total monocytes as well as monocyte subsets is shown in Supplementary figure 1.

Fig. 2 depicts differential increases of chemokine receptors among TB and HIV within total monocytes. The density of CCR2 ($p =$

Table 1
Characteristics of study participants (n = 120).

Characteristic	HC (n = 39)	HIV (n = 35)	TB (n = 34)	TB/HIV (n = 12)
Age in years, median (IQR)	28 (23–32)	33 (27–37)	30 (23–35)	41 (22–47)
Male sex at birth (%)	58.3	42.4	69.2	50.0
Body mass index	21.9	20.6	18.3	19.6
HIV clinical stage (%)				
Stage I & II	N/A	83.3	N/A	0
Stage III & IV	N/A	16.6	N/A	100
HIV viral load, median (IQR)	N/A	5.3×10^4 ($1–17.9 \times 10^4$)	N/A	3.6×10^5 ($LDL^{\#} – 2 \times 10^6$)
Absolute CD4, median (IQR)	N/A	270 (94–599)*	N/A	210 (85–289)**

Lower detectable limit (LDL): HIV-1 viral load 20 copies/ml. Number of samples with CD4 result: *24, **7.

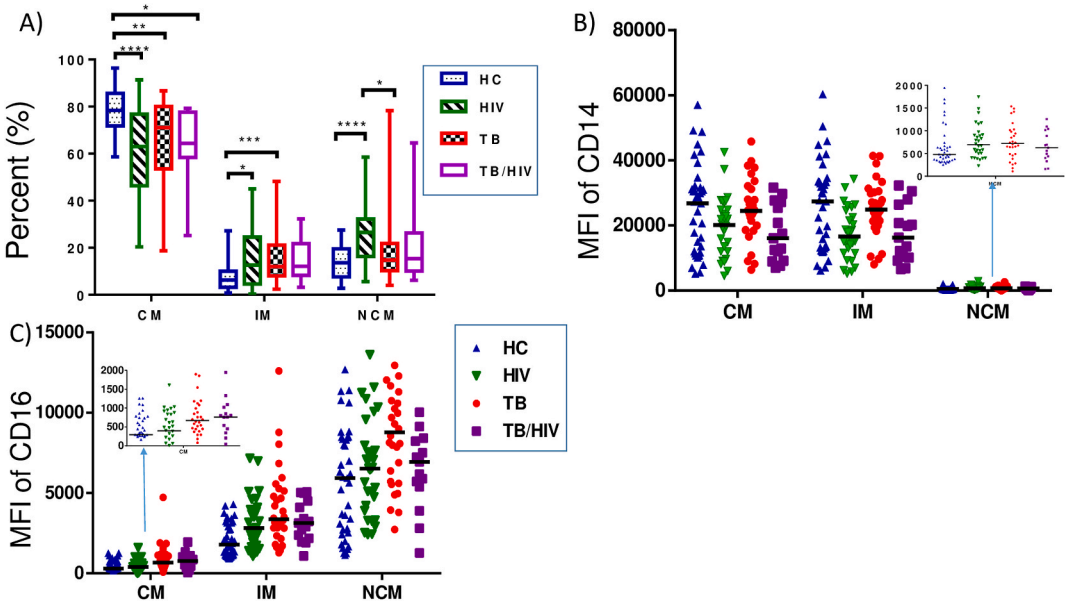

Fig. 1. Frequency of monocyte subsets and expression intensity of CD14 and CD16 in each subset. The graphs represent the four study groups: HC (n = 39), TB (n = 34), HIV (n = 35), TB/HIV (n = 12). A) Monocytes subset frequency in health and dis disease, B) MFI of CD14 and, C) MFI of CD16 within CM, IM and NCM subsets in HC, TB, HIV and TB/HIV co-infection. The insert figures in the direction of the arrow (B, C) are zoomed pictures of the same figure to enhance the visibility of low-frequency subpopulations. Asterisks represent *P*-values of: * 0.05, **0.01, ***0.001, ****0.0001.

0.0052) and CX3CR1 (p = 0.0025) on total monocytes was significantly higher in TB patients compared to HC (Fig. 2A and B). Both CCR2 and CX3CR1 densities were slightly increased in HIV patients compared to HC but it did not reach statistical significance. TB patients had significantly higher CX3CR1 density compared with HIV patients (p = 0.0142).

On the other hand, the MFI of CCR4 on total monocytes was significantly increased in HIV (p = 0.0138) and TB/HIV patients (p = 0.0252) compared to HC (Fig. 2C). Similarly, the density of CCR5 on total monocytes was higher in HIV (p = 0.0001) and TB/HIV (p =

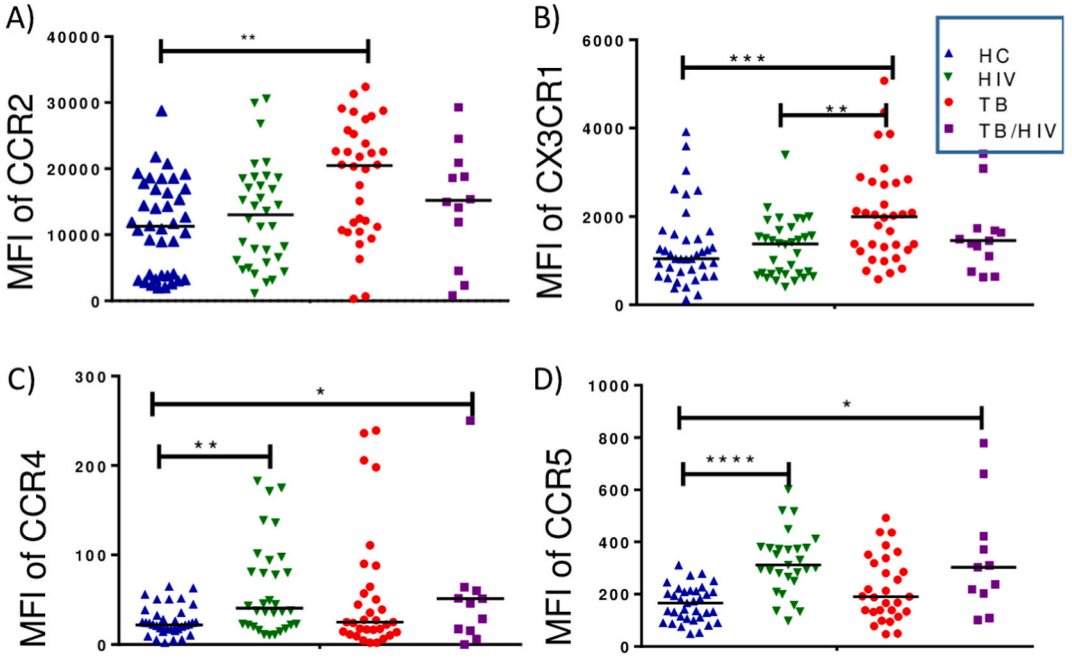

Fig. 2. Expression of chemokine receptors on total monocytes in HC, TB, HIV and TB/HIV patients MFI of A) CCR2, B) CX3CR1 C) CCR4 and D) CCR5 on total monocytes was determined. The middle line in each dot plot represents median values. Group comparison was computed by the Kruskal-Wallis test followed by Dunn's multiple comparison test. Asterisks represent p-value of 0.05*, 0.01**, 0.001***, and 0.0001****.

0.0132) patients compared to HC (Fig. 2D). Conversely, the densities of CCR4 and CCR5 in TB patients were no different from their levels in HC.

TB/HIV co-infected patients had a distinct chemokine receptor expression pattern with moderate to high levels of the four chemokine receptors (Fig. 2A–D). Thus, CCR2, CCR5 and CX3CR1 levels in co-infected patients were intermediate to levels in HIV and TB patient groups, whereas CCR4 expression was higher than in both TB and HIV cohorts.

As $CD4^+$ T cell count and HIV-1 plasma RNA viral load are established indices for HIV disease monitoring, we assessed their correlation with CCR4 and CCR5 levels in HIV patients (Fig. 3). CCR5 ($r = 0.3469$; $p = 0.0652$) but not CCR4 ($r = -0.3066$; $p = 0.1450$) had weak, though not statistically significant, correlation with HIV-1 plasma RNA viral load while no significant correlation with $CD4^+$ T cell count for both chemokine receptors. Chemokine receptors data were converted to categorical data based on HIV clinical stage, HIV-1 plasma RNA viral load and $CD4^+$ T cell count then comparisons were made between categories (Supplementary table 2). There was no significant difference in the expression levels of CCR2, CX3CR1, CCR4 and CCR5 between categories except higher CCR5 in clinical stage I/II.

3.4. Chemokine receptors have similar expression patterns among monocyte subsets in TB and HIV

Following the determination of chemokine receptors expression on total monocytes, we assessed their expression pattern at the monocyte subset level (Fig. 4A–D, and Supplementary table 3). Accordingly, first, we defined the density of CCR2, CCR4, CCR5 and CX3CR1 among HC. As has been well documented, the highest CCR2 expression was on the CM followed by moderate expression on IM but nearly absent on the NCM subset. In contrast, the highest densities of CCR4, CCR5, and CX3CR1 were on the IM subset. Second, we assessed the expression of the four markers among monocyte subsets in disease cohorts. In TB patients, the intensity of CCR2 was significantly higher on CM ($p = 0.0001$), IM ($p = 0.0230$), and NCM ($p = 0.0003$) compared with corresponding monocyte subsets in HC. Likewise, the density of CX3CR1 was significantly elevated on CM ($p = 0.0104$) and IM ($p = 0.0146$) but not on NCM in TB patients compared with matching subsets in HC. By the same token, the increase in density of CCR4 and CCR5 on total monocytes amongst HIV seropositive patients was also apparent on all monocyte subsets. The density of CCR5 was significantly higher in CM ($p < 0.0001$), IM ($p = 0.0050$) and NCM ($p = 0.0050$) while CCR4 density was significantly higher in CM ($p = 0.0047$) and NCM ($p = 0.0278$) compared with corresponding subsets in HC. In general, the IM subset expresses the highest level of the aforementioned chemokine receptors except for CCR2.

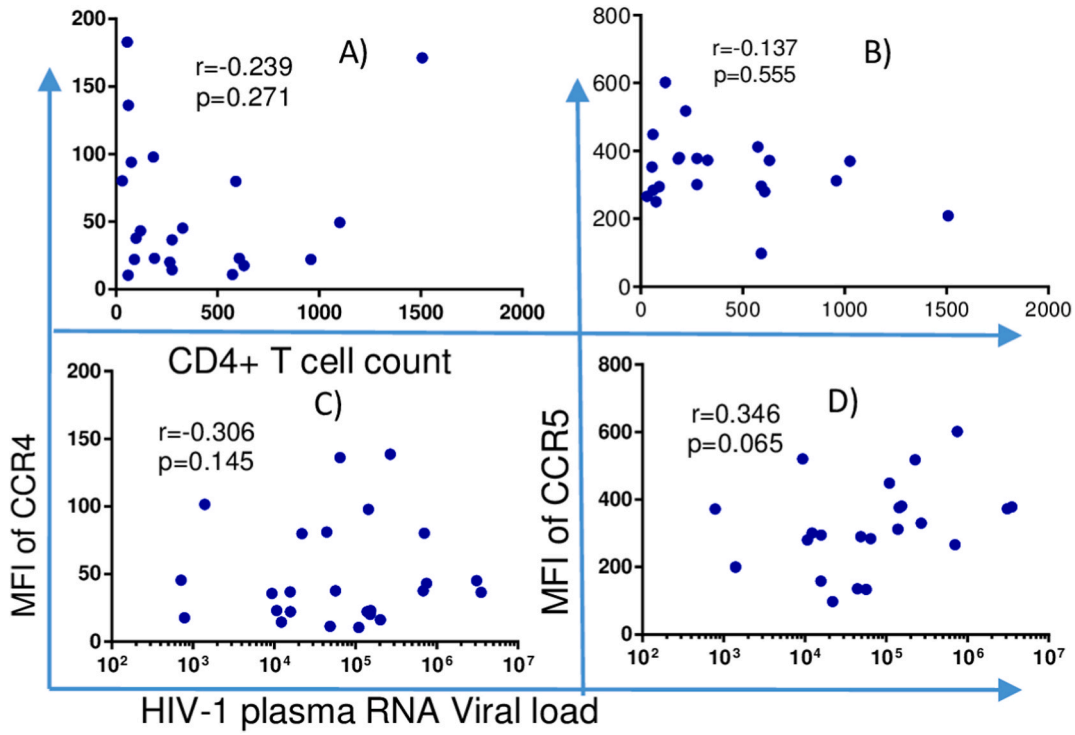

Fig. 3. Correlation of CCR4 and CCR5 with $CD4^+$ T cell count and HIV-1 plasma RNA viral load in HIV patients. Correlation of A) CCR4 vs CD4, B) CCR5 vs CD4, C) CCR4 vs HIV-1 RNA viral load and D) CCR5 vs HIV-1 RNA viral load. $CD4^+$ T cell count presented as number of cells/mm3, HIV-1 plasma RNA viral load as number of viral copies/milliliter are depicted. Non-parametric Spearman correlation test used to assess correlation.

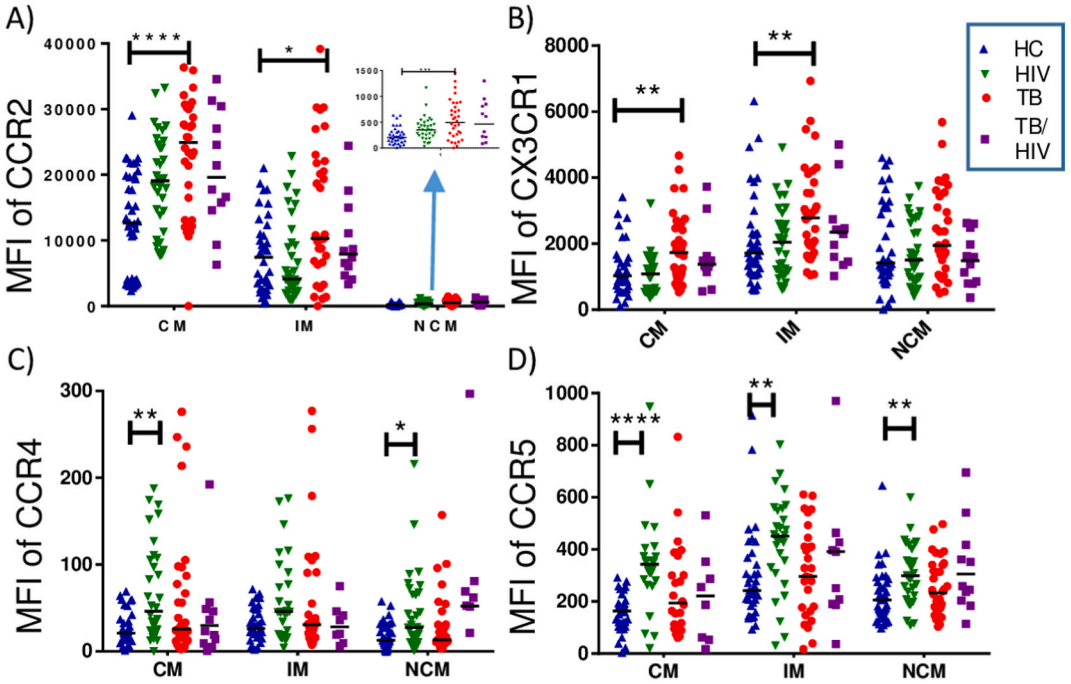

Fig. 4. Chemokine receptors expression on CM, IM and NCM in health and disease. A) CCR2, B) CX3CR1, C) CCR4 and D) CCR5. The y-axis represents MFI of each marker. Each symbol in the plots represents patient specific values and the middle line represents the median. Comparisons across cohorts was made by the Kruskal-Wallis test followed by Dunn's adjustment. Asterisks represent p-value of 0.05*, 0.01**, 0.001*** and 0.0001****.

3.5. Association of chemokine receptors with monocyte subsets in TB and HIV

Given the variable expression of CCR2, CCR4, CCR5 and CX3CR1 within the different monocyte subsets, we determined whether levels were correlated with the frequencies of these subsets (Fig. 5A–D). Among pooled subjects from all cohorts, we observed significant positive and negative correlations between CCR2 and frequency of CM ($r = 0.255$; $p = 0.005$) and NCM ($r = -0.317$; $p = 0.000$) subsets, respectively. Conversely, we observed a significant negative correlation between CX3CR1 and CM ($r = -0.290$; $p = 0.001$) and a positive correlation between CX3CR1 and IM ($r = 0.380$; $p = 0.000$) subsets. CCR5 had a positive correlation with NCM ($r = 0.3016$; $p = 0.0023$) though negatively correlated with CM ($r = -0.2219$; $p = 0.0265$) which solidifies our finding in section 3.2. However, CCR4 did not show a direct correlation with any of the monocyte subsets.

4. Discussion

Enhanced frequencies of IM and NCM subsets have been observed both in TB, HIV and other inflammatory and non-inflammatory diseases [3,11] and our result confirmed these findings. Expansion in the frequency of $CD16^+$ cell populations is associated with increased susceptibility and pathology in these diseases [29,30]. $CD16^+$ monocytes preferentially harbor HIV and are capable of transmigrating into deeper organs such as the central nervous system [30–32]. The increase in IM and NCM in our patients was accompanied by significant increases in CD16 density and a decrease in CD14 density especially in HIV and TB/HIV groups. The decrease in the CD14 density could be due to the shedding of CD14 from monocytes stimulated by LPS translocated from the gut to circulation in chronic HIV infection [33]. Soluble CD14 in HIV positive individuals is associated with disease progression, adverse events and comorbidities.

In addition to the change in the distribution of subsets' proportion, expansion in the numbers of monocytes has been reported in TB patients [34]. One factor contributing to the expansion of monocytes as well as the perturbation of subset distribution could be chemokine receptors. Hence, we evaluated the expression of chemokine receptors on monocytes in HIV and/or TB cohorts as well as its correlation with changes in monocyte subset frequency. Our finding demonstrated an elevated density of chemokine receptors in both TB and HIV disease. All monocyte subsets expressed significantly higher levels of CCR2 in TB and slightly increased CCR2 levels in HIV infection. We speculate that once monocytes are recruited to circulation in CCR2 dependent manner, the IM and NCM stay there for an extended period due to elevated CX3CR1 and CCR5 expression which -promote greater viability, apart from their roles in adhesion and migration [35–38]. The positive correlation of CCR2 density with CM, while CX3CR1 and CCR5 associations with IM and NCM, respectively strengthen the above possibility.

Increased frequency of $CD16^+$ monocyte in HIV and TB/HIV patients was associated with disease severity in chronic obstructive

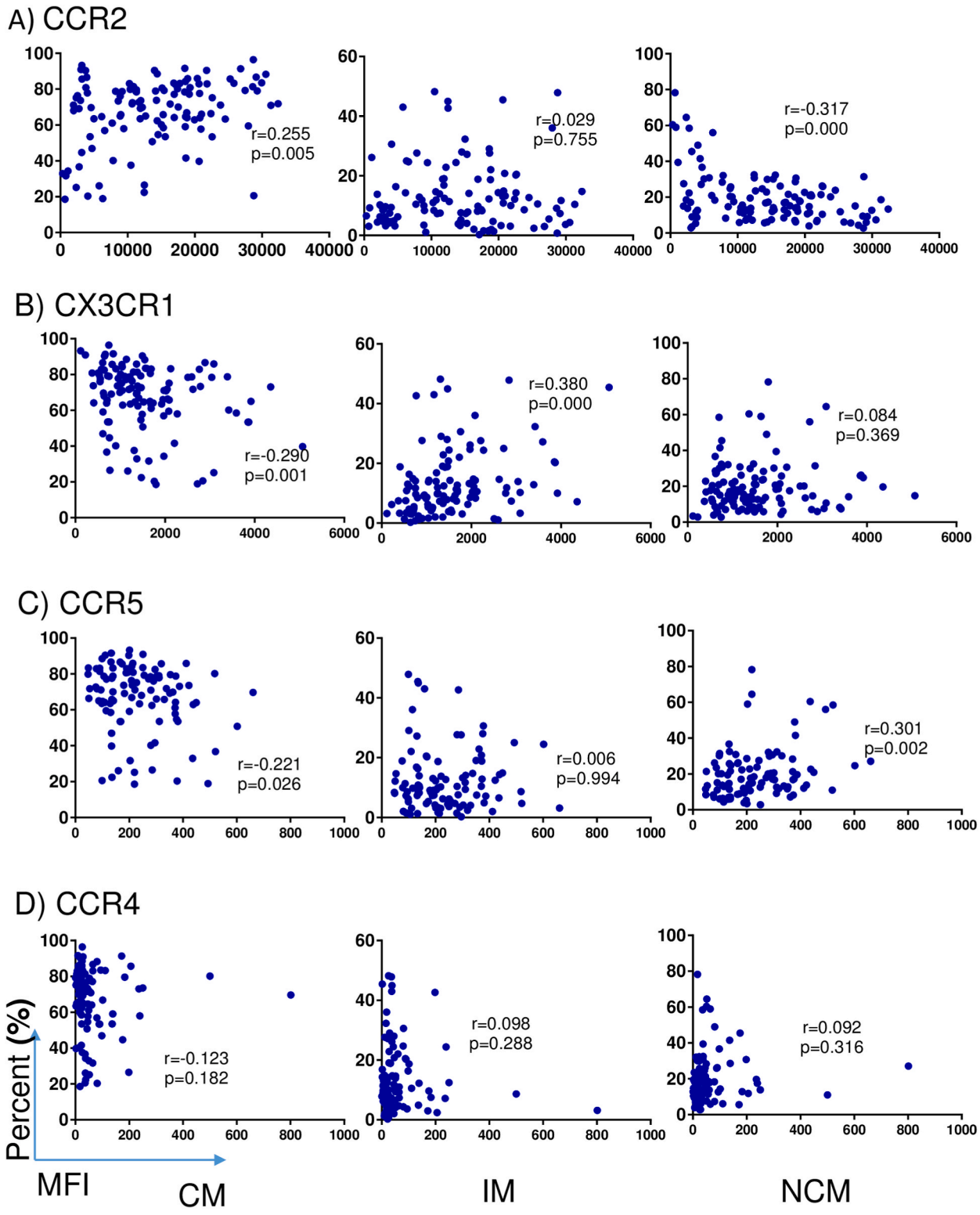

Fig. 5. Correlation of chemokine receptors with monocyte subsets. Figures in row represent chemokine receptors: A) CCR2, B) CX3CR1, C) CCR4 and D) CCR5. Figures in a column are from a single monocyte subset either: CM-classical monocytes, IM-intermediate monocyte, NCM-non-classical monocyte In figures, the y-axis represents percentage of monocyte subsets; the x-axis represents median fluorescence intensity (MFI) of chemokine receptors. Correlations were made on total participants without stratifying the data into study groups.

pulmonary disease (COPD) [39], HIV associated dementia (HAD) [40] and viral spreading [41,42]. Similarly, increased expression of chemokine receptors was associated with TB disease severity [27,43–45]. Portales et al. reported a positive correlation of CCR5 expressed in T cells with HIV disease severity. CCR5 level remained similar despite declining HIV-1 plasma RNA viral load levels after the successful commencement of ART [22]. Likewise, our result showed a positive correlation between CCR5 and HIV-1 plasma RNA viral load while a negative correlation with $CD4^+$ T cell count in participants with HIV and TB/HIV. Studies associating CCR5 levels with increased infiltration of monocytes into the central nervous system in HIV patients [46–48]. Similarly, there might be increased mobility and accumulation of functionally impaired monocytes in the lung due to increased chemokine receptors expression, which possibly results in increased susceptibility of HIV patients to MTB and other pulmonary diseases.

Importantly, the enhanced chemokine receptors expression we observed in our participants was disease-specific. CCR2 and CX3CR1 were significantly elevated on monocytes from TB patients relative to healthy controls, whereas CCR4 and CCR5 levels were higher among HIV and TB/HIV co-infected patients. Conversely, the variability in chemokine receptor levels appeared to be largely independent of the monocyte subset, so that if a given chemokine receptor levels were upregulated in association with disease, the same change was seen in all subsets. For example, CCR2 levels were upregulated in TB, and this was apparent in CM, IM and NCM even though the intensity of CCR2 was substantially lower in the NCM subset. Still, CCR2 on NCM was significantly increased in TB disease compared to HCand HIV patients. The same patterns were seen for CX3CR1 in TB, and CCR4 and CCR5 in HIV, though disease-specific changes did not reach statistical significance for every monocyte subset in all cases. These observations illustrate that disease is not simply impacting the relative frequencies of monocyte subsets each with fixed phenotypes, but rather the phenotypes themselves are being altered. In addition, this implies that at least some of the underlying factors are acting on all monocyte subsets coordinately. This type of mechanism could occur before their differentiation, for example during bone marrow maturation, perhaps in a fashion similar to that proposed for trained immunity, or once the subsets have appeared in circulation.

We demonstrated increased $CD16^+$ monocytes and chemokine receptors expression in TB/HIV co-infected patients. Similarly, Vanham et al. reported an increased frequency of $CD16^+$ monocytes in TB patients both with and without HIV [16]. In contrast, Huaman et al. reported no difference in the monocyte subset frequency between HIV patients with and without prior TB experience [24]. The discrepancy between our findings and Huaman et al. might be attributed to the population difference as our TB/HIV co-infected patients had active TB while theirs were HIV with previous TB experience. The same group also reported an association of HIV with CX3CR1 but no difference in the proportion of CX3CR1 expressing cells due to TB status. In agreement, we demonstrated a comparable increase in CX3CR1 expression on total monocytes in HIV and TB/HIV. To our knowledge, this is the first study to report several chemokine receptor expression phenotypes in the TB/HIV cohort.

Several factors have been associated with the direct modulation of chemokine receptors on monocytes. First, microbe products can influence monocytes through direct interaction with these cells. For example, HIV nef gene products are shown to down-regulate CCR4 and CCR5; whereas HIV tat upregulates CCR5 [49]. Secondly, cytokines either from the monocytes themselves or other sources modulate monocytes subsets as well as their chemokine receptor expression. For example, multiple signaling pathways activated by IFNγ mediate the down-regulation of CCR2 during monocyte differentiation into macrophages [50]. Thirdly, multiple hormones show differing effects on chemokine receptor expression. Cortisol increased CCR2, androgens increase CX3CR1 but steroids decreased CX3CR1, epinephrine impacted the distribution of monocyte subsets, and dopamine increased CCR5 [51–53]. Therefore, studies exploring which factors modulate particular chemokine receptors on monocyte subsets, and how such factors might be over or under expressed in different disease states need further exploration.

One limitation of this study is the small sample size of the TB/HIV cohort. Despite higher expression of chemokine receptors, the highest in some cases, often statistical significance was not reached when comparing with other cohorts. However, we managed to show altered monocyte subsets and chemokine receptor expression in HIV patients with active TB. Several factors contributed to the small sample size in TB/HIV cohort. Reduced prevalence of active TB in HIV due to full-scale ART service, the obligatory use of fresh blood for flow cytometry and difficulty obtaining sufficient cells in collected blood are some of the factors to mention. The other limitation of this study is the lack of follow-up data. Including after treatment data could have completed the study as HIV test-and-treat approach is widely adopted.

In summary, we confirm the widely observed elevated frequency of IM and NCM subsets in disease states such as TB and/or HIV. However, further comprehensive analysis of chemokine receptor levels on these subsets revealed a surprising underlying complexity, implying the presence of factors operating in a disease-specific but not monocyte subset-specific fashion. Further research into the microbial and host factors is warranted to better understand the role and mechanisms of monocyte diversity in these diseases.

Author contribution statement

WEGENE TAMENE: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Vincent C. Marconi; Ulrich Sack; Rawleigh Howe: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Meseret Abebe: Performed the experiments; Analyzed and interpreted the data.

Liya Wassie: Analyzed and interpreted the data; Wrote the paper.

Yohannes Belay: Performed the experiments.

Amha Kebede: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Data availability statement

Data will be made available on request.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e17202.

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