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# Sources of heterogeneity in human monocyte subsets

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ABSTRACT

Human monocytes are commonly defined and discriminated by the extent of their cell surface expression of CD14 and CD16, with associated differences in function and phenotype related to the intensity of expression of these markers. With increasing interest into the function and behaviour of monocytes, it is important to have a clear understanding of how differing strategies of analysis can affect results and how different protocols and population backgrounds can affect this highly morphogenic cell type.

Using PBMCs from populations with differing ethnicities and histories of parasite exposure we have characterized monocyte phenotype based on intensity of CD14 and CD16 expression. Using the surface markers HLA-DR, CCR2 and CX3CR1, we compared monocyte phenotype between populations and further assessed changes in monocytes with freezing and thawing of PBMCs.

Our results reveal that there is a progression of surface marker expression based on intensity of CD14 or CD16 expression, stressing the importance of careful gating of monocyte subtypes. Freezing and thawing of the PBMCs has no effect generally on the monocytes, although it does lead to a decrease in CD16 and CX3CR1 expression. We show that there are differences in the monocyte populations based on ethnicity and history of exposure to the common parasites *Plasmodium falciparum* and *Schistosoma haematobium*.

This study highlights that blood monocytes consist of a continuous population of cells, within which the dominant phenotype may vary dependent on the background of the study population. Comparing results from monocyte studies therefore needs to be done with great care, as ethnic background of donor population, gating strategy and processing of PBMCs may all have an effect on outcome of monocyte phenotype.

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## 1. Introduction

Peripheral blood monocytes, which represent around 10% of circulating leukocytes in humans, are recognized as the largest pool of circulating progenitor cells and form a vital part of the immune system [1,2]. The enormous heterogeneity in human monocyte size, morphology, phagocytic function and cell adhesion was first described in 1989 [3] and was quickly followed by multiple attempts to discriminate monocyte subtypes. Recently new nomenclature was suggested by an expert panel in Brescia, Italy to define three subsets according to expression of CD14 and CD16 [4]. The major subset consists of CD14highCD16negative monocytes (CD14++CD16−), while the CD16 expressing monocytes are usually divided into a CD14highCD16low (CD14++CD16+) and a CD14lowCD16high (CD14+CD16++) subset. These groupings can identify monocytes that differ in surface expression of chemokine markers, major histocompatibility complex (MHC) class II expression and in their capacity to produce cytokines and phagocytose microbial particles [1,5–8]. However, while there have been some in-depth genetic and proteomic analyses of monocyte functions and cell markers [1,6,9], there is still no universally accepted demarcation of these subsets based on phenotypic markers [9]. Furthermore, there is no visible clustering of the cell subsets based on the CD14 and CD16 surface markers, instead the two markers form a spectrum of expression levels potentially contributing to variation between experiments [4,7,10]. Differential expression of chemokine and scavenger receptors indicates a functional

*Abbreviations:* LPS, lipopolysaccharide; MHC, major histocompatibility complex; PBMC, peripheral blood mononuclear cell; LN2, liquid nitrogen; MFI, mean fluorescence index; FSC, forward scatter; SSC, side scatter; NK, natural killer; ELISA, enzyme linked immunosorbent assay; SWAP, soluble worm antigen preparation; ANOVA, analysis of variance; DP, double positive; SEM, standard error of mean.

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**Table 1**
Study cohorts and description.

| Study | Donor ethnicity | Origin (urban/rural) | *N* |
|---|---|---|---|
| Whole monocyte phenotype | African (Zimbabwe) | Rural | 62 |
| Effects of cryopreservation on monocyte phenotype | African (other) | Urban | 5 |
| | Caucasian | | 4 |
| Effects of genetics or exposure on monocyte phenotype | Europe | Urban | 21 |
| | African (Zimbabwe) | Rural | 21 |

potential in terms of trafficking to sites of infection and inflammation. Indeed, monocyte migration and trafficking has been observed to vary between subsets based on expression of CCR2 and CX3CR1 [11]. Another feature of monocytes is their ubiquitous expression of the MHC class II surface receptor, HLA-DR, which is frequently used to distinguish between CD16 expressing monocytes and CD16 expressing NK cells [12]. As a receptor that is involved in antigen presentation [9], it is often considered an activation marker [13–15] and indicates functional differences for the monocyte subsets as well as subset activation status [12].

Thus far, the majority of human monocyte studies have taken place using volunteers of Caucasian background and in high income countries where pathologies arising from non-communicable diseases such as atherosclerosis, liver cirrhosis and asthma dominate [16]. This means that, despite the demonstration of the importance of monocytes in experimental models of parasitic diseases [2,17,18], comparatively little is known about the nature, phenotype and development of monocytes in people exposed to tropical infectious diseases. Furthermore the majority of studies investigating monocyte phenotype and function use whole blood or fresh PBMCs rather than cryopreserved peripheral blood mononuclear cells (PBMCs). Cryopreservation of PBMCs is an indispensable tool for longitudinal clinical studies as well as during fieldwork when samples have to be stored and transported from the collection point to a laboratory. Furthermore, the capability to retrospectively analyze specimens from the same patient allows analysis of large sample populations, monitoring of clinical status over time or after treatment and improves accuracy while reducing within-patient as well as interassay variability [19,20]. To date, studies determining the effects of cryopreservation on PBMCs have focused on cell compartment changes [21] or maturation pathways [22], but no studies have been conducted on the effects of cryopreservation on the cell phenotype which is central to the function of the monocytes.

In this study our aims were (1) to determine changes in expression levels of cell surface markers occurring within the monocyte cell population dependent on CD14 and CD16 expression intensity, (2) to assess the stability of these markers during processes involved in freezing and storage, and finally, (3) to determine if differences occur in the proportion and phenotype of monocytes in the different sub-populations between Caucasian individuals who have been exposed to a typical western lifestyle, and African individuals who are lifelong residents of a rural helminth endemic area.

## 2. Methods

### 2.1. Ethical statement

Written consent was obtained from all participants or their guardians prior to enrolling in the study. Local ethical approval was given and local review board guidelines adhered to. The cohort of rural Africans was part of a larger study investigating the epidemiology and immunology of human schistosomiasis that was conducted in the Mashonaland East Province of Zimbabwe. Permission to conduct the study in the region was obtained from the Provincial Medical Director. Institutional and ethical approval was received from the University of Zimbabwe and the Medical Research Council of Zimbabwe respectively. At the beginning of the study, parents and guardians of participating children had the aims and procedures of the project explained fully in the local language, Shona, and written consent was obtained from participants' parents/guardian before enrolment into the study. After collection of all samples, all participants and their parents/guardians were offered anthelmintic treatment with the recommended dose of praziquantel (40 mg/kg of body weight).

### 2.2. Study populations

To address the different questions, three different cohorts were used, which are described in Table 1. For the purposes of phenotyping monocytes for cell surface expression patterns of the markers CCR2, CX3CR1 and HLA-DR, a cohort of 62 individuals living in a rural area where *Schistosoma haematobium* is prevalent was used. All participants were from the Murehwa district in north eastern Zimbabwe. All individuals recruited into each study were *S. haematobium* and co-infection negative and had never received anti-helminthic treatment. In addition there is little or no infection with *Schistosoma mansoni*, soil transmitted helminths and malaria transmission is sporadic and seasonal [23]. The residents of the area are subsistence farmers with frequent contact with infected water for purposes of irrigation, bathing, washing and collecting water (assessed by questionnaire) [24].

In order to investigate the effects of cryopreservation on monocyte phenotype and cell numbers, peripheral blood from nine African or Caucasian volunteers, currently living in urban environments, was used to compare monocytes from freshly isolated PBMCs to those from cryopreserved PBMCs. For evaluation of differences that genetics and lifetime exposure to infection may have on monocyte phenotype, PBMCs from 21 Africans who were exposed to, but negative for, helminth, malaria and HIV co-infections were compared to 21 age and sex matched Caucasians with no reported exposure to these pathogens. Table 2 shows the ages (mean, median and range) of each of the populations (rural African and Caucasian) used in background comparisons. In order to check for effects of genetic background vs pathogen exposure, five donors of African origin (Kenya ($n = 4$) and Zimbabwe ($n = 1$)) were recruited to the study. All five donors had grown up in an urban environment.

### 2.3. Parasitology

Parasite infection status was determined in the Zimbabwean participants through examination of at least 2 stool and 2 urine samples collected on 3 consecutive days and a single blood sample. The urine samples were used for microscopic diagnosis of *S. haematobium* infection using the standard urine filtration method [25], while the stool sample was used for microscopic diagnosis of intestinal helminths and *S. mansoni* infection using the Kato–Katz method [26]. Blood smears were microscopically examined for *Plasmodium falciparum* infection, and results confirmed using the rapid Paracheck test, (Orchid Biomedical Systems, Goa, India) and serologically tested for HIV status using the DoubleCheckGold™ HIV1&2 test kit (Orgenics, Ltd., Yavne Israel). All Zimbabwean

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