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Monocyte inflammatory profile is specific for individuals and associated with altered blood lipid levels

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ABSTRACT

Background and aims: Atherogenesis is dependent upon monocyte influx into the vessel wall. In humans, three monocyte subsets exist, the number and function of which are significantly altered in cardiovascular disease (CVD). Whether such alterations arise in individuals with a perturbed lipid profile remains largely unanswered, but is important to delineate, as adoption of a pro-inflammatory state may promote plaque formation. Here, we compared the inflammatory status of monocyte subsets and determined whether monocyte inflammatory changes are evident in individuals with a perturbed lipid profile.
Methods: Monocyte subset cytokine production, inflammatory and anti-inflammatory marker expression were determined by whole blood flow cytometry and related to participants' lipid levels.
Results: The intermediate and non-classical monocytes were more inflammatory than classicals as seen by their higher cytokine production (TNF-α, IL-1β, IL-6) and M1 marker (CD86) expression, but lower levels of M2 markers (CD93, CD163). More importantly, a considerable variation was seen between participants, with all monocytes of one individual being more inflammatory than those of another. Many inter-individual differences were related to participants' lipid levels. IL-1β production correlated negatively with Apo A1 and HDL-C. CD86 and TLR2 correlated positively with Chol:HDL-C but negatively with HDL-C and Apo A1:Apo B. Interestingly, CD163 expression correlated positively with Chol:HDL-C but negatively with Apo A1:Apo B.
Conclusions: Our data indicates that priming of all monocytes to an inflammatory state occurs in individuals with a perturbed lipid profile, overriding the normal functional distinction attributed to the different monocyte subsets. As such, all monocytes may be important in CVD.

1. Introduction

Cardiovascular disease (CVD) remains a major problem worldwide [1]. Despite optimal treatment of CVD, there remains a significant residual risk of patients experiencing a clinical event [2]. A major contributor to this residual risk is thought to be inflammation as it is fundamental in plaque development, progression and, ultimately, plaque destabilisation/rupture [3]. Large clinical trials are currently underway to assess the effect of anti-inflammatory agents on CVD event rates [4]. It is recognised, however, that such agents will be associated with their own risks [5], and thus more specific approaches may be required.

A key potential target is the monocyte, as monocyte-derived cells (macrophages) in the plaque are pivotal in both lipid handling and inflammation [6]. In murine models, the continuous influx of monocytes into the plaque is associated with increased plaque size [7] and, accordingly, inhibiting monocyte influx into the plaque reduces its progression [8].

Monocytes are heterogeneous with three different subsets identified in humans, a major subset, the classical: CD14++CD16- (~85%) and two minor subsets, the intermediate: CD14++CD16^{+} (~5%) and non-classical: CD14+CD16++ (~10%) [9]. Note, this subset division is

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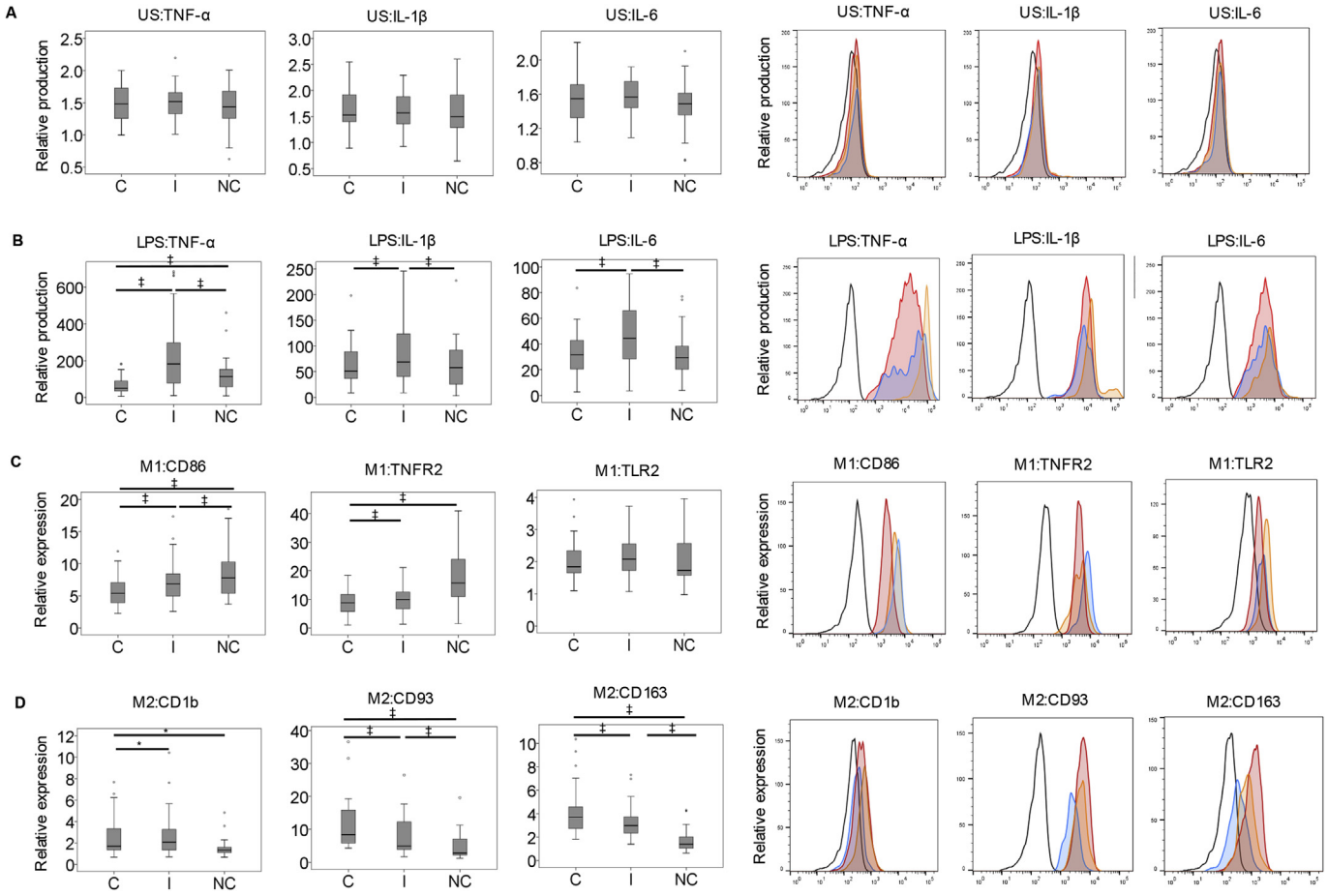

Fig. 1. Comparison of intracellular cytokine production and M1 and M2 marker expression between monocyte subsets.
(A) TNF-α, IL-1β and IL-6 production by unstimulated (US) monocyte subsets. (B) TNF-α, IL-1β and IL-6 production by LPS (1 μg/ml) stimulated monocyte subsets, measured by flow cytometry, n = 30. (C) Expression of M1 markers, CD86, TNFR2 and TLR2 by monocyte subsets, n = 26. (D) Expression of M2 markers, CD1b (n = 23), CD93 and CD163 (n = 26) by monocyte subsets, measured by flow cytometry. C: classical; I: intermediate; and NC: non-classical. Data are presented as box and whisker plots, with outliers denoted by circles and representative histograms. Black lined histograms: isotype control, red histograms: classical monocytes, orange histograms: intermediate monocytes and blue histograms: non-classical monocytes. Statistical calculations of significance were performed using ANOVA followed by the *post hoc* Tukey's test for significant differences between any 2 monocyte subsets: $^{\ddagger}p < 0.01$; $^{*}p < 0.05$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

equivalent to Mon1, Mon2 and Mon3, respectively, as per the recent consensus document [10]. Of these subsets, the proportion of intermediate monocytes is elevated in CVD patients [11,12] and is associated with lipid levels [11,13], plaque morphology [14,15] and occurrence of major clinical events [11,16]. The intermediate subset is, therefore, thought to be a potential treatment target in CVD, akin to other inflammatory conditions [12,16–18]. It is unclear however, whether these associations translate to changes in their function and whether mechanistically, intermediates are the only subset contributing to atherosclerosis.

As the intermediates remain a minor monocyte subset in CVD (<10%), the function of the other subsets, particularly the classical subset given it is the largest proportion, may be important in atherosclerosis progression. Despite speculation in the literature that the intermediate subset is 'pro-atherogenic' [19], in part due to its inflammatory nature [20–22], we found that it was no more inflammatory in CVD patients than in controls, whereas the classical subset was; suggesting that the classical subset also contributes to CVD [23]. With low-density lipoprotein cholesterol (LDL-C) instigating CVD development, here we compared monocyte subset inflammatory profile in a cohort of individuals who were otherwise healthy but differed in their lipid profile. This was done by assessing the expression of inflammatory/pro-atherogenic factors such as cytokine production and expression of CD86, TNFR2 and TLR2 (which are also found on M1 macrophages) [24]. This was counterbalanced by assessing expression of M2 markers, including CD1b, CD93 and CD163 [24]. Results were assessed between subsets, and between participants, as well as for participants relative to their lipid levels.

2. Materials and methods

2.1. Study population

This study was approved by the Western Sydney Local Health District (WSLHD) Human Research Ethics Committee. Informed signed consent was obtained from all participants. We recruited individuals (n = 30) who were in generally good health but differed in lipid levels -with a wide range of lipid levels achieved by including individuals visiting the Westmead Lipid Clinic. Exclusion