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Pro-inflammatory monocyte-macrophage polarization imbalance in human hypercholesterolemia and atherosclerosis

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ABSTRACT

Monocyte-macrophages (MoMas) play a major role in atherosclerosis. In mice, hypercholesterolemia increases pro-inflammatory monocytes that promote plaque growth, but whether this is true also in humans in unknown. We herein analyzed monocyte subsets and MoMa phenotypes in familiar (FH, $n = 22$) and non-familiar (NFH, $n = 20$) hypercholesterolemic compared with normocholesterolemic (CTRL, $n = 20$) patients. We found that FH and NFH had higher circulating pro-inflammatory $CD68^+CCR2^+$ M1 MoMas than CTRL, while anti-inflammatory $CX_3CR1^+CD163^+/CD206^+$ M2 MoMas were reduced only in NFH. As a result, the M1/M2 polarization balance was increased in FH and, more markedly in NFH. M1 MoMas and the M1/M2 polarization ratio were directly correlated to pre-treatment LDL cholesterol levels and strongly associated with the presence of atherosclerotic plaques. In conclusion, we show for the first time that human hypercholesterolemia is associated with a pro-inflammatory imbalance of circulating monocytic cells, which can predispose to the development of atherosclerosis.

1. Introduction

Monocyte-macrophage lineage cells actively participate in the pathogenesis of atherosclerosis [1,2]. They are recruited following chemokine gradients generated in the diseased vasculature [3]. Once in the vessel wall, monocytes contribute to the inflammatory milieu and differentiate into macrophages. Peripheral blood monocytes can be distinguished based on CD14 and CD16 expression into traditional subsets, which reflect the inflammatory status [4]. In tissues, macrophages can be polarized into a pro-inflammatory (M1) phenotype prone to foam cell differentiation, or into an anti-inflammatory (M2) status with scavenger activity [5]. This M1/M2 spectrum is a simplistic representation of true macrophage heterogeneity [6], but is instrumental to study cell-mediated inflammation ex vivo. In animal models of hypercholesterolemia, monocytosis strongly contributes to the development of atherosclerotic lesions, by expanding the pool of pro-inflammatory cells [7–9], but human data in support of this pathway are very limited. The aim of this study was to quantify circulating monocyte-macrophages (MoMas) subsets in patients with familiar and non-familiar hypercholesterolemia compared to healthy subjects, and to detect eventual associations with atherosclerosis.

2. Methods

2.1. Patients

The study was conducted in accordance with the principles of the Declaration of Helsinki and all subjects provided informed consent. Subjects were recruited consecutively at the Lipid and Metabolism Clinics of the University Hospital of Padova. Male and female subjects aged 18–80 were eligible if they did not report any of the following condition: acute disease or infection; recent trauma, surgery or cardiovascular event; immunological disorders or organ transplant; pregnancy or lactation; inability to provide informed consent. For all patients, we recorded the following data: age, sex, height, weight, body mass index (BMI), prevalence of diabetes, hypertension and smoking habit, current and pre-treatment lipid profile, medical history and medications. A carotid and femoral artery ultrasound examination was performed to detect atherosclerotic plaques, and the intima media thickness (IMT) in the common carotid and common femoral arteries were also measured. Based on the Dutch Lipid Clinic Network criteria for the diagnosis of familial hypercholesterolemia in adults [10], the probable and definite familiar hypercholesterolemia FH categories

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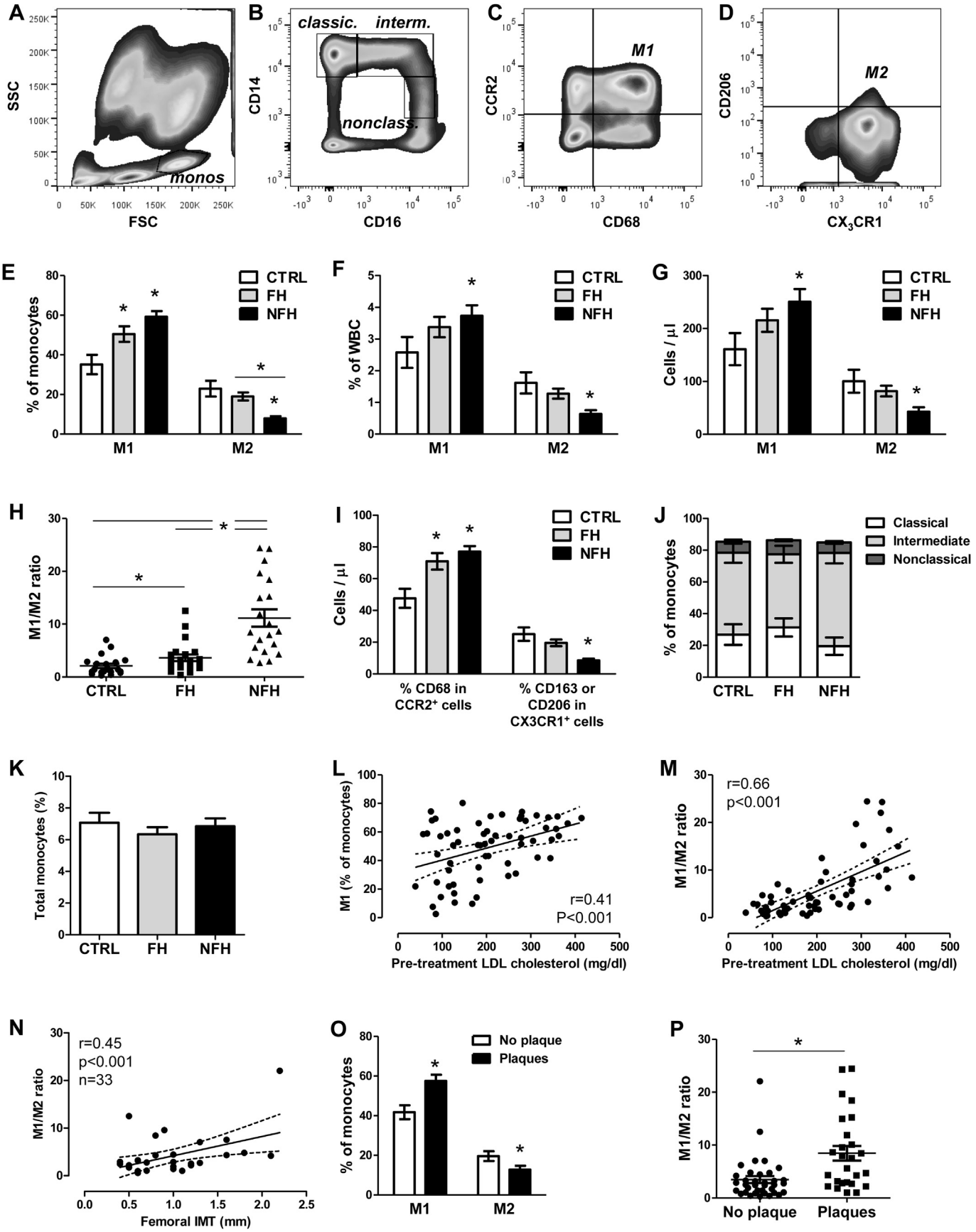

Fig. 1. A–D) Representative FACS plots illustrating the gating strategy used to identify and enumerate monocyte-macrophage subpopulations in a NFH patient. First, monocytes were gated in the SSC vs FSC plot (A) and then examined for the expression of CD14/CD16 to identify classical, intermediate and nonclassical monocyte subsets (B), of CD68/CCR2 to