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Altered leukocyte distribution under hypercholesterolemia: A cross-sectional study in children with familial hypercholesterolemia

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ABSTRACT

Background and aims: Children with familial hypercholesterolemia (FH) have elevated LDL cholesterol from the first year of life, and represent a model of early-stage atherosclerosis. Data suggest that adults with FH have alterations in circulating monocyte subpopulations towards a more pro-inflammatory phenotype, but it is not known whether FH children have similar perturbations. In addition, there are no data on the distribution of lymphocyte subpopulations in FH children. The objective of the present study was to characterize the distributions of circulating monocyte and lymphocyte subpopulations in children with FH and healthy, normocholesterolemic children.
Methods: Using flow cytometry analysis, we analyzed whole blood B- and T-cell subpopulations and monocyte subpopulations in FH ($n = 23$) and healthy ($n = 20$) children. Moreover, we measured serum markers of leukocyte and endothelial cell activation using EIA.
Results: We found that FH children had monocytosis as well as a shift in the monocyte subpopulations. This shift was characterized by higher circulating pro-inflammatory and non-classical monocytes, and lower levels of classical monocytes, and seemed to be present only in FH children with low HDL cholesterol (HDL-C, below 1.3 mmol/L). Additionally, monocytes expressing CD18 and serum E-selectin were higher in FH children, in particular FH children with low HDL-C.
Conclusions: FH children with low HDL-C had monocytosis as well as a shift in monocyte subpopulations towards a more pro-inflammatory phenotype. Our results suggest activation of monocytes at a very early stage of atherosclerosis in humans.

1. Introduction

Cardiovascular disease (CVD) is the main cause of mortality worldwide [1]. It is primarily caused by atherosclerosis, a pathological process of the arterial wall with the bi-directional interaction between lipids and inflammation as a phenotypic hallmark [2]. Monocytes and lymphocytes are key players in this inflammatory process and contribute to plaque progression and rupture [3,4].

There is a large degree of monocyte and lymphocyte heterogeneity [5]. Cell-surface expression of various proteins may define distinct phenotypical, and in some degree also functional, subpopulations of these cells [6]. It has been suggested that some of these subpopulations may be of particular importance in

Abbreviations: CRP, C reactive protein; CVD, cardiovascular disease; FH, heterozygous familial hypercholesterolemia; HDL-C, high-density lipoprotein cholesterol concentration; LDL-C, low-density lipoprotein cholesterol concentration; RTE, recent thymic emigrant.

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atherosclerosis. Specifically, monocytes characterized by expression of both CD14 and CD16 proteins on the membrane surface are regarded as pro-inflammatory and have been suggested to be among the most important foam cell progenitors [7–9]. Also lymphocyte subpopulations, characterized by certain membrane-molecule profiles as assessed by flow cytometry, may differ in their inflammatory and immunological potential, and thereby also in their pro-atherogenic potential [3].

Subjects with heterozygous familial hypercholesterolemia (FH) have elevated plasma LDL cholesterol concentration (LDL-C) from the first year of life, accompanied by accelerated progression of atherosclerosis and increased risk of premature CVD morbidity and mortality. Children with FH may therefore represent a model of early-stage atherosclerosis. These children have greater intima media thickness than sex- and age-matched healthy controls [10]. In addition, FH children may have increased concentrations of C-reactive protein (CRP) and neopterin in plasma, reflecting systemic inflammation and monocyte activation, respectively [10,11]. Additionally, FH children have increased inflammatory potential in circulating immune cells, suggesting that the inflammatory arm of atherosclerosis is activated at an early stage of disease [12–14].

Data suggest that adults with FH or hypercholesterolemia have alterations in circulating monocyte subpopulations towards a more pro-inflammatory phenotype, but it is not known whether FH children have similar perturbations [15,16]. Moreover,, to the best of our knowledge, there are no data on the distribution of lymphocyte subpopulations in FH children.

The objective of the present study was to characterize the distributions of circulating monocyte and lymphocyte subpopulations in children with FH and healthy, normocholesterolemic children.

2. Patients and methods

2.1. Study design and participants

In the present cross-sectional study, we compared children with FH and healthy control children to describe the distributions of circulating monocyte and lymphocyte subpopulations using flow cytometry, as well as circulating markers of inflammation using commercially available ELISA kits.

During the period April to October 2015, we recruited non statin-treated children with heterozygous FH from the Lipid Clinic, Oslo University Hospital Rikshospitalet, Oslo, Norway. All children had a definite FH diagnosis based on either genetic (n = 22, 96%) or clinical diagnosis (n = 1, 4%), where the latter was determined using the Dutch Lipid Clinic network classification (World Health Organization publication no WHO/HGN/FH/CONS/99.2) [14]. All the 22 FH children with a genetically verified mutation had mutations in the LDL receptor. Thirteen of the 22 FH children had LDL receptor negative mutations and nine FH children had LDL receptor defective mutations. “LDL-receptor negative mutations” were defined as class 1 and 2A mutations according to (probable) classification at Jojo-genetics (http://www.jojogenetics.nl/wp/database/). Similarly, “Other mutations” were defined as class 2B, 3, 4 and 5. Healthy control children without FH were recruited in the same time period. All children were healthy, and none were using lipid-lowering or anti-inflammatory medications at the time of attendance. We obtained approval from the South East Norway Regional Ethics Committee, and we obtained written informed consent from all participants or the parents when the child was less than 16 years old, before any study procedure was undertaken.

2.2. Variables

Clinical measurements, blood sampling and standard plasma biochemistry were performed as previously described [14].

Concentration of CD25, CD163, CD14, ICAM-1, VCAM-1 and E-selectin was determined by enzyme immunoassays from R&D Systems (Minneapolis, MN). The intra-assay coefficient of variation was <12% (except for 4 samples).

2.3. Flow cytometry

B- and T-cell subpopulations and monocyte subpopulations were analyzed by flow cytometry. For B-cell analysis the blood samples were washed twice before incubation with antibodies. T-cell and monocyte analysis was performed in unwashed blood sample. Briefly, EDTA-blood was incubated with optimally titrated antibodies for 15 min at room temperature, followed by erythrocyte lysis (BD FACSLysing Solution, Beckman Dickinson, CA). Data acquisition was performed on a Gallios Flow cytometer (Beckman Coulter, San Diego, CA) or a Canto II Flowcytometer (BD). For T-cells and monocytes, 1×10^5 cells was acquired; for B-cells, 1×10^6 cells, if possible. The following antibodies were used: CD31, CD45RO, CD28, CD45RA, CD127, CD19, CD27; CD11b, CD14, CD11a, CD18 (Becton Dickinson); CD4, CD8, CD3, CD25, CD38, IgM, IgD (Beckman Colter); TCR alfa/beta, CD21 (R&D Systems); CXCR5 (eBioscience, San Diego, CA); CD45 (Invitrogen, Waltham, MA); CD27 (Dako, Glostrup, Denmark); CD16 (LSBio, Seattle, WA); HLA-DR (Biolegend, San Diego, CA). B-cell were gated as CD19 + and further sub-classified as naive (IgD+, IgM+, CD27−), IgM memory (CD27+, IgD+, IgM+), class switched (CD27+, IgM-, IgD-), plasmablasts (CD19 + dim, CD27++, CD38++), transitional (IgM++, CD38++, CD24+) and CD21 low B cells (CD38 low, CD21 low). T-cells were gated as CD3+ and further as naive CD4+ (CD4+, CD45RA+), recent thymic emigrants (CD4+, CD45RA+, CD31+), CD4+ memory (CD4+, CD45RO+), follicular like CD4+ (CD4+, CD45RO+, CCR5+), regulatory T-cells (CD4+, CD25++, CD127-), naive CD8+ (CD8+, CD27+, CD28+), CD8+ early effector memory (CD8+, CD27+, CD28−), CD8+ late effector memory (CD8+, CD27−, CD28−). Monocytes were separated in classical (CD14++, CD16−), intermediate/pro-inflammatory (CD14++, CD16+) and non-classical (CD14+, CD16++).

2.4. Statistical analyses

All data are presented as mean (standard deviation) or median (25th–75th percentile). Independent Samples T test was used for parametric data. For nonparametric data we log-transformed the variables and used Independent Samples T test. Correlations were calculated using Pearson's correlation coefficient when there were no apparent outliers, whereas Spearman's rank correlation coefficient was used for variables with clear outliers. Alpha level of significance was set to 5%. Statistical package for the social sciences (SPSS, v.20, IBM) was used for all calculations.

3. Results

3.1. Study population

We included 43 children, of which 23 had FH and 20 were normocholesterolemic controls (Table 1). Fifty-seven and 50% were girls in the FH children and healthy children groups, respectively. The median age was 11.3 (min-max: 6.3–15.6) years and 9.6 (min-max: 6.9–13.1) years in the FH children and control children groups, respectively. The groups were similar in terms of age, gender, height, weight and BMI. The FH children had higher total and LDL-C. CRP and triglycerides were also higher in FH children, and there was a tendency that FH children had lower HDL cholesterol (HDL-C).