International Journal of Cardiology 190 (2015) 214–216

Contents lists available at ScienceDirect

International Journal of Cardiology

journal homepage: www.elsevier.com/locate/ijcard

Letter to the Editor

Monocyte dysfunction as a previously unrecognized pathophysiological mechanism in ApoE −/− mice contributing to impaired arteriogenesis

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ARTICLE INFO

Article history:
Received 19 April 2015
Accepted 21 April 2015
Available online 23 April 2015

Keywords:
Hyperlipidemia
Atherosclerosis
Arteriogenesis
Monocytes
Cell function
Growth factors

Hyperlipidemia (HL) is a major cardiovascular risk factor promoting atherogenesis leading to coronary and peripheral artery disease. Collateral vessel development, a crucial compensatory mechanism in peripheral artery disease, is negatively influenced by HL [1]. Monocytes and tissue-resident macrophages accelerate both atherogenesis and arteriogenesis by supplying growth factors, cytokines and proteolytic enzymes [2]. The decreased monocyte/macrophage accumulation around growing collateral vessels in HL is associated with jeopardized arteriogenesis [1].

Ligand-induced monocyte extravasation to sites of collateral growth is a crucial step. This involves several factors including monocyte chemoattractant protein-1 (MCP-1) and vascular endothelial factor-A (VEGF-A), which facilitate monocyte chemotaxis in arteriogenesis, a short lasting and well defined process [2,3]. Previous studies in humans indicated that cardiovascular risk factors such as HL severely hamper ex vivo monocyte chemotaxis to VEGF-A and MCP-1 [4]. Based on these findings, we proposed that monocytes can be used as circulating "biosensors" to detect metabolic deregulation and elevated cardiovascular risk [4]. However, HL often co-exists with other cardiovascular risk factors such as diabetes mellitus (DM) or smoking, all known to negatively affect monocyte chemotaxis [4,5].

We now demonstrate that monocytes from ApoE −/− mice with HL show a completely abrogated chemotaxis towards VEGF-A and a significantly decreased one towards MCP-1. Experiments were approved by the local animal ethical committee of Maastricht University. Blood was sampled [6] and white blood cell counts were performed. Lipid values were measured enzymatically. Plasma lipid levels were significantly elevated in ApoE −/− mice (Table 1). ApoE −/− mice had significantly higher numbers of CD11b + monocytes ($p < 0.05$).

For chemotaxis analysis blood from sex, age and genetically (C57Bl/6 or ApoE −/−) matched mice (avg. 5–6 mice per experimental condition, each condition was repeated 3–6 times — "n" in Fig. 1) was pooled and chemotaxis analysis of isolated monocytes was performed [7]. Monocytes from ApoE −/− mice show significantly impaired chemotactic responses to VEGF-A and MCP-1 compared to wild-type (WT) mice (Fig. 1A, B). In fact, chemotaxis to VEGF-A was not distinguishable from chemokinesis. In contrast, MCP-1-induced chemotaxis was reduced with only a moderate stimulation at the optimal concentration of 10 ng/mL ($p < 0.05$). Furthermore, monocytes from ApoE −/− mice had a significantly elevated chemokinesis (Fig. 1C) as compared to WT mice ($p < 0.05$).

The pioneering finding of this study is that HL-conditioned monocytes from ApoE −/− mice are dysfunctional, as chemotaxis towards both VEGF-A or MCP-1 is severely impaired. These data imply that *i.)* monocytes show a functional defect in the presence of HL, and that *ii.)* monocyte dysfunction is likely to contribute to pathological changes observed in ApoE −/− mice [1].

Besides stimulation of atherogenesis [8], chronic HL impairs arteriogenesis [1]. Monocytes contribute to arteriogenesis by VEGFR-1- [4] or CCR-2-mediated [2] migration from the blood stream to the growing vessel [2]. Reduced expression of arteriogenic factors is an unlikely cause for impaired arteriogenesis in HL as therapeutic rescue attempts with either VEGF-A or MCP-1 largely failed [1,9]. Furthermore, impaired arteriogenesis in HL mice was accompanied by decreased

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http://dx.doi.org/10.1016/j.ijcard.2015.04.188

Table 1
Characteristics of mice, plasma lipid levels and monocyte numbers.

	WT (n = 34)	ApoE −/− (n = 34)	p-Value
Age of mice (weeks)	38.9 ± 8.4	37.8 ± 10.1	n.s.
Total cholesterol (mg/dL)	55.07 ± 7.97	399.94 ± 85.8	$p < 0.05$
LDL cholesterol (mg/dL)	5.92 ± 2.89	278.46 ± 64.29	$p < 0.05$
HDL cholesterol (mg/dL)	41.37 ± 6.55	98.18 ± 16.24	$p < 0.05$
Triglicerides (mg/dL)	46.05 ± 12.71	116.53 ± 56.29	$p < 0.05$
Mononuclear cells (MNC, $\times 10^6$/mL)	1.47 ± 0.32	1.59 ± 0.47	n.s.
CD11b + monocytes (% of MNC)	6.35 ± 0.86	11.97 ± 2.26*	$p < 0.05$

LDL — low density lipoprotein, HDL — high density lipoprotein.

recruitment of macrophages both under the HL conditions and following the arteriogenic stimulation [1]. This previous finding implies – in the light of our novel data – that both native as well as growth factor-stimulated arteriogenesis are reduced in HL due to a reduced/delayed accumulation of dysfunctional blood-derived monocytes at sites of vascular repair [1]. Indeed, bone marrow-derived cells from WT mice alleviate hindlimb ischemia in ApoE −/− mice by improving blood flow and promoting arteriogenesis [10].

Our previous work on human monocytes documented an impaired chemotactic response to arteriogenic ligands VEGF-A and MCP-1 [4] in HL. Our novel findings proof the same in ApoE −/− mice. Impaired monocyte chemotaxis to VEGF-A contributes to impaired arteriogenesis in DM [4]. DM induces unspecific monocyte activation secondary to increased oxidative stress and advanced glycation of functionally relevant molecules [5]. The described monocyte dysfunction in HL may be due to monocyte activation following lipid overload-induced oxidative stress and leads to up-regulation of adhesion molecules [11]. This activation of monocytes may explain the observed elevated chemokinesis in ApoE −/− mice (Fig. 1C) and extravasation to inflamed endothelium of atherosclerotic plaques. Altogether, increased adhesive properties, increased monocyte numbers (Table 1) and extensive intraplaque angiogenesis will promote atherogenesis by progressive monocyte migration to the lesion [8] despite partially impaired migration to pro-atherogenic stimuli (here tested MCP-1). A broad spectrum of chemokines is responsible for monocyte recruitment in atherosclerosis. Modified lipoproteins may serve as chemoattractants [8] as well. Therefore, therapeutic strategies may need to target multiple targets.

In the process of compensatory arteriogenesis the stimuli for monocyte recruitment and the therapeutic time window (days/weeks) are rather short compared to atherosclerosis (years/decades) [2]. Therefore a decreased monocyte response demonstrated in our study may provide a functional basis for impaired/delayed arteriogenesis while atherogenesis continues.

Lowering monocyte numbers can block the progression of atherosclerosis [12]. The applicability, however, is limited by the fact that post-infarction monocytosis is physiologically required for both healing the damaged myocardium [13] and for neovascularisation [2]. These findings point towards the dilemma of why and how monocytes promote tissue healing in infarcted myocardium and in parallel worsen atherosclerosis. Monocytosis is an accompanying condition featuring several cardiovascular risk factors (e.g., HL, DM) in which the healing capability of monocytes is disturbed. Our findings stress the importance of therapeutically improving monocyte responsiveness to "healing" stimuli such as VEGF, which then could allow to therapeutically lower monocyte counts as a second, independent step.

HL may have an even more detrimental effect on arteriogenesis than DM [14]. Therefore, it will be of utmost importance to further investigate the mechanisms hampering monocyte chemotaxis in HL. This should provide important insight into the pathophysiology of HL and into the basis for therapeutic correction of monocyte function to improve arteriogenesis in HL. It is tempting to speculate that the HL-related monocyte defect is different from the DM-related phenotype as monocyte chemokinesis is significantly elevated in HL.

This is the first description of monocyte dysfunction in ApoE −/− mice, namely an impaired chemotactic response. This is likely to explain

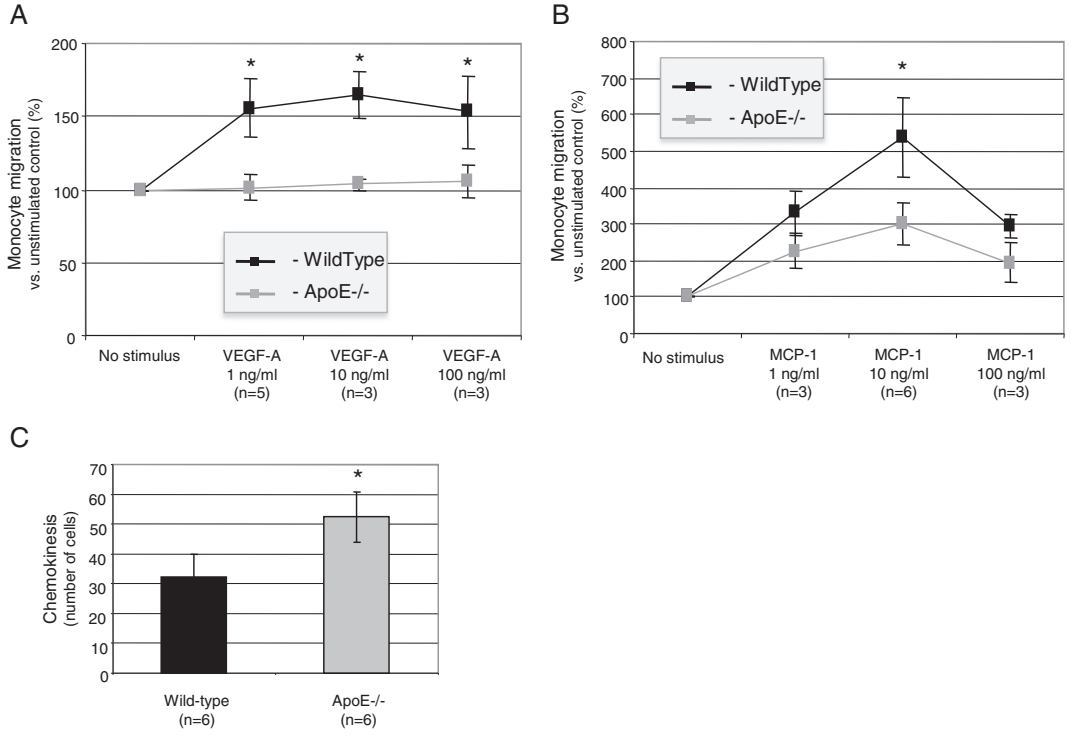

Fig. 1. Monocyte motility from WT and ApoE −/− mice to VEGF-A and MCP-1. Chemotactic response to VEGF-A (A) and MCP-1 (B) presented as percentage of unstimulated control; C, baseline migration (chemokinesis, 3 h) presented as cell number/power field (mean ± standard error of mean). Statistical analysis was performed using SPSS 18.0.1 software. The probability of difference between the groups was evaluated using Kruskal–Wallis test. Subsequently, Mann–Whitney test was performed to estimate the level of significance. * — $p < 0.05$, comparing corresponding conditions of two mouse phenotypes.

some of the pathogenetic consequences of HL including the activated atherogenesis as well as the hampered angiogenesis/arteriogenesis.

Conflict of interest

Nothing to disclose.

Acknowledgment

This study was supported in part by grant from Cells-in-Motion Cluster of Excellence (EXC 1003—CiM), University of Münster, Münster, and by grant from the Cardiovascular Research Institute Maastricht (30983187N).

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