Heart Vessels (2013) 28:135–142
DOI 10.1007/s00380-011-0218-1

ORIGINAL ARTICLE

A decrease in the percentage of circulating mDC precursors in patients with coronary heart disease: a relation to the severity and extent of coronary artery lesions?

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Received: 30 November 2010 / Accepted: 8 December 2011 / Published online: 7 January 2012

Abstract Inflammation plays a pivotal role in coronary heart disease. Dendritic cells (DCs) are principal players in inflammation and atherosclerosis. Although the percentage of circulating DC precursors in coronary heart disease have been investigated, circulating myeloid DC (mDC) and plasmacytoid DC (pDC) precursors have not been extensively studied, particularly in relation to the severity of coronary artery lesions in patients with coronary heart disease. In this study, we recruited controls ($n = 29$), patients with stable angina pectoris (SAP, $n = 30$), patients with unstable angina pectoris (UAP, $n = 56$), and patients with acute myocardial infarction (AMI, $n = 50$). The severity and extent of coronary artery lesions was evaluated by Gensini score, following coronary angiograms. The percentage of circulating mDC and pDC precursors was determined by fluorescence-activated cell sorting (FACS). Plasma levels of MCP-1 and MMP-9, which correlate with atherosclerosis and DC migration, were also measured. The percentage of circulating mDC precursors was reduced in patients with AMI and UAP compared with control and SAP patients, respectively ($p < 0.01$ for AMI vs. SAP and Control, $p < 0.05$ for UAP vs. SAP and Control). The percentage of circulating pDC precursors was not significant changed. The levels of plasma MMP-9 and MCP-1 and Genisi score were all increased in patients with AMI and UAP, compared to control and SAP patients, respectively ($p < 0.01$ for AMI vs. SAP and control, $p < 0.05$ for UAP vs. SAP and control). Overall, the percentage of circulating mDC precursors was negatively correlated with MCP-1 ($p < 0.001$), MMP-9 ($p < 0.001$) and Genisi scores ($p < 0.001$). Genisi scores were positively correlated with the levels of MCP-1 ($p < 0.001$) and MMP-9 ($p < 0.001$). Our study suggested that the percentage of circulating mDC precursors is negatively correlated with the severity and extent of coronary artery lesions in patients with coronary heart disease.

Keywords Dendritic cell · Coronary heart disease · Monocyte chemoattractant protein-1 · Matrix metalloproteinase · Genisi score

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Introduction

Atherosclerosis is an important pathologic driver of coronary heart disease, which is a major cause of morbidity and mortality in the world, particularly in developed countries [1]. Recent evidence has indicated that inflammation and immunity are involved in mediating all stages of atherosclerosis, from low-density lipoprotein (LDL) cholesterol accumulation within the sub-endothelial space to atherosclerotic plaque progression, rupture, and thrombosis. These patients often present with acute coronary syndrome (ACS) [2]. Immune cells, including monocytes, macrophages, T-lymphocytes, mast cells, foam cells, and dendritic cells, have been observed in atherosclerotic lesions

[3]. Among these cells, dendritic cells (DCs) are highly potent antigen-presenting cells (APCs) uniquely able to initiate primary immune responses to various antigens by activation of naive T-cells.

DCs are central to the regulation of inflammation. DCs migrate as precursors from bone marrow, circulate in the peripheral blood, and penetrate peripheral tissues. There, they give rise to immature DCs and carry out a sentinel-like function to monitor the microenvironment. Upon acquiring "maturation/danger" signals, i.e., components of pathogens, cytokines, and other molecules associated with inflammation or tissue damage, immature DCs rapidly undergo differentiation and maturation and migrate along chemotactic gradients to lymphatic tissues, where they form contacts with T-cells to initiate a primary immune response [4].

The population of DCs is heterogenous [5]. Two generally accepted types of DCs, which have been described in both mice and humans, are the plasmacytoid DCs (pDCs) and myeloid DCs (mDCs) [6]. It has been suggested that mDCs and pDCs have distinct morphology, surface molecules and functions [7], and represent two different lineages. pDCs, often referred to as interferon-producing cells, show low expression of CD11c and high expression of CD123. mDCs, often referred to as conventional DCs, show high expression of CD11c and low expression of CD123.

Available evidence suggests that DCs play an important part in the pathogenesis of atherosclerosis. DCs are found in the arterial intima in humans, an area commonly thickened by atherosclerosis [8]. Also, DCs have been shown to preferentially accumulate in regions predisposed to atherosclerosis in the normal murine aortic intima. There, they initiate nascent foam cell lesions at very early stages, as the atherosclerotic plaque starts to develop [9–11]. In advanced atherosclerotic plaques, DCs are present and accumulate preferentially within the vulnerable plaque shoulder by co-localizing with T-cells [12, 13]. The number of accumulated DCs is directly parallel to plaque complexity and inflammation [14]. Several previous studies have also demonstrated that in patients with ACS, the overall number of circulating mDC precursors was significantly decreased, while the number of plaque-associated mDCs was increased [15, 16]. Also, reduction of circulating DC precursors may reflect enhanced expression of DCs in atheromatous lesions, thereby reflecting a higher burden of atherosclerotic disease.

The accumulation of mDCs in plaques is reversible, as the number of plaque-associated mDCs was lowered after statin treatment [12]. This effect may be attributed to statin suppressing the maturation and migration of DCs [17, 18].

Earlier studies confirmed that IFN-α played an important role in plaque instability in human atheromas. IFN-α is mainly produced by pDCs. It can induce marked upregulation of TRAIL on CD4+ T-cells, thereby weakening the scaffold of the lesion and rendering the plaque instable [19]. Moreover, IFN-α can amplify the effects of lipopolysaccharide on mDCs by up-regulating TLR4 on their surface. When exposed to IFN-α, mDCs produce markedly higher amounts of the pro-inflammatory cytokines TNF-α, IL-12, and IL-23, and boost their MMP-9 production. All of these factors are mediators implicated in destabilizing plaques [20] and may be the primary mechanism linking DCs to plaque rupture, the underlying pathophysiologic cause of ACS. However, the relation between the percentage of circulating mDC and pDC precursors with the severity of coronary artery lesions in patients with coronary heart disease (CHD) has not been extensively studied.

Circulating DC precursors ultimately form atherosclerotic lesions [21], after being recruited from the blood by chemokines and induced by several atherogenic factors, such as oxidized LDL-cholesterol [22]. Besides inducing DCs, oxidized LDLs also promote the interaction of NK cells and DCs via CD48-2B4 contact-dependent mechanisms, thereby contributing to the occurrence and development of atherosclerosis [23]. Chemokines are known to induce leukocyte migration, growth, and activation through seven transmembrane domain G protein-coupled cell-surface receptors on target cells. Monocyte chemoattractant protein-1 (MCP-1), a member of the chemokine family, is highly expressed in human atherosclerotic lesions [21]. Deletion of MCP-1, or its corresponding receptor CCR2, attenuated atherosclerosis in experimental mouse models [24–27]. Clinical evidence has also shown that the plasma levels of MCP-1 have independent prognostic value in acute and chronic phases after ACS [28–30]. Additionally, MCP-1/CCR2 is critical for DC cell migration and maturation [31]. Furthermore, extracellular matrix metalloproteinases (MMPs), especially MMP-9, are essential for DC migration through the extracellular matrix in response to pro-inflammatory factors and chemokines [32, 33]. Interestingly, MMP-9 is found in the vulnerable shoulder regions of atherosclerotic plaques [34]. Earlier studies found that the plasma levels of both MMP-9 and MCP-1 were increased in patients with ACS, and were decreased after treating with angiotensin-converting enzyme inhibitor [35, 36].

To shed further light on the roles of circulating DC precursors in the pathogenesis of atherosclerosis, we examined the circulating number of mDC and pDC precursors, the plasma levels of MMP-9 and MCP-1, and the severity of coronary artery lesions in patients with different stages of CHD, in order to further evaluate the relationship between circulating levels of DC precursors with the severity of coronary disease.

Methods

Subjects

The study protocol conforms to the principles of the Declaration of Helsinki and was performed with approval of the Ethics Committee of South Medical University. Subjects were selected from individuals who underwent coronary angiography to investigate ischemic heart disease based on clinical indications (typical and atypical chest discomfort) from September 2006 to December 2009. All subjects are Han Chinese. All subjects gave informed consent, both verbally and in writing, for participation in the study, and underwent coronary artery angiography at Zhujiang Hospital of South Medical University before entering the study. According to clinical standards, routine blood analyses were performed in our hospital clinical laboratory.

In total, 165 subjects (114 men and 51 women, age range from 32 to 84 years with mean age of 63 ± 9.2 years) were studied. Patients diagnosed with CHD diagnosis had to have had at least one severe stenosis (>50%) in a major coronary artery, as determined by diagnostic coronary angiography.

The patients were divided into three study groups. The first group included patients with stable angina pectoris (SAP) that had a long-term, stable effort angina that had lasted at least 3 months and a positive exercise test. The second group included patients with unstable angina pectoris (UAP), as defined by as either angina with a progressive crescendo pattern or angina that occurred at rest without a recent myocardial infarction. In those patients, transient ST–T segment depression and T-wave inversion often were present, but no significant elevation of cardiac enzymes was detected. Patients with AMI had typical angina associated with ST-segment elevations in electrocardiogram and/or elevated plasma troponin-I. The third group, controls, consisted of patients with normal coronary artery angiographies. In total, we recruited 29 controls, 30 patients with SAP, 56 patients with UAP, and 50 patients with AMI.

Exclusion criteria were established for patients with autoimmune, neoplastic, liver, hematological or renal diseases, diabetes mellitus, recent surgery or recent trauma, and/or chronic inflammatory conditions. In addition, patients with valvular heart disease, nonischemic cardiomyopathy, and/or cerebrovascular disease were also excluded. Also, patients who took medications, such as immunosuppressive agents, statins, angiotensin-converting enzyme inhibitors, and angiotensin receptor blockers (before enrollment) were also excluded.

Fluorescence-activated cell sorting analysis

Fasting blood samples were obtained prior to coronary angiography. Blood was collected in tubes containing EDTA and samples were analyzed by flow cytometry (FACS-CALIBUR, CellQuest software, BD Biosciences, USA). The four-color Dendritic Value Bundle Kit (BD Biosciences San Jose, California, USA) was used for DC analysis according to the manufacturer's instructions. The four-color Dendritic Value Bundle Kit included FITC-conjugated anti-lineage 1 (lin1) cocktail antibodies, anti-human leukocyte antigen (HLA)-DR-PerCP, anti-CD11c-APC, anti-CD123-PE, and isotype control mouse IgG2a-APC and mouse IgG1-PE antibodies. The lin1 cocktail contains monoclonal antibodies against CD3 (T-cells), CD16 and CD56 (natural killer cells), CD19 and CD20 (B cells), and CD14 (monocytes/macrophages). DCs were defined as cells positive for PerCP-conjugated anti-HLA-DR, negative for FITC-conjugated anti-lin1 and positive for either PE-conjugated anti-CD11c (mDC precursors) or APC-conjugated anti-CD123 (pDC precursors; Fig. 1).

Detecting plasma concentrations of MCP-1 or MMP-9

Concentrations of MCP-1 or MMP-9 in plasma were determined simultaneously using enzyme-linked immunosorbent assay kits (Bender Medsystems, Vienna, Austria) according to the manufacturer's instructions.

Determining the severity of coronary artery lesions by Gensini score

Selective coronary angiography was conducted by two experienced interventional cardiologists blinded to the patients' clinical characteristics and biochemical results. The extent of coronary artery stenosis was assessed by quantitative coronary angiography. Gensini score was used to assess the severity and extent of coronary artery lesions. According to the degree of luminal narrowing and its location, the Gensini score was calculated by assigning a value to each coronary stenosis. Details of Gensini score are as follows: 1–25, 26–50, 51–75, 76–90, 91–99, and 100% of coronary luminal narrowing were given scores of 1, 2, 4, 8, 16, and 32 respectively, which were then multiplied by a factor that represents the importance of the lesion's position in the coronary arterial system: 5 for the left main coronary artery, 2.5 for the proximal segment of the left anterior descending coronary artery (LAD) or the circumflex artery (LCX), 1.5 for mid-segment of LAD, 1 for distal segment of the CHD or mid-distal of LCX or right coronary artery, and 0.5 for all others.

Statistical analysis

Statistical analysis was performed using SPSS software, version 13.0 (SPSS Inc., Chicago, IL, USA). Continuous

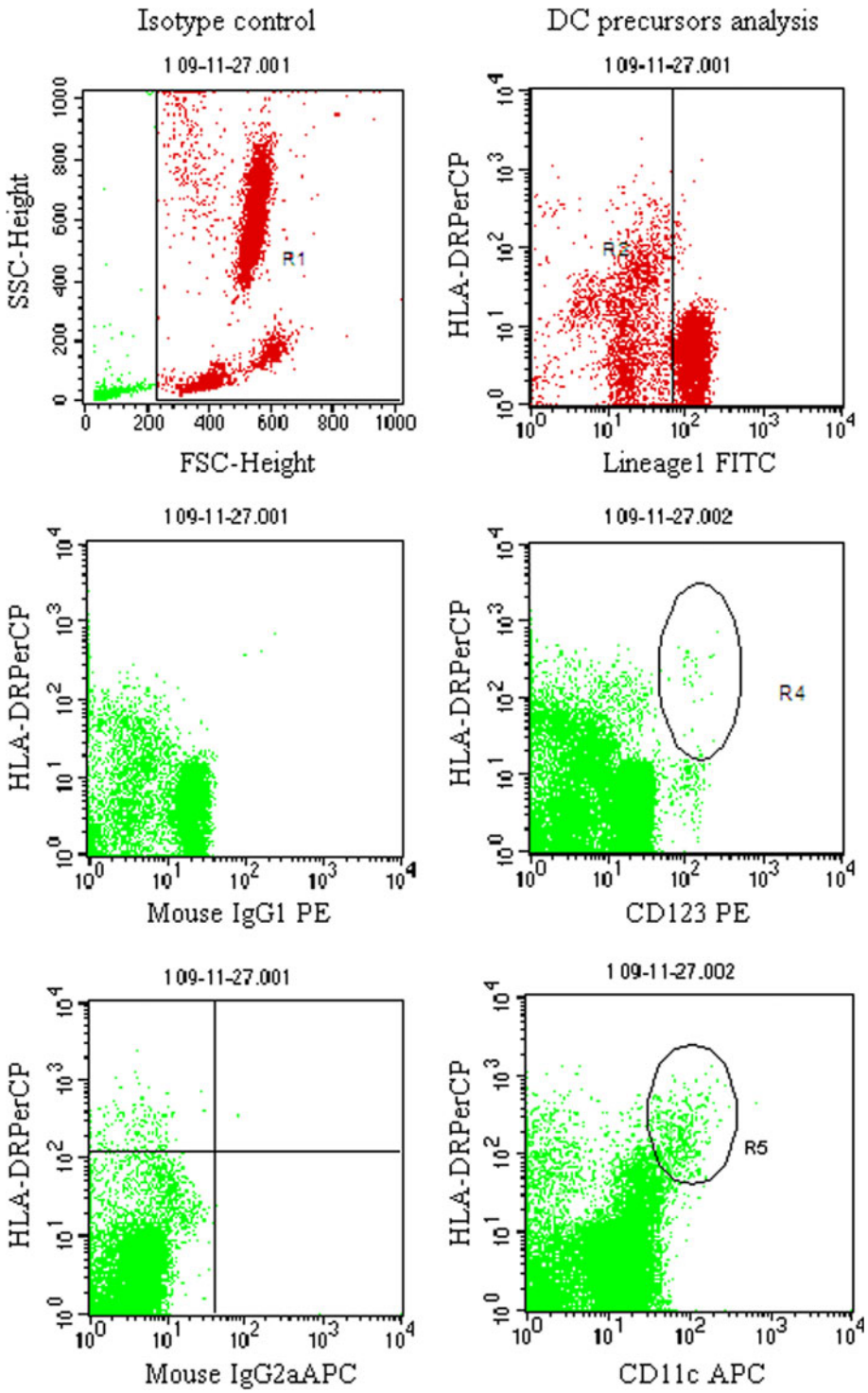

Fig. 1 Detection of dendritic cell precursors (mDC and pDC) in peripheral blood by four-color flow cytometry. R1: region based on forward and side light scatter properties to exclude debris. R2: region containing DC, defined as HLA-DR^+ and lineage cells. R4 and R5: regions containing cells gated on R1 and R2. R4 identifies HLA-DR^+CD123^+ cells (pDC precursors), R5 identifies HLA-DR^+CD11c^+ cells (mDC precursors)

variables were expressed as mean ± SD and categories were expressed as percentages. Data distribution was assessed by the Shapiro–Wilks test. Variables were compared by ANOVA or χ^2 test. Proportions were compared by χ^2 test. Correlation coefficients were assessed by Pearson's product–moment correlation. A p value of less than 0.05 was considered statistically significant.

Results

The clinical characteristics and laboratory data of subjects are summarized in Table 1. The percentage of circulating pDC precursors in peripheral blood mononuclear cells was similar in all groups. The percentage of circulating mDC precursors in peripheral blood mononuclear cells was lower

Table 1 Clinical characteristics of patient cohort

	C	SAP	UAP	AMI	*p*
Age (years)	65 ± 6.9	61 ± 10.4	63 ± 10.1	63 ± 8.7	0.49
Male gender, *n* (%)	21 (72)	22 (73)	39 (70)	32 (80)	0.80
Risk factors, *n* (%)					
Hypertension	9 (31)	9 (30)	20 (36)	11 (26)	0.49
Current smoking	12 (41)	14 (47)	27 (48)	21 (53)	0.86
Diabetes mellitus	5 (17)	7 (23)	12 (21)	13 (33)	0.81
Hypercholesterolemia	11 (38)	13 (43)	30 (54)	19 (48)	0.24
Medication, *n* (%)					
Ca-antagonist	3 (10.3)	5 (17)	11 (20)	4 (10)	0.29
Aspirin	7 (24)	10 (33)	17 (30)	18 (45)	0.39
β-Blockers	5 (17)	4 (13)	8 (14)	5 (13)	0.81
Other antiplatelet agents	2 (7)	3 (10)	6 (11)	10 (25)	0.26
HbA1c (%)	5.40 ± 0.72	5.79 ± 1.59	5.58 ± 0.98	5.82 ± 1.14	0.04
TC (mmol/l)	5.17 ± 1.36	4.81 ± 1.20	4.87 ± 1.24	4.86 ± 1.16	0.65
LDL-C (mmol/l)	2.40 ± 0.68	2.98 ± 1.06[a]	2.80 ± 0.68[a]	2.95 ± 0.86[a]	0.02
TG (mmol/l)	1.56 ± 1.50	1.32 ± 0.47[a]	1.39 ± 0.70[a,b]	1.43 ± 0.60[a, b]	0.74
Leukocytes (g/l)	7.39 ± 1.88	7.83 ± 2.59	7.30 ± 2.09	8.08 ± 3.50	0.44
Creatinine (μmol/l)	114.03 ± 110.29	104.29 ± 57.36	96 ± 20.69[a]	95.86 ± 20.67[a]	0.54
Uric acid (μmol/l)	257.76 ± 116.17	328.33 ± 136.76	340.98 ± 148.12	346.42 ± 121.52	0.03
cTnI (μg/l)	0.012 ± 0.004	0.012 ± 0.011[a]	0.056 ± 0.115[a,b]	14.408 ± 15.818[a,b,c]	<0.01

Values are expressed as percentages or mean ± SD

C controls, *SAP* stable angina pectoris, *UAP* unstable angina pectoris, *AMI* acute myocardial infarction, *HbA1c* hemoglobin A1C, *TC* total cholesterol, *LDL-C* low-density lipoprotein cholesterol, *TG* triglycerides, *cTnI* cardiac troponin I

[a] $p < 0.05$ versus control subjects

[b] $p < 0.05$ versus stable angina pectoris patients

[c] $p < 0.05$ versus unstable angina pectoris patients

in AMI and UAP than in control and SAP, respectively ($p < 0.01$ for AMI vs. SAP and control, $p < 0.05$ for UAP vs. SAP and control; Table 2). The levels of plasma MMP-9 and Genisi scores were higher in AMI and UAP than in Control and SAP, respectively ($p < 0.01$ for AMI vs. SAP and control, $p < 0.05$ for SAP vs. SAP and control; Table 2). Overall, the percentage of mDC, but not pDC, precursors in peripheral blood mononuclear cells, was negatively correlated with MCP-1, MMP-9 and Genisi scores, respectively (Table 3). Also, the levels of MCP-1 and MMP-9 were positively correlated with Genisi scores (Table 4).

Table 2 DC subsets, MMP-9 levels, MCP-1 levels, and Genisi score of subjects in each group

	C	SAP	UAP	AMI	*p*
MMP-9 (ng/ml)	0.12 ± 0.05	0.23 ± 0.14[a]	0.32 ± 0.12[a,b]	0.38 ± 0.12[a,b]	<0.01
MCP-1 (pg/ml)	0.18 ± 0.10	0.37 ± 0.12[a]	0.46 ± 0.14[a,b]	0.55 ± 0.18[a,b]	<0.01
mDC-p (%)	1.26 ± 0.35	0.75 ± 0.31[a]	0.61 ± 0.22[a]	0.52 ± 0.22[a,b]	<0.01
pDC-p (%)	0.15 ± 0.05	0.18 ± 0.05	0.17 ± 0.08	0.15 ± 0.16	0.12
Genisi score	2.00 ± 2.89	39.35 ± 28.71[a]	62.25 ± 36.54[a,b]	72.32 ± 35.90[a,b]	<0.01

Values are expressed as mean ± SD

C controls, *SAP* stable angina pectoris, *UAP* unstable angina pectoris, *AMI* acute myocardial infarction, *MMP-9* matrix metalloproteinase-9, *MCP-1* monocyte chemoattractant protein-1, *mDC-p* myeloid dendritic cell precursors, *pDC-p* plasmacytoid dendritic cell precursors

[a] $p < 0.05$ versus control subjects

[b] $p < 0.05$ versus stable angina pectoris patients

Table 3 Correlation analysis of the percentage of DC precursors in peripheral blood mononuclear cells with MCP-1, MMP-9 and Genisi score in the overall population

	mDC-p (%)		pDC-p (%)	
	r	*p*	*r*	*p*
MCP-1	−0.631	<0.001	0.080	0.309
MMP-9	−0.799	<0.001	−0.014	0.863
Genisi score	−0.707	<0.001	0.073	0.350

r correlation coefficient, *MCP-1* monocyte chemoattractant protein-1, *MMP-9* matrix metalloproteinase-9

Table 4 Correlation analysis of the levels of MCP-1 and MMP-9 with Genisi score in the overall population

	Genisi score	
	r	*p*
MCP-1	0.416	<0.001
MMP-9	0.807	<0.001

r correlation coefficient, *MCP-1* monocyte chemoattractant protein-1, *MMP-9* matrix metalloproteinase-9

Discussion

In this study, we demonstrate that the percentage of mDC precursors in peripheral blood mononuclear cells is lower in AMI and UAP than in Control and SAP, and correlate with Genisi score and the levels of MMP-9 and MCP-1. These data suggest that a decrease in the circulating mDC precursors may relate to the severity and extent of coronary atherosclerotic lesions in patients with CHD.

Previous studies have shown that circulating mDC, but not circulating pDC, precursors are decreased in CHD and increased in atherosclerotic carotid plaques [15]. Recently, however, Fukunaga et al. [37] demonstrated that circulating pDC precursors are significantly lower in ACS than in SAP and Control. Furthermore, recent studies suggest that the decreased circulating mDC precursors may be recruited from blood into atherosclerotic lesions [12, 14, 21, 38, 39]. What remains to be seen is whether decreased circulating mDC precursors are related to the severity and extent of coronary atherosclerotic lesions in patients with CHD. For this reason, we examined the percentage of circulating mDC and pDC precursors and the severity and extent of coronary atherosclerotic lesions in patients with different stages of CHD, to determine their relationship.

Herein, we report that the percentage of circulating mDC precursors was lower in AMI and UAP than in SAP and Control, but similar in AMI and UAP. We also found that the percentage of circulating percentage pDC precursors was not significantly different, a discrepancy between our study and previous studies. One of the reasons for this difference may be due to racial/ethnic or environmental disparities of the studied subjects. Additionally, we may have seen different results because of the use of different diagnostic kits or because of differences in the severity and extent of coronary atherosclerotic lesions in our patient cohort.

In our study, we decided to focus on Han Chinese as the study participants. Even in recent years, partly as the result of the spread of Western lifestyles, the incidence of CHD is rising in China [40], but has not yet reached the incidence levels of developed countries. Furthermore, our study showed that the levels of LDL-cholesterol and HbA1c are increased in CHD. The fact that LDL-cholesterol promotes DCs maturation and migration has been reported previously in in vivo studies [41]. It has also been shown that the number of circulating pDCs, but not circulating mDCs, is decreased in older woman with type 2 diabetes and high HbA1c levels [42]. Furthermore, the effect of immunosuppressive agents [43], statins [17], angiotensin-converting enzyme inhibitors [44], and angiotensin receptor blockers [44] on DCs maturation and migration has been confirmed using in vivo or in vitro experiments. Thus, we selected participants who were not taking these agents at the time.

The interaction of MCP-1 interaction with its receptor, CCR2, is critical for the migration of cells on which CCR2 is expressed: monocytes, macrophages, and DCs. All these immune cells are involved in the pathogenesis of atherosclerotic plaques. Deletion of *MCP-1* or *CCR2* in apolipoprotein E-deficient mice are protected from the development of diet-induced atherosclerosis [25, 26]. Furthermore, CCR2 knock-out mice provide strong evidence that CCR2 is critical for the maturation and migration of DCs [31]. In addition, and consistent with previous results [30], our findings showed that MCP-1 levels were elevated in patients with ACS, and positively correlated with Genisi score, suggesting that high serum MCP-1 levels may reflect a higher burden of coronary atherosclerotic lesions. In our study, the association between high levels of MCP-1 and low percentage of mDC precursors may indicate that MCP-1 plays an important role in the recruitment of circulating mDC precursors to atherosclerotic lesions.

MMP-9, a proteolytic enzyme, is secreted from polymorphonuclear leukocytes. Recently, studies have demonstrated the expression and secretion of MMP-9 by activated monocytes and monocyte-derived DCs [45, 46]. It has been shown that MMP-9 is essential for DCs to migrate in response to CCL19, both in vitro and in vivo [33]. Consistently, we found that the serum levels of MMP-9 were increased in CHD, specifically in patients with ACS [47]. Notably, we found that the serum levels of MMP-9 were negatively correlated with the percentage of mDC

precursors, and positively correlated with Genisi score. The above results indicate that the decreased circulating mDC precursors might be partly recruited from blood into atherosclerotic lesions by circulating MMP-9 in CHD.

To determine the relationship between the subsets of circulating mDC and pDC precursors with the severity of coronary atherosclerotic lesions, Genisi score was used to evaluate the total coronary atherosclerotic burden, as determined by coronary angiography. In our study, we found that the percentage of circulating mDC precursors, but not circulating pDC precursors, was negatively correlated with the Genisi score in patients with CHD. This result, along with previous studies, may indicate that the percentage of mDC precursors reflects the total coronary atherosclerotic burden and that decreased circulating mDC precursors are recruited from blood into the atherosclerotic lesions. Emerging evidence indicates that DCs contribute to promoting plaque inflammation as well as vulnerable plaque formation and rupture [38], indeed the major cause of AMI. So the percentage of circulating mDC precursors may be a promising potential marker for the severity and extent of coronary atherosclerotic lesions.

There are some limitations in our study. First, because of abiding by the necessarily stringent inclusion and exclusion criteria, the sample size is relatively small. Second, we did not determine the levels of CCR2 or CCR7 expression on circulating DCs, which would have helped to better understand the underlying mechanisms of circulating DCs and coronary atherosclerotic lesions. Third, this study is not powered to prove a direct causal relationship between DCs and the formation of vulnerable plaque. Fourth, we did not collect data on a number of molecules that are known to play a role in the migration of DCs, including P-selectin, E-selectin, VCAM-1, CCL5, CX3CL1, CCL19, or CCL21.

In conclusion, we found that the percentage of circulating mDC precursors is negatively correlated with the severity and extent of coronary atherosclerotic lesions. Further clinical studies are required to demonstrate whether regulation of the percentage of circulating mDC precursors in CHD might yield new therapies.

Acknowledgments This study was support by Grant number 2009B030801206 from 2009 Guangdong technology project.

Conflict of interest None declared.

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