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Interleukin-32 increases in coronary arteries and plasma from patients with coronary artery disease

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ABSTRACT

Background: Interleukin-32 (IL-32) is a cytokine associated with higher risk of cardiovascular diseases in inflammatory environments. This study aimed to investigate the IL-32 levels in coronary artery disease (CAD) patients.

Methods: IL-32 expression in coronary arteries from both normal donors and CAD patients were analyzed. Plasma IL-32, IFN-γ and IL-17 levels in stable angina pectoris (SAP, n = 80) patients, unstable angina pectoris (UAP, n = 96) patients, acute myocardial infarction (AMI, n = 72) patients and patients exhibiting chest pain unrelated to coronary artery disease (NCAD, n = 72) were measured. Additionally, whether plasma IL-32 levels were independent correlated with the presence of CAD was analyzed.

Results: IL-32 was high expressed in atherosclerotic plaques of CAD patients when compared with normal coronary arteries, and macrophages were the major sources of IL-32. Compared with the NCAD group, IL-32, IFN-γ and IL-17 levels were increased in the CAD group and gradually increased through the SAP, UAP and AMI groups. Plasma IL-32 levels were positively correlated with the Gensini score, IFN-γ levels and IL-17 levels in CAD patients. The results of linear regression showed that IL-32 was independently associated with the occurrence of CAD.

Conclusion: Both the coronary artery and circulating IL-32 levels were increased in CAD patients and IL-32 may be a marker of noninvasive diagnosis of CAD.

1. Introduction

Coronary artery disease (CAD) is one of the most common and complex clinical diseases and can result in many serious complications. Although the special mechanisms of CAD remain unclear, it is known that local inflammation of the blood vessels is a critical mechanism of atherosclerosis (AS) and is thus critical in CAD as the two diseases are closely related [1,2].

Interleukin-32 (IL-32) is a newly discovered inflammatory cytokine with eight isoforms in most mammals, including IL-32α, IL-32β, IL-32γ, IL-32δ, IL-32ε, IL-32ζ, IL-32η, IL-32θ, and IL-32 s [3]. IL-32 is widely distributed throughout the body, and its expression can be observed both in immune and non-immune cells [4–8]. Previous studies have demonstrated that IL-32 is involved in a variety of diseases in which it amplifies inflammatory response, such as rheumatoid arthritis and inflammatory bowel disease [9,10]. Interestingly, anti-inflammatory responses and protective roles of IL-32 were also observed in liver fibrosis, lipopolysaccharide-induced arthritis and airway inflammation in a mouse model of asthma [8,11,12].

However, recent studies have also demonstrated that IL-32 participates in cardiovascular diseases. In an earlier article, IL-32 found to be highly expressed in human atherosclerotic plaques, and its expression in macrophages and human umbilical endothelial cells could be increased by both Poly I and LPS treatment; furthermore, transgenic mice fed a normal-fat diet exhibited vascular abnormalities resembling AS [13]. In another study, IL-32 levels were significantly increased in patients with heart failure after myocardial infarction, and elevated IL-32 result in poor patient outcome; in addition, infarct size in a mouse model of MI was exacerbated by treatment with recombinant rat IL-32α and IL-32γ [15]. Due to its regulatory effect in inflammatory pathways and several pro-inflammatory cytokines, IL-32 was considered to be closely associated with the risk of cardiovascular diseases [14]. CAD is a common cardiovascular disease and has a mutual nosogenesis with AS; however, the levels of plasma IL-32 associated with CAD are still unknown. This study aims to detect the circulating IL-32 levels in CAD patients and explore possible mechanisms of its pathology.

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2. Materials and methods

2.1. Collection of human coronary artery specimen and blood samples

Normal coronary arteries were collected from (n = 6) normal donors who were declared brain-dead because of a car accident or stroke by two doctors with > 25 years of clinical experience. The donor had no apparent history of cardiovascular disease, and the coronary arteries were not damaged in car accident and without pathology. Diseased coronary arteries (n = 8) were obtained from CAD patients who underwent heart transplantation surgery. All the coronary artery specimens were collected by the surgeons during heart transplantation procedures.

Blood samples were collected from patients (n = 347) who suffered from chest pain and were hospitalized from July 2015 to February 2017. According to major exclusion criteria, some of these patients (n = 27) were screened out of the study [16]. The remaining patients (n = 320) were divided into a non-CAD (NCAD, n = 72) group, stable angina pectoris (SAP, n = 80) group, unstable angina pectoris (UAP, n = 96) group and acute myocardial infarction (AMI, n = 72) group according to the characteristics of the patients' chest pain, results of electrocardiogram and results of coronary angiography [17]. Fasting peripheral venous blood samples were collected into vacutainers with sodium heparin and were centrifuged for 15 min at 3000g; the supernatants were collected and stored at −80 °C. All procedures from collection to preservation of the blood samples were completed within 1 h.

Written informed consent was obtained from patients themselves or their families, and this study protocol was approved by the Medical Ethics Committee of the People's Hospital of Guangxi Zhuang Autonomous Region.

2.2. Histological analysis

Coronary artery specimen were immediately fixed for 5–7 days with 4% neutral paraformaldehyde, and then were embedded in paraffin and cut into 5 μm slices and mounted onto slides. IL-32 expression in each sample was detected by immunofluorescence staining. In addition, double immunofluorescence staining with anti-CD4 antibody and anti-IL-32 antibody, anti-CD68 antibody and anti-IL-32 antibody, anti-CD31 antibody and anti-IL-32 antibody, anti-α-SMA antibody and anti-IL-32 antibody was performed to determine the source of IL-32.

2.3. Measurement of plasma IL-32, IFN-γ and IL-17

Blood samples above were thawed at room temperature and diluted by 1:5, and plasma IL-32 (R&D Systems, USA), IFN-γ and IL-17 (both from eBioscience, USA) levels of each sample were measured using enzyme-linked immunosorbent assay (ELISA) kits following the manufacturer's instructions. The lower limit of detection of IL-32 ELISA kit is 50 pg/ml, and the Intra-assay and inter-assay coefficients of variation for ELISA were < 5% and < 10%, respectively.

2.4. Estimation the severity of coronary stenosis

Gensini scores were used to estimate the severity of coronary stenosis of each patient according to their coronary angiography results. The calculations of a Gensini score for each patient were performed as described in our previous study [16].

3. Statistical analyses

We first analyzed whether the collected data for the cytokine expression levels and the clinical characteristics of the patients' conformed to a normal distribution. The mean ± standard deviation (SD) was calculated for data with a normal distribution. Student's *t*-tests were performed to analyze the differences between two groups, and one-way analysis of variance (ANOVA) with Tukey's post hoc analysis was performed to compare between multiple groups. The median (lower quartile to upper quartile) was calculated for data with a non-normal distribution and were compared using Mann-Whitney *U* tests. The categorical variables are presented as counts (percentages) and compared using chi-square tests. Spearman's correlation analysis was used to calculate correlations between IFN-γ, IL-17, Gensini score and IL-32. All the data were analyzed by SPSS 22.0 software, and a P value < .05 was considered significant.

4. Results

4.1. Basic clinical characteristics of patients

During the patients who provided coronary artery specimen, the CAD group had higher C-reactive protein (CRP) and cardiac troponin I (cTnI) levels when compared with normal group. No significant differences of other clinical characteristics were found between these two groups, including age, male, smoking, lipid levels, and fasting glucose (Glu). Parts of the blood pressure of patients in normal group was maintained by vasoactive drugs and we can't get the confirmed blood pressure values, therefore, the blood pressure data in normal group was not provided. The clinical data of each group are listed in Table 1.

Compared with the NCAD group, the CAD group had an increased percentage of patients who had a history of smoking, diabetes and/or hypertension. Additionally, low-density lipoprotein cholesterol (LDL-C), Glu, CRP, N-terminal B-type natriuretic peptide (BNP), left ventricular ejection fraction (LVEF), creatinine (CREA), cTnI and Gensini score were observed to be higher in CAD patients. There were no differences found in other measured characteristics between the NCAD and CAD groups, including age, sex, occurrence of obesity, hyperlipidemia, body mass index (BMI), blood pressure, total cholesterol (TC), total triglycerides (TG) and high-density lipoprotein cholesterol (HDL-C). The clinical parameters of each group are listed in Table 2.

4.2. IL-32 expression in CAD patients

The IL-32 expression was significantly increased in CAD group when compared with normal group (Fig. 1A). In addition, CD4+ T lymphocytes, macrophages, endothelial cells and smooth muscle cells all secrete IL-32, of which the macrophages were the main source, followed by CD4+ T lymphocytes, smooth muscle cells and endothelial cells secrete less (Fig. 1B).

Table 1
Clinical characteristics who provided aortic samples.

Group	Normal	CAD	P value
Age (years)	54 ± 10	58 ± 8	0.477
Male (n, %)	4 (66.7)	5 (62.5)	0.877
Smoking (n, %)	2 (33.3)	4 (50.0)	0.548
SBP (mmHg)	–	111 ± 13	–
DBP (mmHg)	–	70 ± 9	–
TC (mmol/L)	4.6 ± 0.7	4.7 ± 0.5	0.796
TG (mmol/L)	1.7 ± 0.8	2.0 ± 0.9	0.519
HDL-C (mmol/L)	1.5 ± 0.5	1.4 ± 0.5	0.606
LDL-C (mmol/L)	2.4 ± 0.6	2.0 ± 0.8	0.272
Glu (mmol/L)	5.8 ± 1.2	6.3 ± 1.2	0.437
CRP (mg/L)	1.4 ± 1.1	8.3 ± 5.0	0.005
cTnI (10^{-3} ng/L)	24 (12, 31)	113 (87, 253)	0.007

SBP: systolic blood pressure; DBP: diastolic blood pressure; TC: total cholesterol; TG: total triglycerides; HDL-C: high-density lipoprotein cholesterol; HDL-C: low-density lipoprotein cholesterol; Glu: fasting glucose; CRP: C-reactive protein; BNP: N-terminal B-type natriuretic peptide; LVEF: left ventricular ejection fraction; cTnI: cardiac troponin I.

Table 2
Information of clinical characteristics in NCAD and CAD group.

Characteristics	NCAD	CAD			
		Total	SAP	UAP	AMI
Elderly (n, %)	32 (44.4)	126 (50.1)	38 (47.5)	50 (52.1)	38 (52.1)
Gender (M/F)	40/32	148/100	49/31	56/40	43/29
Smoking (n, %)	17 (23.6)	100 (40.3)[a]	35 (43.8)[a]	33 (33.3)	32 (44.4)[a]
Obesity (n, %)	26 (36.1)	98 (39.5)	26 (40.0)	40 (41.7)	32 (44.4)
Diabetes (n, %)	8 (7.9)	78 (31.5)[a]	21 (26.3)[a,b]	30 (31.3)[a]	27 (37.0)[a]
Hyperlipidemia (n, %)	22 (30.6)	78 (31.5)	28 (35.0)	25 (26.4)	25 (34.7)
Hypertension (n, %)	35 (48.6)	158 (63.7)[a]	53 (66.3)[a]	56 (58.3)	49 (68.1)[a]
Age (years)	57 (46, 66)	61 (53, 68)	58 (48, 68)	62 (53, 67)	61 (53, 63)
BMI (Kg/m^2)	24.5 (22.7, 26.3)	24.7 (22.7, 27.4)	24.3 (22.6, 27.3)	24.8 (22.8, 27.4)	24.8 (22.4, 27.5)
SBP (mmHg)	152 (135, 164)	145 (130, 160)	145 (126, 157)	141 (129, 159)	151 (136, 163)[d]
DBP (mmHg)	84 (78, 97)	84 (75, 96)	80 (72, 95)	80 (74, 90)	90 (80, 100)[c,d]
TC (mmol/L)	4.7 (3.9, 5.1)	4.5 (3.9, 5.0)	4.6 (3.9, 5.0)	4.5 (3.8, 4.8)	4.7 (4.1, 5.1)[d]
TG (mmol/L)	1.3 (1.1, 1.7)	1.3 (1.0, 1.7)	1.2 (0.9, 1.9)	1.2 (1.0, 1.6)	1.4 (1.0, 1.8)
HDL-C (mmol/L)	1.1 (0.9, 1.4)	1.1 (0.9, 1.4)	1.1 (1.0, 1.5)	1.1 (0.9, 1.4)	1.1 (1.0, 1.4)
LDL-C (mmol/L)	2.1 (1.8, 2.6)	2.0 (1.5, 2.6) [a]	2.0 (1.6, 2.7)	1.9 (1.6, 2.5) [a]	2.1 (1.6, 2.6)
Glu (mmol/L)	5.4 (4.9, 5.9)	5.7 (5.0, 6.7) [a]	5.4 (5.0, 6.6)	5.6 (4.9, 6.6)	5.9 (5.3, 7.6)[a,b,c,d]
CREA (μmol/L)	77 (69, 91)	86 (77, 98)[a]	84 (76, 96)[a]	87 (74, 99)[a]	89 (79, 101)[a]
CRP (mg/L)	1.4 (0.6, 2.7)	5.7 (1.8, 12.5)[a]	3.7 (2.5, 17.6)[a]	5.7 (0.9, 9.6) [a]	10.7 (3.0, 19.1)[a,c,d]
BNP (pg/ml)	73 (58, 107)	96 (74, 140)[a]	84 (59, 160)	94 (80, 114)[a]	109 (95, 146)[a,b,c,d]
LVEF (%)	59 (57, 62)	55 (49, 60)[a]	56 (51, 60)	54 (50, 62)	53 (48, 57)[a,b,c,d]
cTnI (10^{-3} ng/L)	9 (6, 17)	17 (8, 1452)[a]	10 (5, 18)[b]	14 (7, 17)[b]	3426 (2173, 8563)[a,b,c,d]
Gensini score	-	32 (18, 51)[a]	21 (10, 34)[a,b]	33 (20, 48)[a,c]	45 (32, 59)[a,b,c,d]
Medications, (n, %)					
ACEI/ARB	19 (26.4)	112 (45.2)[a]	38 (47.5)[a]	42 (43.8)[a]	32 (44.4)[a]
β blockers	4 (5.6)	99 (39.9)[a]	24 (30.0)[a]	28 (29.2)[a]	47 (65.3)[a,b,c,d]
CCB	21 (29.2)	122 (49.2)[a]	44 (55.0)[a]	44 (45.8)[a]	34 (47.2)[a]
Diuretics	6 (8.3)	62 (25.0)[a]	24 (30.0)[a]	24 (25.0)[a]	14 (19.4)[a]
Oral hypoglycemics	6 (8.3)	62 (25.0)[a]	17 (21.3)[a]	23 (24.0)[a]	22 (30.6)[a]
Insulin	4 (5.6)	45 (18.1)[a]	13 (16.3)[a]	18 (18.8)[a]	14 (19.4)[a]
Aspirin	8 (10)	145 (58.5)[a]	42 (52.5)[a]	59 (61.5)[a]	44 (61.1)[a]
Statin	17 (23.6)	178 (71.8)[a]	62 (77.5)[a]	65 (67.7)[a]	51 (70.8)

BMI: body mass index; CREA: creatinine; BNP: N-terminal B-type natriuretic peptide; LVEF: left ventricular ejection fraction; ACEI: angiotensin-converting enzyme inhibitor; ARB: angiotensin receptor blocker; CCB: calcium channel blocker.
[a] $p < 0.05$ vs. control group.
[b] $p < 0.05$ vs. Total CAD group.
[c] $p < 0.05$ vs. SAP group.
[d] $p < 0.05$ vs. UAP group.

4.3. Plasma levels of IL-32, IFN-γ, and IL-17 in CAD patients

Compared with the NCAD group, IL-32 levels were significantly increased in the CAD group (Fig. 2A). In addition, IL-32 levels gradually increased as CAD progressed from SAP to UAP to AMI (Fig. 2A). Similar trends in IFN-γ and IL-17 levels as found in IL-32 levels (Fig. 2B and C). The Spearman's correlation analysis showed that IL-32, IFN-γ, and IL-17 levels were all positively correlated with the Gensini score in CAD patients (Fig. 2D–F). Additionally, in CAD patients, IFN-γ and IL-17 levels were positively correlated with IL-32 levels (Fig. 2G and H). Plasma IL-32, IFN-γ and IL-17 levels in each group are listed in Table 3.

4.4. Simple linear regression analysis and binary logistic regression analysis

To investigate whether IL-32 was correlated with the presence of CAD, simple linear regression analysis and binary logistic regression analysis were performed. The results of simple linear regression analysis showed that IL-32, IFN-γ, IL-17, Glu, BNP, LVEF and cTnI levels exhibited a trend ($p < .05$) toward an association with the onset of CAD, whereas smoking, LDL-C and CREA showed no obvious trend toward this association ($p > .05$). Several of these variables, including IL-32, IFN-γ, IL-17, Glu, BNP, LVEF and cTnI, were used to perform binary logistic regression analyses. The results demonstrated that elevated IL-32 (β 0.118, 95% CI 0.004 to 0.232; $p = .043$), IFN-γ (β 0.205, 95% CI 0.080 to 0.331; $p = .001$) and IL-17 (β 0.237, 95% CI 0.117 to 0.358; $p < .001$) may have an association with the onset of CAD (as shown in Table 4).

5. Discussion

In this study, we found that for the first that higher IL-32 expression was observed in coronary plaque of CAD patients, Both immune cells and non-immune cells, including CD4+ T lymphocytes, macrophages, smooth muscle cells and endothelial cells can secrete IL-32, especial the macrophages, followed by CD4+ T lymphocytes. In addition, plasma IL-32 levels were significantly increased in CAD patients and positively correlated with the severity of CAD. IL-32 levels were also positively correlated with IFN-γ and IL-17 levels in CAD patients. Furthermore, IL-32 was independently associated with the occurrence of CAD.

Currently, more than thirty interleukins have been identified, and evidence from clinical experiments and animal studies has demonstrated their critical role in the progression of CAD and AS. In an AS mouse model, exogenous recombinant IL-4 promoted M2 macrophage differentiation and protected against an increase in atherosclerotic plaque area [18]. In angiotensin II-infusion Apolipoprotein (APO) E knockout mice, the neutralization of IL-5 abrogated the protective effect of Valsartan on AS [19]. Another study reported that regulatory B cells alleviated inflammatory response and AS via promoting IL-10 secretion [20]. Liu J et al. reported that transgenic overexpression of IL-37 promoted the differentiation of T helper cells toward an anti-inflammatory phenotype, increased plaque stability and exhibited significant improvements in atherosclerotic burden [21]. The above cytokines play a protective role in AS, while others have been shown to aggravate the progression of AS. A recent study found that anti-IL-6 receptor antibody treatment was useful in preventing AS induced by

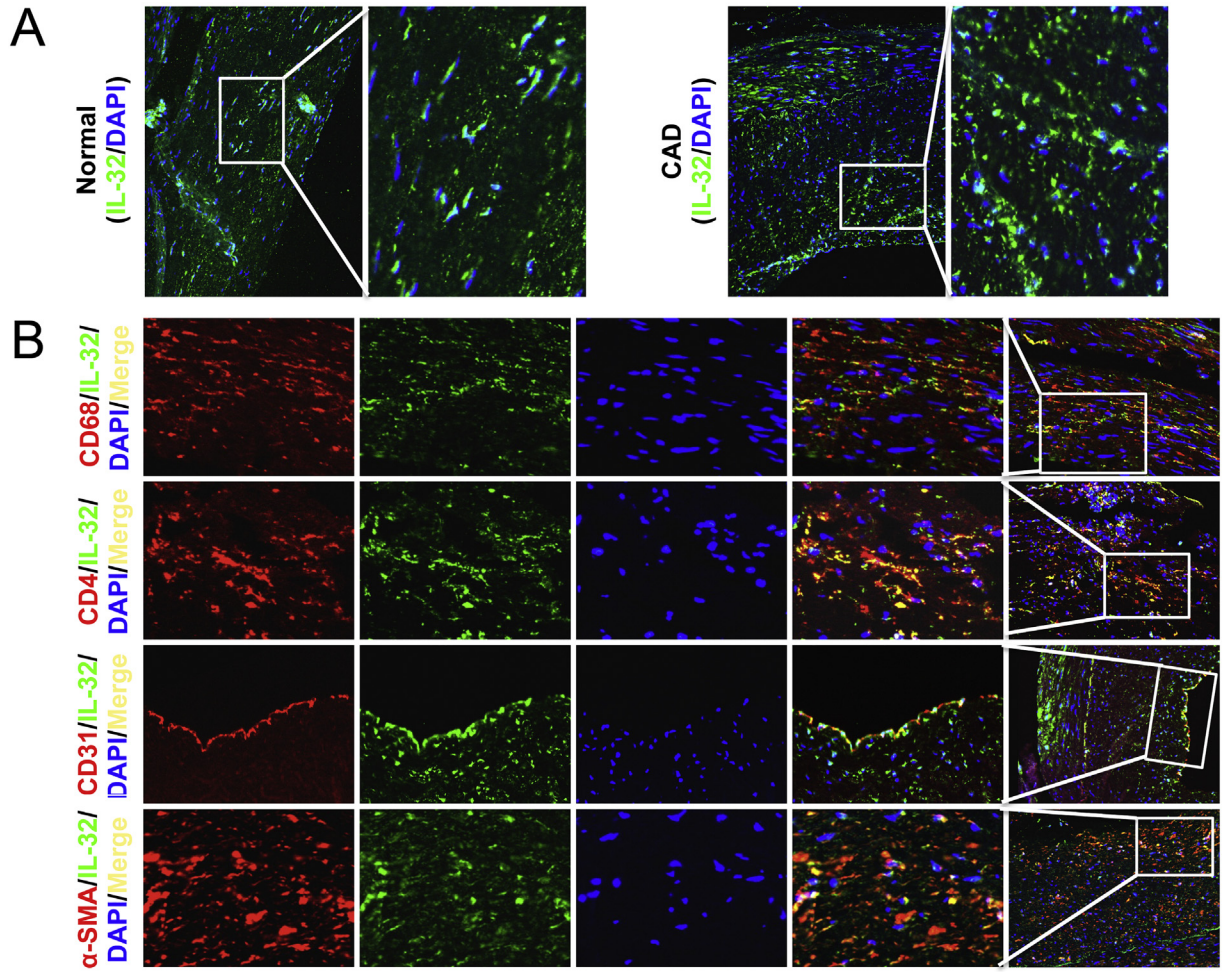

Fig. 1. IL-32 expression in human coronary artery specimen. (A). IL-32 expression in coronary arteries from normal donors and CAD patients were measured by immunofluorescence staining (200×). (B). The source of IL-32 in CAD group was detected by double immunofluorescence staining (200×).

dyslipidemia and/or inflammation [22]. Zhang W et al. reported that in ApoE−/− mice, exogenous recombinant IL-4 treatment activated a STAT3 pathway, up-regulated levels of vascular endothelial adhesion molecule-1, further promoted the infiltration of T cells and macrophages and exerted pro-atherosclerotic effects [23]. A knockout of IL-22 in ApoE−/− mice fed with a high-fat diet exhibited a reduction of plaque area through an effect on smooth muscle phenotype conversion [24]. In addition, the role of some cytokines in AS, such as IL-17, is controversial [25]. Previous studies have also reported that IL-32c transgenic mice fed a normal-fat diet exhibited vascular abnormalities resembling atherosclerosis [13]. In light of the fact that CAD is known to be a further development of AS and to investigate whether IL-32 is involved in CAD, we investigated the plasma levels of IL-32 in CAD patients. The results showed that IL-32 levels were significantly increased in CAD patients. In addition, the Gensini score was used to assess the severity of CAD, and our results showed that IL-32 levels were positively correlated with the Gensini score. These data suggest that IL-32 may participate in the progression of CAD.

CD4+ T lymphocytes and macrophages are the two most important immune cells, which had been demonstrated to be found at all stages of AS and is closely related to the development and progression of AS [26]. In our study, we found that immune cells, including CD4+ T lymphocytes and macrophages, were the main source of IL-32. These results may suggest that IL-32 may participate in AS/CAD via regulating inflammatory response, although non- immune cells also secrete a small amount of IL-32. At present, few studies have been conducted on IL-32 and the exact mechanisms involved in the regulation of CAD are unclear. However, it was determined that the regulation of downstream inflammatory mediators is an important mechanism participating in the disease pathology, including TNF-α, IL-6, IL-1β, CCL2/5 and MMP1/9/13 [13,15]. IFN-γ and IL-17 are functional cytokines of Th1 and Th17, respectively, which have been demonstrated to be closely related to AS and CAD. We therefore investigated whether IL-32 regulates IFN-γ and IL-17 expression and measured IFN-γ and IL-17 levels. The results indicated that both IFN-γ and IL-17 levels were increased in CAD patients and positively correlated with their Gensini score. In addition, a positive correlation of IFN-γ, IL-17 and IL-32 was observed. Furthermore, the binary logistic regression analysis showed that elevated IL-32 was correlated with the onset of CAD. These data may suggest that IL-32 could participate in CAD via regulating the secretion of IFN-γ and IL-17.

Compelling evidence suggests that CD4+ T lymphocytes, which are an important agent of cell-mediated immune response and includes T helper (Th) cells Th2, Th17 and Treg, play an important role in the progression of CAD and AS [27]. Enhanced Th1 immune response was observed in local atherosclerotic lesions and circulating lymphocytes in atherosclerotic animal models as well as in CAD patients. These observations suggested that an imbalance of Th1/Th2 cells plays a critical role in the development of plaque rupture, AS and CAD. This finding has been used to explain the mechanism of AS and CAD development and progression in the past [28,29]. CD4 + CD25+ regulatory T (Treg) cells and Th17 cells were later discovered as novel subtypes of CD4+ Th cells and are distinct from Th1 and Th2 cells. With further research, increased Th1 levels and decreased Treg expression were found in both human CAD patients and animal AS models [30,31], leading some

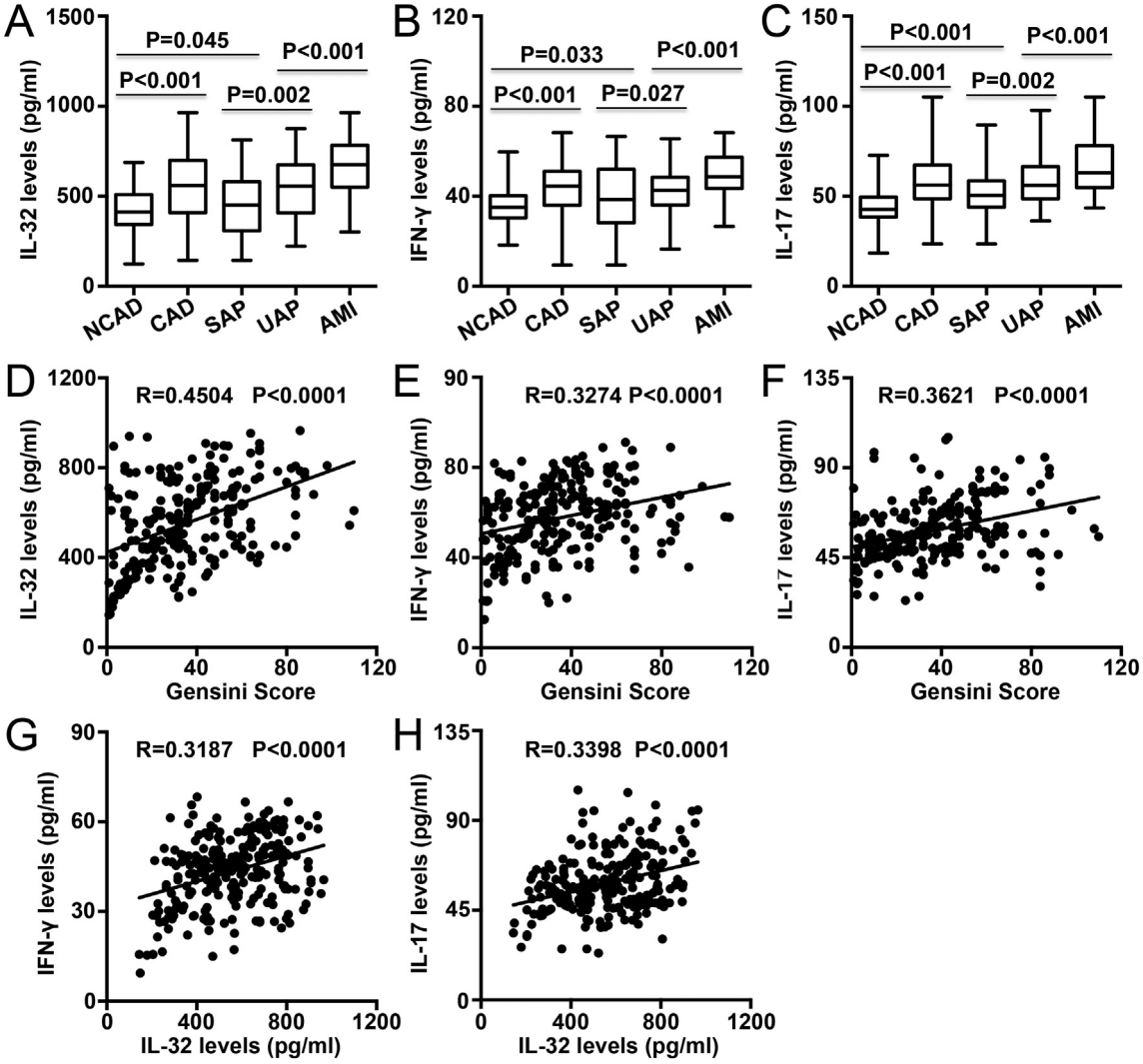

Fig. 2. Plasma IL-32, IFN-γ, and IL-17 levels in the NCAD and CAD groups. (A–C). Plasma IL-32 IFN-γ, and IL-17levels in each group were detected by ELISA. (D–F). Spearman's correlation between IL-32, IFN-γ, IL-17 levels and Gensini score in CAD patients. (G, H). Correlations of IFN-γ and IL-17 levels with IL-32 in CAD patients were assessed by Spearman's correlation analysis.

researchers to suggest that an imbalance of Th17 and Treg may be another mechanism of AS and CAD. Because IFN-γ and IL-17 are the functional cytokines of Th1 and Th17, respectively, and our data showed that both IFN-γ and IL-17 were positively correlated with IL-32 in CAD patients, we speculated that IL-32 may be involved in AS or CAD via regulation of Th1 or Th17 differentiation. While IL-32 has several isoforms, Heinhuis B et al. found that IL-32β, IL-32γ and total IL-32, rather than IL-32α and IL-32δ, were increased in human atherosclerotic vascular tissue [13]. In another study, Xuan W et al. reported that both rIL-32α and rIL-32γ treatment amplified inflammatory response and exacerbated infarct size in a mouse MI model [14]. These data suggest that the biological roles of IL-32 are complex and that different IL-32 isoforms may have a pro-inflammatory effect in specific inflammatory environments.

There were some limitations in the present study. First, evidence has demonstrated that many inflammatory cells participate in the AS and CAD process, such as macrophages and dendritic cells; however, our study only investigated Th1 and Th17 differentiation in vitro without

Table 3
Plasma cytokines in NCAD and CAD group.

Characteristics	NCAD	CAD			
		Total	SAP	UAP	AMI
IL-32 (pg/ml)	398 (325, 464)	561 (409, 700)[a]	451 (308, 581)[a,b]	556 (408, 675)[a,c]	676 (551, 784)[a,b,c,d]
IFN-γ (pg/ml)	34.5 ± 6.9	43.4 ± 11.6[a]	38.7 ± 13.3[a,b]	43.1 ± 8.9[a,c]	49.1 ± 9.6[a,b,c,d]
IL-17 (pg/ml)	42.7 (38.5, 49.6)	56.3 (48.6, 67.4)[a]	50.6 (44.0, 58.5)[a,b]	56.1 (48.6, 66.6)[a,c]	63.0 (54.9, 78.2)[a,b,c,d]

[a] $p < 0.05$ vs. Control group.
[b] $p < .05$ vs. Total CAD group.
[c] $p < .05$ vs. SAP group.
[d] $p < .05$ vs. UAP group.

Table 4
Association between cytokines, clinical characteristics and the presence of CAD were assessed by simple linear regression analysis and subsequent binary logistic regression analysis.

Variables	Simple linear			Binary logistic		
	β	95% CI	P value	β	95% CI	P value
IL-32	0.346	0.242 to 0.449	< 0.001	0.169	0.057 to 0.280	0.003
IFN-γ	0.333	0.229 to 0.437	< 0.001	0.155	0.080 to 0.331	0.006
IL-17	0.409	0.308 to 0.509	< 0.001	0.252	0.137 to 0.367	< 0.001
Glu	0.138	0.029 to 0.248	0.013	0.059	−0.042 to 0.160	0.253
BNP	0.148	0.039 to 0.257	0.008	0.088	−0.020 to 0.197	0.111
LVEF	−0.094	0.204 to 0.016	0.015	−0.009	−0.115 to 0.097	0.868
cTnI	0.221	0.114 to 0.329	< 0.001	−0.008	−0.121 to 0.105	0.888
Smoking	0.142	0.032 to 0.251	0.011	0.075	−0.023 to 0.173	0.133
LDL-C	−0.091	−0.201 to 0.020	0.107			
CREA	0.111	0.031 to 0.191	0.192			

assessing macrophages, dendritic cells, or expression of other cell types. Second, while we speculated that IL-32 participates in AS and CAD by regulating Th1 and Th17 differentiation in vitro, we did not perform in vivo investigations such as determining plaque area in IL-32-treated ApoE knockout mice.

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References

[1] A.M. Lundberg, G.K. Hansson, Innate immune signals in atherosclerosis, Clin. Immunol. 134 (2010) 5–24.
[2] S. Taleb, A. Tedgui, Z. Mallat, Adaptive T cell immune responses and atherogenesis, Curr. Opin. Pharmacol. 10 (2010) 197–202.
[3] M.B. Khawar, M.H. Abbasi, N. Sheikh, Il-32: a novel pluripotent inflammatory interleukin, towards gastric inflammation, gastric cancer, and chronic rhino sinusitis, Mediat. Inflamm. 2016 (2016) 8413768.
[4] M.S.M.A. Damen, C.D. Popa, M.G. Netea, C.A. Dinarello, L.A.B. Joosten, Interleukin-32 in chronic inflammatory conditions is associated with a higher risk of cardiovascular diseases, Atherosclerosis 264 (2017) 83–91.
[5] K. Ota, M. Kawaguchi, J. Fujita, F. Kokubu, S.K. Huang, Y. Morishima, S. Matsukura, M. Kurokawa, Y. Ishii, H. Satoh, T. Sakamoto, N. Hizawa, Synthetic double-stranded RNA induces interleukin-32 in bronchial epithelial cells, Exp. Lung Res. 41 (2015) 335–343.
[6] C.A. Nold-Petry, M.F. Nold, J.A. Zepp, S.H. Kim, N.F. Voelkel, C.A. Dinarello, Il-32-dependent effects of IL-1beta on endothelial cell functions, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 3883–3888.
[7] D. Li, D. Chen, X. Zhang, H. Wang, Z. Song, W. Xu, Y. He, Y. Yin, J. Cao, C-Jun nterminal kinase and akt signalling pathways regulating tumour necrosis factor-alpha-induced interleukin-32 expression in human lung fibroblasts: implications in airway inflammation, Immunology 144 (2015) 282–290.
[8] A.R. Moschen, T. Fritz, A.D. Clouston, I. Rebhan, O. Bauhofer, H.D. Barrie, E.E. Powell, S.H. Kim, C.A. Dinarello, R. Bartenschlager, J.R. Jonsson, H. Tilg, Interleukin-32: a new proinflammatory cytokine involved in hepatitis c virus-related liver inflammation and fibrosis, Hepatology 53 (2011) 1819–1829.
[9] Y. Shimizu, M. Yamamoto, H. Yajima, C. Suzuki, Y. Naishiro, H. Takahashi, K. Imai, Y. Shinomura, Role of interleukin-32 in the mechanism of chronic inflammation in igg4-related disease and as a predictive biomarker for drug-free remission, Mod. Rheumatol. 26 (2016) 391–397.
[10] Y.E. Park, G.T. Kim, S.G. Lee, S.H. Park, S.H. Baek, S.I. Kim, J.I. Kim, H.S. Jin, IL-32 aggravates synovial inflammation and bone destruction and increases synovial natural killer cells in experimental arthritis models, Rheumatol. Int. 33 (2013) 671–679.
[11] B.R. Bang, H.S. Kwon, S.H. Kim, S.Y. Yoon, J.D. Choi, G.H. Hong, S. Park, T.B. Kim, H.B. Moon, Y.S. Cho, Interleukin-32γ suppresses allergic airway inflammation in mouse models of asthma, Am. J. Respir. Cell Mol. Biol. 50 (2014) 1021–1030.
[12] M.H. Park, D.Y. Yoon, J.O. Ban, D.H. Kim, D.H. Lee, S. Song, Y. Kim, S.B. Han, H.P. Lee, J.T. Hong, Decreased severity of collagen antibody and lipopolysaccharide-induced arthritis in human IL-32β overexpressed transgenic mice, Oncotarget 6 (2015) 38566–38577.
[13] B. Heinhuis, C.D. Popa, B.L. van Tits, S.H. Kim, P.L. Zeeuwen, W.B. van den Berg, J.W. van der Meer, J.A. van der Vliet, A.F. Stalenhoef, C.A. Dinarello, M.G. Netea, L.A. Joosten, Towards a role of interleukin-32 in atherosclerosis, Cytokine 64 (2013) 433–440.
[14] W. Xuan, W. Huang, R. Wang, C. Chen, Y. Chen, Y. Wang, X. Tan, Elevated circulating IL-32 presents a poor prognostic outcome in patients with heart failure after myocardial infarction, Int. J. Cardiol. 243 (2016) 367–373.
[15] M.S.M.A. Damen, C.D. Popa, M.G. Netea, C.A. Dinarello, L.A.B. Joosten, Interleukin-32 in chronic inflammatory conditions is associated with a higher risk of cardiovascular diseases, Atherosclerosis 264 (2017) 83–91.
[16] J. Ye, M. Wang, Y. Xu, J. Liu, H. Jiang, Z. Wang, Y. Lin, J. Wan, Sestrins increase in patients with coronary artery disease and associate with the severity of coronary stenosis, Clin. Chim. Acta 472 (2017) 51–57.
[17] Y. Lin, Y. Huang, Z. Lu, C. Luo, Y. Shi, Q. Zeng, Y. Cao, L. Liu, X. Wang, Q. Ji, Decreased plasma IL-35 levels are related to the left ventricular ejection fraction in coronary artery diseases, PLoS ONE 7 (2012) e52490.18.
[18] K. Meng, Q. Zeng, Q. Lu, Y. Lin, B. Wu, K. Yu, Z. Dong, J. Zhang, M. Chai, Y. Liu, Q. Ji, Y. Zhou, Valsartan attenuates atherosclerosis via upregulating the Th2 immune response in prolonged angiotensin II-treated ApoE(−/−) mice, Mol. Med. 21 (2015) 143–153.
[19] H. Rincón-Arévalo, J. Villa-Pulgarín, J. Tabares, M. Rojas, G. Vásquez, J.R. Ramírez-Pineda, D. Castaño, L.M. Yassin, Interleukin-10 production and T cell-suppressive capacity in B cell subsets from atherosclerotic apoE −/− mice, Immunol. Res. 65 (2017) 995–1008.
[20] L. Cardilo-Reis, S. Gruber, S.M. Schreier, M. Drechsler, N. Papac-Milicevic, C. Weber, O. Wagner, H. Stangl, O. Soehnlein, C.J. Binder, Interleukin-13 protects from atherosclerosis and modulates plaque composition by skewing the macrophage phenotype, EMBO Mol. Med. 4 (2012) 1072–1086.
[21] J. Liu, J. Lin, S. He, C. Wu, B. Wang, J. Liu, Y. Duan, T. Liu, S. Shan, K. Yang, N. Dong, Q. Ji, K. Huang, D. Li, Transgenic overexpression of IL-37 protects against atherosclerosis and strengthens plaque stability, Cell. Physiol. Biochem. 45 (2018) 1034–1050.
[22] K. Akita, K. Isoda, Y. Sato-Okabayashi, T. Kadoguchi, K. Kitamura, F. Ohtomo, K. Shimada, H. Daida, An Interleukin-6 receptor antibody suppresses atherosclerosis in Atherogenic mice, Front Cardiovasc. Med. 4 (2017) 84.
[23] W. Zhang, T. Tang, D. Nie, S. Wen, C. Jia, Z. Zhu, N. Xia, S. Nie, S. Zhou, J. Jiao, W. Dong, B. Lv, T. Xu, B. Sun, Y. Lu, Y. Li, L. Cheng, Y. Liao, X. Cheng, IL-9 aggravates the development of atherosclerosis in ApoE−/− mice, Cardiovasc. Res. 106 (2015) 453–464.
[24] S. Rattik, K. Hultman, U. Rauch, I. Söderberg, L. Sundius, I. Ljungcrantz, A. Hultgårdh-Nilsson, M. Wigren, H. Björkbacka, G.N. Fredrikson, J. Nilsson, IL-22 affects smooth muscle cell phenotype and plaque formation in apolipoprotein E knockout mice, Atherosclerosis 242 (2015) 506–514.
[25] S. Taleb, A. Tedgui, Z. Mallat, IL-17 and Th17 cells in atherosclerosis: subtle and contextual roles, Arterioscler. Thromb. Vasc. Biol. 35 (2015) 258–264.
[26] C. Wu, S. He, Y. Peng, K.K. Kushwaha, J. Lin, J. Dong, B. Wang, J. Lin, S. Shan, J. Liu, K. Huang, D. Li, TSLPR deficiency attenuates atherosclerotic lesion development associated with the inhibition of TH17 cells and the promotion of regulator T cells in ApoE-deficient mice, J. Mol. Cell. Cardiol. 76 (2014) 33–45.
[27] X. Zhou, A.K.L. Robertson, C. Hjerpe, G.K. Hansson, Adoptive transfer of CD4+ T cells reactive to modified low-density lipoprotein aggravates atherosclerosis, Arterioscler. Thromb. Vasc. Biol. 26 (2006) 864–870.
[28] J. Frostegård, A.K. Ulfgren, P. Nyberg, U. Hedin, J. Swedenborg, U. Andersson, K.H. Göran, Cytokine expression in advanced human atherosclerotic plaques: dominance of proinflammatory (Th1) and macrophage-stimulating cytokines, Atherosclerosis 145 (1999) 33–43.
[29] X. Cheng, Y. Chen, J.J. Xie, R. Yao, X. Yu, M.Y. Liao, Y.J. Ding, T.T. Tang, Y.H. Liao, Y. Cheng, Suppressive oligodeoxynucleotides inhibit atherosclerosis in ApoE (−/−) mice through modulation of Th1/Th2 balance, J. Mol. Cell. Cardiol. 45 (2008) 168–175.
[30] J.J. Xie, J. Wang, T.T. Tang, J. Chen, X.L. Gao, J. Yuan, Z.H. Zhou, M.Y. Liao, R. Yao, X. Yu, D. Wang, Y. Cheng, Y.H. Liao, X. Cheng, The Th17/Treg functional imbalance during atherogenesis in ApoE (−/−) mice, Cytokine 49 (2010) 185–193.
[31] X. Cheng, X. Yu, Y.J. Ding, Q.Q. Fu, J.J. Xie, T.T. Tang, R. Yao, Y. Chen, Y.H. Liao, The Th17/Treg imbalance in patients with acute coronary syndrome, Clin. Immunol. 127 (2008) 89–97.