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BRIEF REPORT

Platelets from patients with chronic inflammation have a phenotype of chronic IL-1β release

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Abstract

Background: Chronic inflammation is a cardiovascular risk factor, and interleukin-1β (IL-1β) is central to the inflammatory host response. Platelets contain the NLRP3 inflammasome and are able to translate IL-1β messenger RNA (mRNA) and secrete mature IL-1β upon activation. However, the role of a chronic inflammatory environment in platelet IL-1β mRNA and protein content remains unclear.

Objectives: The aim of the current study was to investigate intracellular platelet IL-1β and IL-1β mRNA in a chronic inflammatory state.

Methods: Sixty-five patients with stable inflammation (ie, high-sensitivity C-reactive protein within predefined margins in 2 separate measurements) were stratified according to high-sensitivity C-reactive protein levels in low (0.0-0.9 mg/L), medium (1.0-2.9 mg/L), and high (3.0-9.9 mg/L) risk groups. Platelet reactivity as well as platelet IL-1β protein synthesis were studied.

Results: The highest risk group was characterized by a distinct cardiovascular risk profile and approximately 20% higher platelet counts. While platelet reactivity was not different, a reduction in intracellular platelet IL-1β mRNA and IL-1β protein levels was observed in the highest risk group and was linked to decreased platelet size and granularity. This signature suggests a phenotype of chronic IL-1β secretion and could be experimentally phenocopied by stimulation of platelets from healthy volunteers with either TRAP-6 or collagen related peptide (CRP-XL).

Conclusion: Our data suggest a phenotype of chronic IL-1β secretion by platelets in patients with chronic sterile inflammation.

KEYWORDS
caspase-1, high sensitive C-reactive protein, inflammation, interleukin-1beta, platelets

Essentials

- The role of platelet IL-1β release in chronic inflammation is currently unclear.
- Platelets from 65 patients with varying degrees of chronic inflammation were studied.
- Chronic inflammation linked to reduced levels of intracellular IL-1β and IL-1β release.
- Chronic inflammation induces a phenotype that indicates chronic IL-1β release from platelets.

1 | INTRODUCTION

Chronic inflammation is a cardiovascular risk factor that predisposes individuals to cardiovascular disease [1]. Interleukin-1β (IL-1β) is central to the inflammatory host response, and pharmacological inhibition by canakinumab and colchicine is an effective therapeutic approach to reduce atherothrombotic and cardiovascular events [2,3]. Leucocytes are thought to be the main source of IL-1β, which is synthesized as pro–IL-1β in the cell and gets released in its mature form after proteolytic cleavage by caspase-1. When leucocytes are exposed to certain danger-associated molecular patterns (ie, lipopolysaccharide), intracellular transcription and synthesis of pro–IL-1β is triggered [4]. A second signal such as adenosine triphosphate initiates assembly and activation of an intracellular multimeric inflammasome complex that consists of the NACHT leucine-rich repeat pyrin domain containing protein 3 (NLRP3) and the apoptosis-associated speck-like protein [4]. Together, these events lead to cleavage of pro–caspase-1 into its active form caspase-1 and subsequent cleavage and release of active IL-1β.

Platelets are at the crossroads of thrombosis and inflammation, and megakaryopoiesis is in part regulated by IL-1β plasma levels [2,5,6]. Several lines of evidence demonstrate that platelets process and translate IL-1β messenger RNA (mRNA) to mature IL-1β, which is cleaved and secreted upon activation [7–10]. In addition, the presence of the NLRP3 inflammasome in platelets has been demonstrated by various studies [11–13]. These data suggest a close relationship between the IL-1β pathway and platelet biology. While there is a plethora of experimental data to suggest IL-1β production, the role in cardiovascular disease is unclear. Therefore, the aim of the current study was to investigate intracellular IL-1β protein content, platelet IL-1β mRNA levels, and platelet activity in the context of a chronic inflammatory state.

2 | METHODS

2.1 | Material and reagents

Human IL-1β enzyme-linked immunosorbent assay kit was from Aviva System Biology. Anti–IL-1β antibody (3A6) and anti-GAPDH antibody (14C10) were from Cell Signaling. Primers for IL-1β (HS01555410), Caspase-1 (HS00354836), TLR2 (HS02621280), TLR4 (HS00152939), MYD88 (HS01573837), and GAPDH (HS02786624) as well as TaqMan Gene Expression Master Mix and complementary DNA Reverse Transcription Kit were from Applied Biosystems. Custom-made pre–IL-1β primer was from Eurofins. FAM-FLICA was from Immunochem Technologies. PAC-1, anti–P-selectin (clone AK4), and anti–IL-1β (clone AS10) were from BD Bioscience. TRAP-6 was from Anaspec. CRP-XL was from Cambcol. All other reagents were from Sigma Aldrich.

2.2 | Patient population

Sixty-five patients were recruited between June 2018 and June 2019 while admitted to the Department of Internal Medicine 1 at University Hospital Aachen (Supplementary Figures S1 and S4). All patients were required to have stable coronary artery disease (ie, diagnosed by angiography). Previous myocardial infarction was allowed if it occurred ≥30 days before inclusion. In addition, patients with an estimated glomerular filtration rate of <30 mL/min/1.73 m^2, prior/current malignancy other than basal cell carcinoma, history of tuberculosis and/or history of ongoing chronic or recurrent infectious disease were excluded from the study. To minimize the impact of infectious diseases, patients were required to have stable high-sensitivity C-reactive protein (hsCRP) levels, which was defined as hsCRP within predefined margins (low, 0.0-0.9 mg/L; medium, 1.0-2.9 mg/L; high, 3.0-9.9 mg/L) [14] in 2 separate (≥24 hours) measurements. For some additional experiments, blood was taken from drug-free healthy volunteers. Human samples were processed and stored in accordance with the regulations of the Rheinisch Westfälische Technische Hochschule-Biobank and approved by the Ethics Committee of the Medical Faculty (EK 206/09).

2.3 | Platelet preparation

Citrated blood was taken as previously described [15]. Platelet-rich plasma (PRP) was obtained by centrifugation of whole blood at 200*g* at 20 °C for 15 minutes. For some experiments, washed platelets were needed. PRP was treated with citric acid (0.3 M) and centrifuged for 12 minutes at 800*g*. The platelet pellet was then resuspended in a wash buffer (36-mM citric acid, 10-mM EDTA, 5-mM d-glucose, 5-mM KCl, and 9-mM NaCl; pH 6.5) and centrifuged again for 12 minutes at 800*g*. Platelets were finally resuspended at indicated concentrations in modified Tyrode's buffer (150-mM NaCl, 5-mM HEPES, 0.55-mM

NaH_2PO_4, 7-mM $NaHCO_3$, 2.7-mM KCl, 0.5-mM $MgCl_2$, and 5.6-mM glucose; pH 7.4).

2.4 | Flow cytometry and intracellular IL-1β staining

PRP was stimulated with increasing concentrations of TRAP-6 for 20 minutes. The reaction was stopped by addition of 20:1 Fix/Lyse Buffer (BD Bioscience) for 10 minutes. Ten thousand events were gated by characteristic platelet side and forward scatter properties and assessed for P-selectin and PAC-1 expression as median fluorescence intensity (MFI) on a BD-FACS Canto II (BD Bioscience). For assessment of intracellular IL-1β, platelets were stained for CD42b, fixed in 20:1 Fix/Lyse Buffer, and permeabilized with Triton X-100 0.1% (v/v in phosphate-buffered saline) for 5 minutes at 4 °C. Subsequently platelets were stained for intracellular IL-1β (BD Bioscience) and washed 2 times with ice-cold phosphate-buffered saline, and CD42b-positive cells were collected on a BD-FACS Canto II.

2.5 | Caspase-1 activity assay (FAM-FLICA)

Platelets were incubated with FAM-FLICA for 5 minutes, and the reaction was subsequently quenched with 450 μL of ice-cold modified Tyrode's buffer. In some cases, platelets were pretreated with TRAP-6 (10 μM), CRP-XL (10 μg/mL), or melittin (10 μM, positive control) for 60 minutes (Supplementary Figure S3). The FAM-FLICA signal from platelets was assessed on the BD-FACS Canto II. Data were normalized relative to platelets treated for 60 minutes with 10 μM melittin to induce maximal NLRP3 inflammasome activity [16].

2.6 | Western blot

PRP (5×10^8 platelets/mL) was treated with either CRP-XL (10 μg/mL) or TRAP-6 (10 μM) for 60 minutes. Platelets were then resuspended at 1:1 in wash buffer (36-mM citric acid, 10-mM EDTA, 5-mM d-glucose, 5-mM KCl, and 9-mM NaCl; pH 6.5) and centrifuged for 12 minutes at 800*g*. The platelet pellet was then lysed in Laemmli buffer, separated by Sodium Dodecyl Sulfate - Poly Acrylamid Gel Electrophoresis, transferred to a PVDF membrane, and probed for IL-1β (1:1000) and GAPDH (1:000), representative of $N = 3$ independent experiments. Imaging was performed on a Bio-Rad ChemiDoc MP Imaging System.

2.7 | Polymerase chain reaction

Platelet mRNA was isolated from washed platelets by Trizol (Invitrogen) as previously described [8]. mRNA was then transcribed into complementary DNA by reverse transcription according to the manufacturer instructions (Applied Biosystems). Real-time reverse-transcription polymerase chain reaction was performed according to manufacturer instructions for mRNA for IL-1β (HS01555410), pre–IL-1β, caspase-1 (HS00354836), TLR2 (HS02621280), TLR4 (HS00152939), MYD88 (HS01573837), and GAPDH (HS02786624). The primer for pre–IL-1β was custom-designed to target intron 1 (ie, forward primer; GTAGCCGTCATGGGGAAGTC) and exon 1 (ie, reverse primer; CGAGCTCAGGTACTTCTGCC).

2.8 | Statistical analysis

The baseline characteristics in the Table are reported as means with SD in case of continuous variables, and frequencies with percentages in case of binary variables. Figures are presented as mean with SEM. Two groups were compared by a Student's *t*-test, while comparisons between 2 and more groups were made with the chi-square test with analysis of variance (1-way analysis of variance) for categorical and continuous data, respectively. Post hoc testing was performed by Dunnett's test. All statistical analysis was performed with GraphPad Prism v9.0 and SPSS version v28.0 (IBM).

3 | RESULTS

3.1 | Patient characteristics according to hsCRP levels

Patient characteristics stratified by hsCRP risk groups are presented in the Table. Patients within the highest risk group (ie, CRP_{high}) were characterized by a distinct risk profile including increased interleukin-6 and fibrinogen plasma levels as well as higher rates of previous myocardial infarction and presence of peripheral artery disease (all $P < .05$; Table). Of note, patients within the highest risk group had approximately 20% higher leukocyte and platelet counts compared to the lowest risk group (all $P < .05$; Table).

3.2 | Platelet reactivity and inflammatory phenotype according to hsCRP levels

Platelet reactivity measured by TRAP-6 induced P-selectin expression, and integrin αIIbβ3-activation (PAC-1) was not different between hsCRP risk groups (Figure 1A, B). Next, we assessed the potential of platelets to synthesize the proinflammatory cytokine IL-1β. Intracellular protein levels of platelet IL-1β measured by flow cytometry ($hsCRP_{low}$, 14.1 ± 1.5 MFI; $hsCRP_{intermediate}$, 15.5 ± 2.9 MFI; $hsCRP_{high}$, 7.7 ± 1.2 MFI; $P = .004$ [$hsCRP_{low}$ vs $hsCRP_{high}$]) and platelet IL-1β secretion ($hsCRP_{low}$, 1.0; $hsCRP_{intermediate}$, 0.5 ± 0.4; $hsCRP_{high}$, 0.4 ± 0.2; $P = .008$ [$hsCRP_{low}$ vs $hsCRP_{high}$]) were significantly reduced in the highest risk group (Figure 1C, D). These changes were paralleled by a trend toward an increase of IL-1β plasma levels ($hsCRP_{low}$, 4.8 ± 0.9 pg/mL; $hsCRP_{intermediate}$, 7.2 ± 2.3

TABLE Patient characteristics stratified by stable high-sensitivity C-reactive protein.

		High-sensitivity C-reactive protein			
	Overall, *N* = 65	**0-0.9 mg/L**	**1.0-2.9 mg/L**	**3.0-9.9 mg/L**	***P* value**
Patient characteristics		*N* = 22	*N* = 14	*N* = 29	
Age	72 (10)	72 (10)	72 (9)	73 (10)	>.99
Male/female	47/65 (72%)	16/22 (73%)	11/14 (79%)	20/29 (69%)	.94
BMI	27.3 (4.1)	26.2 (2.9)	27.2 (4.2)	28.2 (4.8)	.23
Laboratory parameters					
hsCRP (mg/L)	2.49 (1.92)	0.54 (0.20)	1.62 (0.55)	4.39 (1.05)	**<.001**
IL-1β (pg/mL)	6.8 (10.5)	4.8 (1.0)	7.2 (8.8)	8.1 (14.6)	.57
IL-6 (pg/mL)	6.2 (3.6)	4.6 (2.1)	5.9 (3.7)	7.8 (4.0)	**.007**
Fibrinogen (g/L)	311 (51)	276 (33)	321 (41)	335 (54)	**<.001**
Leucocytes (/μL)	6.68 (1.64)	6.11 (1.29)	6.37 (1.32)	7.23 (1.87)	**.042**
Platelets (/nL)	220 (62)	196 (47)	212 (37)	240 (75)	**.042**
Hemoglobin (g/L)	13.14 (2.13)	13.40 (1.03)	13.53 (2.09)	12.76 (2.68)	.43
Creatinine (mg/dL)	1.08 (0.40)	0.99 (0.21)	1.02 (0.24)	1.18 (0.54)	.24
eGFR (mL/min/17.3 m^2)	69 (16)	72 (14)	72 (14)	65 (18)	.29
HbA1c, %	6.04 (1.04)	5.80 (0.56)	6.29 (1.49)	6.09 (1.05)	.38
Total cholesterol (mg/dL)	164 (49)	150 (33)	162 (39)	175 (60)	.18
LDL (mg/dL)	101 (44)	91 (30)	94 (33)	112 (54)	.2
HDL (mg/dL)	51 (16)	51 (17)	53 (16)	50 (16)	.83
NTproBNP (pg/ml)	1117 (1208)	538 (294)	1113 (NA)	1522 (1502)	.27
β-thromboglobulin (pg/mL)	92 (86)	88 (56)	84 (35)	98 (118)	.87
Platelet factor 4 (μg/mL)	37 (41)	32 (37)	47 (48)	35 (40)	.57
Medical history					
Carotid stenosis	2/65 (3.1%)	1/22 (4.5%)	0/14 (0%)	1/29 (3.4%)	>.99
Peripheral artery disease	11/65 (17%)	0/22 (0%)	3/14 (21%)	8/29 (28%)	**.014**
Coronary artery disease					.51
<50% stenosis	4/65 (6.2%)	2/22 (9.1%)	0/14 (0%)	2/29 (6.9%)	
1-vessel	17/65 (26%)	3/22 (14%)	5/14 (36%)	9/29 (31%)	
2-vessel	18/65 (28%)	5/22 (23%)	4/14 (29%)	9/29 (31%)	
3-vessel	26/65 (40%)	12/22 (55%)	5/14 (36%)	9/29 (31%)	
Myocardial infarction	15/65 (23%)	3/22 (14%)	1/14 (7.1%)	11/29 (38%)	**.05**
PCI	33/65 (51%)	13/22 (59%)	6/14 (43%)	14/29 (48%)	.65
CABG	15/65 (23%)	4/22 (18%)	6/14 (43%)	5/29 (17%)	.16
Smoke history					.94
Active smoker	5/65 (7.7%)	2/22 (9.1%)	1/14 (7.1%)	2/29 (6.9%)	
Ex-smoker	29/65 (45%)	10/22 (45%)	5/14 (36%)	14/29 (48%)	
Diabetes	21/65 (32%)	3/22 (14%)	8/14 (57%)	10/29 (34%)	.024
Arterial hypertension	50/65 (77%)	17/22 (77%)	11/14 (79%)	22/29 (76%)	>.99
Hyperlipidemia	41/65 (63%)	15/22 (68%)	8/14 (57%)	18/29 (62%)	.85
Atrial fibrillation	21/65 (32%)	9/22 (41%)	3/14 (21%)	9/29 (31%)	.49

(Continues)

TABLE (Continued)

		High-sensitivity C-reactive protein			
	Overall, *N* = 65	0-0.9 mg/L	1.0-2.9 mg/L	3.0-9.9 mg/L	*P* value
COPD	4/65 (6.2%)	1/22 (4.5%)	1/14 (7.1%)	2/29 (6.9%)	>.99
Medication					
Aspirin	44/65 (68%)	18/22 (82%)	9/14 (64%)	17/29 (59%)	.21
DAPT	14/65 (22%)	4/22 (18%)	3/14 (21%)	7/29 (24%)	.93
Vitamin K antagonist	10/65 (15%)	3/22 (14%)	2/14 (14%)	5/29 (17%)	>.99
β-Blocker	48/65 (74%)	16/22 (73%)	9/14 (64%)	23/29 (79%)	.58
Calcium channel blocker	11/65 (17%)	2/22 (9.1%)	2/14 (14%)	7/29 (24%)	.36
Mineral receptor antagonist	18/65 (28%)	5/22 (23%)	2/14 (14%)	11/29 (38%)	.25
Diuretic	40/65 (62%)	13/22 (59%)	8/14 (57%)	19/29 (66%)	.85
Statin	57/65 (88%)	19/22 (86%)	14/14 (100%)	24/29 (83%)	.3

Data are reported as mean (SD) or *n*/*N* (%).

Bold means $P \leq .05$.

BMI, body mass index; CABG, coronary artery bypass graft; COPD, chronic obstructive pulmonary disease; DAPT, dual antiplatelet therapy; eGFR, estimated glomerular filtration rate (Chronic Kidney Disease - Epidemiology Collaboration formula); HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; IL, interleukin; LDL, low-density lipoprotein; NA, not available; NTproBNP, NT pro brain natriuretic peptide; PCI, percutaneous coronary intervention.

pg/mL; $hsCRP_{high}$, 8.1 ± 2.8 pg/mL; $P = .56$; Figure 1E). In addition, reduced intracellular IL-1β protein was matched by significantly reduced levels of platelet IL-1β mRNA ($hsCRP_{low}$, 0; $hsCRP_{intermediate}$, −0.45 ± 0.29; $hsCRP_{high}$, −0.91 ± 0.24; $P = .017$), while other inflammatory transcripts remained unaffected (all $P > .05$; Figure 1F). Of note, contamination of leukocytes was excluded in all preparations by the absence of leukocyte-specific mRNA colony-stimulating factor (Supplementary Figure S2). In line with diminished IL-1β in the highest risk group, these patients also had higher caspase-1 activity; however, that failed to reach statistical significance ($P = .0503$; Figure 1G).

3.3 | Platelet size and granularity according to hsCRP levels

Structural assessment of platelets within the highest risk group (ie, $hsCRP_{high}$) revealed a trend toward reduced platelet size ($hsCRP_{low}$, 4256 ± 156 MFI; $hsCRP_{intermediate}$, 4189 ± 191 MFI $hsCRP_{high}$, 3708 ± 195 MFI; $P = .063$; Figure 2A) and a significantly reduced granular content ($hsCRP_{low}$, 643 ± 25 MFI; $hsCRP_{intermediate}$, 623 ± 34 MFI; $hsCRP_{high}$, 524 ± 28 MFI, $P = .006$ Figure 2B). These data are in line with previous reports on inflammatory diseases, including systemic lupus erythematosus [17], polymyositis [18], and tuberculosis [19]. Platelet IL-1β secretion has been previously demonstrated to be activation-dependent [7,8,12], and the cytokine is suggested to be located in platelet α-granules and the open canalicular system [10]. Therefore, we hypothesize that the low-size/low-granularity phenotype may indicate chronic low-level degranulation/secretion that contributes to platelet IL-1β secretion. Indeed, platelet granularity (ie, side scatter), P-selectin levels (ie, α-granules marker), and intracellular IL-1β levels correlated significantly with each other (Figure 2C, D). These data suggest that low-level platelet activation contributes to the release of IL-1β in patients with increased levels of inflammation.

3.4 | Activation-dependent IL-1β release by platelets

To experimentally demonstrate that IL-1β release is activation-dependent, we used TRAP-6 and CRP-XL as specific platelet agonists. In healthy, drug-free volunteers, platelet stimulation by TRAP-6 and CRP-XL led to a significant decrease in platelet granularity (TRAP6, 643 ± 25 MFI vs 492 ± 18 MFI; $P = .001$; CRP-XL, 696 ± 11 MFI vs 600 ± 21 MFI; $P = .016$; Figure 3 A, B). This decrease in platelet granularity was matched by an increase in caspase-1 activity (basal, 27.2% ± 2%; TRAP-6, 47.7% ± 3%; CRP-XL, 51.5% ± 7%; $P = .046$ [basal vs TRAP-6]; $P = .028$ [basal vs CRP]; Figure 3C) and increased IL-1β secretion (basal, 0 ng/mL, TRAP-6, 2.3 ± 0.2 ng/mL, CRP-XL, 2.0 ± 0.08 ng/mL; $P = .0001$ [basal vs TRAP-6], $P = .0002$ [basal vs CRP-XL]; Figure 3D). Finally, we demonstrated that activation by either TRAP-6 or CRP-XL led to a significant decrease in intracellular IL-1β protein in platelets ($P = .0011$ [basal vs TRAP-6]; $P = .024$ [basal vs CRP-XL]; Figure 3E, F), similar to the phenotype detected in patients with high level of inflammation. In addition, there was a trend toward reduced levels of pro–IL-1β that failed to reach statistical significance (all $P > .05$; Figure 3E, F). Hence, these data support low-level platelet activation as a potential mechanism of IL-1β secretion in patients (Figure 4).

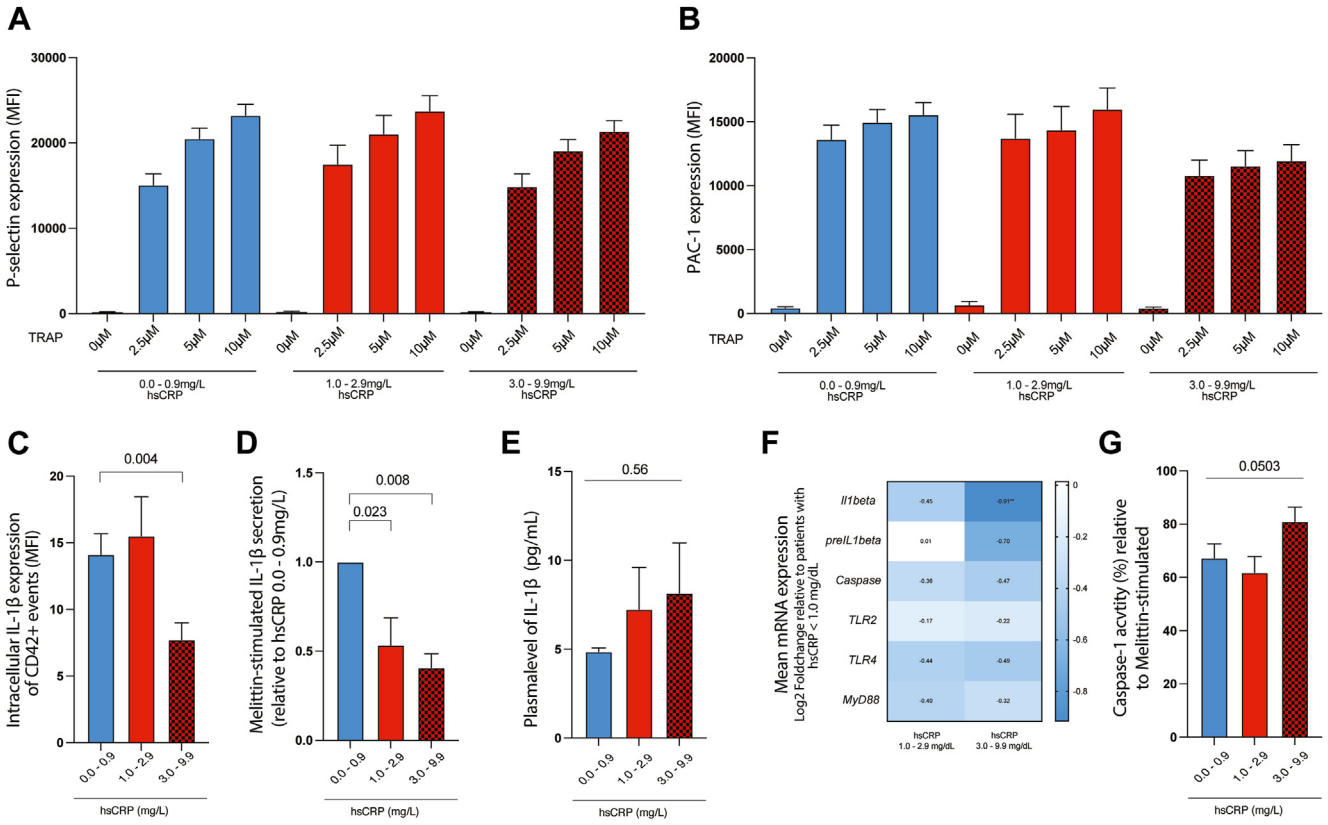

FIGURE 1 Platelet reactivity and inflammatory phenotype according to high-sensitivity C-reactive protein (hsCRP) levels. (A, B) Platelet P-selectin (BD Bioscience) expression and PAC-1 (BD Bioscience) binding in response to TRAP-6 stimulation. Platelet-rich plasma was stimulated with increasing concentrations of TRAP-6 for 20 minutes. Data tested by analysis of variance (ANOVA), all $P > .05$. (C) Median fluorescence intensity (MFI) of intracellular interlukin-1β (IL-1β) of CD42b+ (BD Bioscience) events. Data tested by ANOVA. (D) Melittin-stimulated (10 µM, 60 minutes) IL-1β secretion measured in supernatant from 2×10^8/mL washed platelets. Data relative to patients with a hsCRP level of <1.0 mg/L. Data tested by ANOVA. $N = 7$ patients/group (E) Plasma levels of IL-1β. Data tested by ANOVA. (F) Heatmap for mean platelet messenger RNA (mRNA) expression denoted as Log2-fold change relative to patients with a hsCRP level of <1.0 mg/L. Data tested by ANOVA. (G) Caspase-1 activity in platelet-rich plasma measured by FAM-FLICA. Platelets were gated by characteristic forward and side scatter. Data relative to platelets treated for 60 minutes with 10-µM melittin (positive control). Data tested by ANOVA.

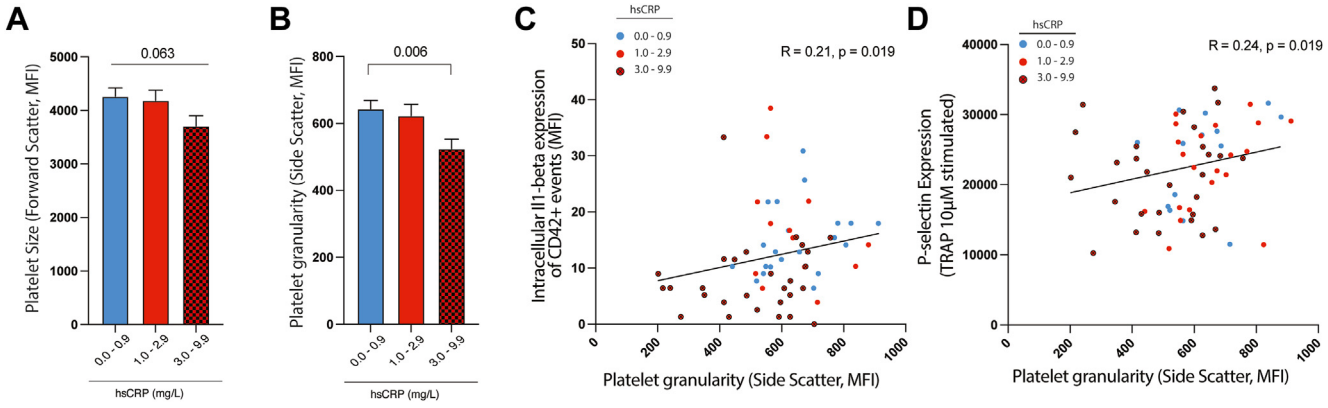

FIGURE 2 Platelet size and granularity according to high-sensitivity C-reactive protein (hsCRP) levels. (A) Platelet size measured in platelet-rich plasma by forward scatter (FSC). Platelets were gated by characteristic FSC and side scatter (SSC). Data tested by analysis of variance. (B) Platelet granularity in platelet-rich plasma assessed by SSC. Platelets were gated by characteristic FSC and SSC. Data tested by analysis of variance. (C, D) Correlation between intracellular platelet interlukin-1β (IL-1β) levels, platelet granularity (SSC), and P-selectin expression. Data points color-coded for low, intermediate, and high hsCRP. Data tested by Spearman Rho. MFI, median fluorescence intensity.

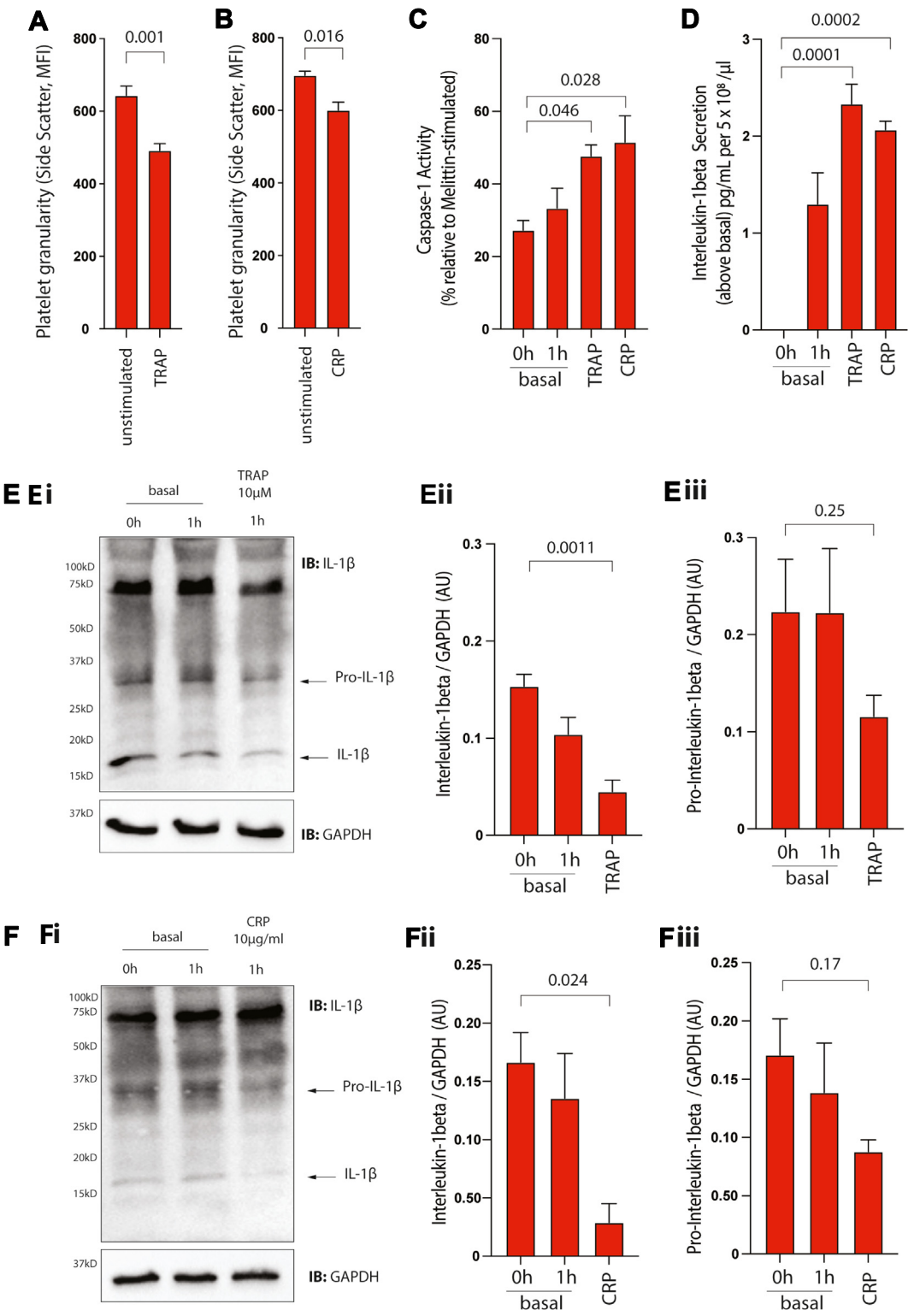

FIGURE 3 Activation-dependent interleukin-1β (IL-1β) release by platelets. (A, B) Platelet granularity in platelet-rich plasma assessed by side scatter in response to TRAP-6 and CRP-XL stimulation. Platelet-rich plasma was treated with or without TRAP-6 or CRP-XL for 60 minutes. Data tested by *t*-test. (C) Caspase-1 activity in platelets measured by FAM-FLICA in response to TRAP-6 and CRP-XL. Data relative to platelets treated for 60 minutes with 10-µM melittin (positive control). Data tested by analysis of variance (ANOVA). (D) IL-1β secretion in response to TRAP-6 and CRP-XL stimulation. Platelets were treated with or without TRAP-6 or CRP-XL for 60 minutes. Data expressed as IL-1β secretion above basal. Data tested by ANOVA. (E, F) Intracellular IL-1β and pro–IL-1β assessed by Western blot after stimulation with (E) TRAP-6 and (F) CRP-XL. Representative of $N = 3$ independent experiments. Data tested by ANOVA.

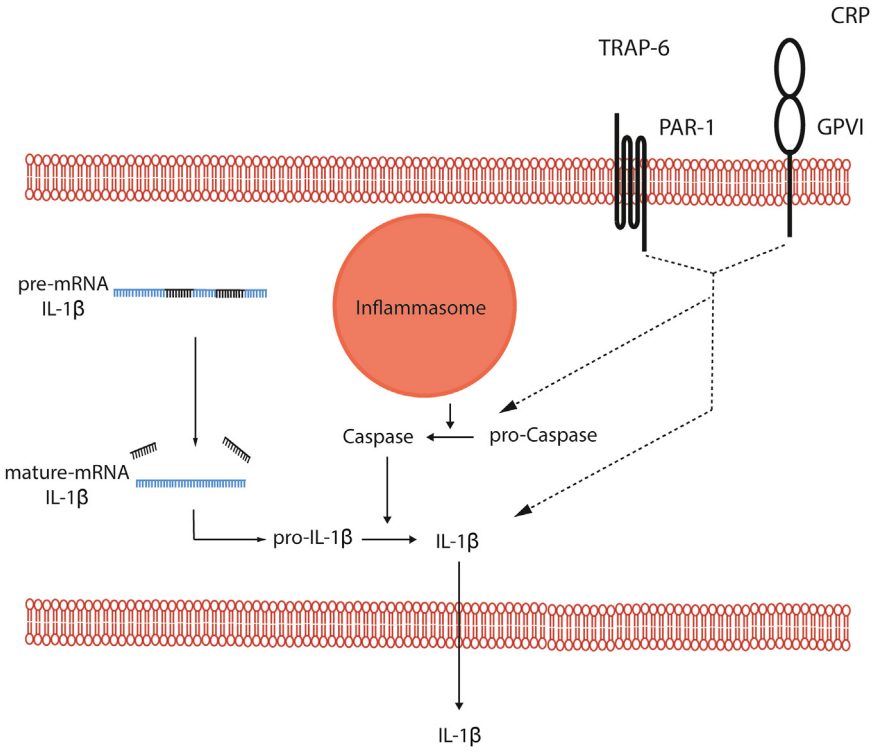

FIGURE 4 Proposed mechanism of activation-dependent interleukin-1β (IL-1β) secretion in platelets. Proposed mechanism of IL-1β secretion in platelets. IL-1β is translated from messenger RNA (mRNA) into pro–IL-1β. Platelet activation through PARs and GPVI and potentially chronic activation lead to activation of caspase-1 and subsequent release of IL-1β. CRP, C-reactive protein; PARs, protease activated receptors.

4 | DISCUSSION

In the current study, we demonstrate that platelets from patients stratified with high hsCRP serum levels express a phenotype that is suggestive of chronic IL-1β release. To our knowledge, this is the first study that investigates platelet IL-1β from mRNA to protein level through secretion in patients with chronic subclinical inflammation.

Elegant studies by Lindemann et al. [7] and Denis et al. [8] first demonstrated that platelet pre-IL-1β mRNA can be actively spliced and translated into pro-IL-1β and subsequently secreted as IL-1β. Our study extends current knowledge and demonstrates that platelets from patients with chronic inflammation are characterized by reduced intracellular IL-1β mRNA and protein levels, which are associated with reduced granularity. IL-1β protein synthesis in anucleate platelets is limited to the predefined content of intracellular IL-1β mRNA [8], and therefore, the parallel reduction in IL-1β mRNA and IL-1β protein levels indicates secretion of the cytokine. This is consistent with a recent report on SARS-CoV-2–infected patients that demonstrated reduced intracellular IL-1β levels linked with α-granule depletion [9]. Of note, while our data suggest a link between α-granule release and intracellular IL-1β levels, the modest correlation suggests additional mechanisms of IL-1β release and identification of these deserves further attention. With respect to the existing literature, our data suggest that an inflammatory environment, either sterile or nonsterile, could facilitate ongoing platelet activation that triggers IL-1β secretion in platelets. We were able to experimentally replicate this phenotype with platelet agonists, indicating that IL-1β secretion may share common pathways with regular platelet function. While the kinetics of IL-1β secretion were beyond the scope of this patient-centered study, our experimental data utilized incubation times for up to 1 hour, which is in line with previous reports on long-term incubations that demonstrate caspase-1 activity, IL-1β cleavage, and IL-1β release [7,11,20]. However, chronic IL-1β secretion in vivo is likely not caused by potent prototypical agonists such as collagen and thrombin because these would trigger full platelet activation, thrombosis, and subsequent thrombocytopenia [21]. In contrast, several low-level platelet agonists including high-mobility group box 1 [22], trimethylamine N-oxide [23], phenylacetylglutamine [24], and oxidized low-density lipoprotein [24] have been identified, which are all linked to a cardiovascular risk profile similar to that found in the current study. These may serve as plausible agonists for chronic IL-1β secretion. Identification of these was beyond the scope of this study but may help to further delineate the role of platelets in inflammation.

Our study has some limitations. First, this study is observational and, therefore, susceptible to bias. In particular, hsCRP is a global inflammatory marker, and the increased levels may point to other yet undefined mechanisms that drive the relationship between IL-1β and platelets. However, while experimental replication of our data is necessary, our data are in line with the current understanding of IL-1β in platelets and supported by previous publications [7,10]. Furthermore, it is likely that the chronic inflammatory environment is influenced by leucocytes; however, while being of interest, the relationship between platelets and leucocytes was beyond the scope of this study. Likewise,

the potential role of cardioprotective medication on the platelet phenotype has not been analyzed due to the low number of patients included. Nevertheless, as the inflammatory phenotype appears to be dependent on canonical platelet activation, it is reasonable to hypothesize that antiplatelet medication may prove effective in the management of this phenotype. In addition, we were unable to investigate the role of megakaryocytes in the context of subclinical inflammation. Therefore, altered platelet biogenesis as a potential confounder in the relationship between IL-1β and platelets cannot be excluded.

5 | CONCLUSION

In the context of inflammation, platelets express a phenotype that is suggestive of activation-dependent IL-1β secretion. If this holds true, platelets may contribute to the chronic inflammatory host response through continuous IL-1β secretion, which deserves further attention in future studies.

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ETHICS STATEMENT

Human samples were processed and stored in accordance with the regulations of the Rheinisch Westfälische Technische Hochschule-Biobank and, the study was approved by the Ethics Committee of the Medical Faculty (EK 206/09).

AUTHOR CONTRIBUTIONS

M.B., K.S., and N.M. designed the research, analyzed data, and wrote the manuscript. K.L. and H.M. performed experiments. S.F.M., K.N., T.S., and M.S.H. analyzed and critically discussed data. E.D. provided essential materials. All authors reviewed the final version of the manuscript.

RELATIONSHIP DISCLOSURE

T.S. reports payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events from and participation on Data Safety Monitoring Boards or Advisory Boards for Amgen, Astellas, AstraZeneca, Bayer, Boehringer Ingelheim, GSK, Novartis, Novo Nordisk, Sanofi, and Vifor. The other authors declare no conflict of interest.

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SUPPLEMENTARY MATERIAL

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