Distinct Subtypes of Microparticle-containing Immune Complexes Are Associated with Disease Activity, Damage, and Carotid Intima-media Thickness in Systemic Lupus Erythematosus

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ABSTRACT. ***Objective.*** Microparticles (MP) are small extracellular vesicles present in body fluids. MP originate from different cellular lineages, principally from platelets in blood, and may expose phosphatidylserine (PS). In systemic lupus erythematosus (SLE), MP harbor immunoglobulin G (IgG), thereby forming MP-containing immune complexes (mpIC). We aimed to verify an association between SLE disease activity, damage, and surrogate markers of atherosclerosis and MP harboring IgG, taking into account the platelet origin and PS exposure of MP.

Methods. MP expressing surface IgG, platelet antigen (CD41+), and PS were quantified using flow cytometry in plasma of 191 women with SLE. Carotid ultrasounds (US) were available in 113 patients. Spearman correlation analysis was used to analyze whether levels of MP were associated with the following outcomes: SLE Disease Activity Index 2000 (SLEDAI-2K), Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index (SDI), and carotid US plaques and intima-media thickness (CIMT) as surrogates for vascular damage.

Results. We found CD41+ MP harboring IgG present in SLE. A positive correlation was found between SLEDAI-2K and levels of CD41+ MP harboring IgG and exposing ($p = 0.027$) and non-exposing PS ($p = 0.001$). Conversely, SDI ($p = 0.024$) and CIMT ($p = 0.016$) correlated with concentrations of CD41– MP harboring IgG and exposing PS. Associations were independent of low-density lipoprotein cholesterol level, body mass index, and antimalarial drug use.

Conclusion. Different subtypes of mpIC are produced in SLE and are associated with distinct clinical characteristics such as disease activity and vascular damage. The assessment of MP subtypes might serve for the design of predictive markers of disease activity and vascular damage in patients. (J Rheumatol First Release September 1 2016; doi:10.3899/jrheum.160050)

Key Indexing Terms:
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Microparticles (MP), also known as microvesicles, are small extracellular vesicles with a diameter of 100–1000 nm produced by cytoplasmic membrane budding and shedding. MP are distinct from exosomes, which display smaller dimensions (10–100 nm in diameter) and originate from a pool of membranes in the multivesicular bodies[1]. Depending on the cellular source and release trigger, MP consist of different components, which originate from the surface and intracellular compartments of the producing cell. Because they can interact with other cells and can be internalized in cell recipients, MP are thought to be involved in intercellular communication[1].

Cellular activation and apoptosis are triggers of MP release. During this process, the membrane asymmetry is generally lost because of major membrane and cytoskeleton rearrangements, engaging exposition of anionic phosphatidylserine (PS) on MP surface[2]. PS can support coagulation factor deposition, suggesting that PS-harboring MP may participate in thrombosis[3]. Further, the recognition of PS by developmental endothelial locus 1, lactadherin, or tyrosine kinase receptors leads to rapid MP clearance from blood circulation[4,5,6]. Although any cellular lineages might shed MP, those from platelets and erythrocytes appear among the most numerous in the blood of healthy individuals, the platelet-derived MP alone representing the majority of them[7,8]. Intriguingly, more than half of the platelet-derived MP present in blood lack surface expression of PS, adding an additional level to the heterogeneity of MP[8,9].

Systemic lupus erythematosus (SLE) is an autoimmune inflammatory disease with an important contribution of immune complexes (IC). Deposition of pathogenic IC in organs such as the kidneys and chronic inflammation all contribute to tissue and vascular damage seen in patients with SLE. In SLE, PS-positive MP expose autoantigens that are recognized by autoantibodies, forming MP-containing IC (mpIC)[10,11,12]. The cellular source of these antigenic MP is unknown, although they seem to originate from apoptotic bodies exposing nuclear antigens[10]. Because platelets do not have a nucleus, they are generally not suspected to contribute to the load of autoantigens hallmarked in SLE. However, platelets represent a significant pool of autoantigens, such as the non-histone nuclear protein high mobility group box 1 (HMGB1), which may be exposed by platelet MP[13,14]. Further, platelets do have RNA, microRNA, and mitochondrial DNA, which are transferred from the megakaryocyte during pro-platelet formation, and which could represent a source of nucleic acid autoantigens[15,16].

There is accumulating evidence pointing to an active contribution of platelets to SLE pathology[15]. Hence, platelet depletion in SLE-prone mice and the treatment of these mice with the antagonist of the P_2Y_{12} receptor clopidogrel (which blocks platelet activation) results in reduced proteinuria, kidney damage, and mortality[17]. In SLE, platelets display surface activation markers. Increased levels of platelet-derived mediators such as soluble CD40 ligand, P-selectin, and thromboxane have been consistently reported in the blood of these patients[15,18]. Annexin V is a protein that binds PS with high affinity. Because MP can expose PS, annexin V-conjugated fluorescent probes are frequently used to detect MP in biological fluids, such as plasma. In SLE, annexin V-positive MP (annexin V+ MP) harbor immunoglobulin G (IgG), forming annexin V+ mpIC. Of clinical relevance, the levels of annexin V+ mpIC correlate with concentrations of autoantibodies and complement activation[10]. Further, platelet MP are present at higher concentrations in patients with SLE[18]. Whether MP of platelet origin are antigenic in SLE, and whether platelet mpIC levels correlate with clinical characteristics of SLE disease have not been investigated. In our present study, our aims were to determine whether platelet-derived MP harbored IgG and whether we could detect associations between disease characteristics and levels of MP subtypes in SLE.

MATERIALS AND METHODS

Patients. Blood was obtained from 191 consecutive women with SLE from the University of Toronto Lupus Clinic between August 2010 and October 2011. Of these, 113 had a carotid ultrasound (US) performed. Those who did not have carotid US either refused the extra research procedure or were living too far away to come back for this test. All patients were women by study design for 2 reasons: (1) to minimize the sex-specific variability in cardiovascular (CV) risks and (2) to account for the sex disparity in SLE because 90% of patients affected are women. Information on demographic variables such as age (yrs), marital status (married/common law vs not), education (completed high school or higher vs not), employment (working vs not), race (white, Asian, black, or other), and menopausal status was collected. In addition, data were collected on clinical variables including CV risk factors [body mass index (BMI), smoking status (current, past, or never), hypertension (Y/N), diabetes (Y/N), lupus disease duration (yrs)] and selected current medication exposures [antimalarials (Y/N), antiplatelet or anticoagulant (Y/N), lipid-lowering drugs (Y/N), or the use of prednisone (average dose in mg/day)]. For control plasma samples, blood from 9 age-matched women was collected and processed using the same procedure during the same period of time. This study has been reviewed and approved by the Research Ethics Board of the University Health Network (#10-0637-BE) and of the CHU de Québec – Université Laval (#B14-08-2108).

Preparation of plasma samples. Blood was collected from study participants after 12-h to 14-h fasts in 4.5 ml citrate (0.109 M)-buffered glass tubes. Blood was centrifuged at 1500 × g for 10 min at room temperature (RT), the upper phase, poor in platelets, was separated, and centrifuged an additional time at 1500 × g for 10 min at RT. The resulting plasma was immediately conserved at –80°C in tube aliquots.

High-sensitivity flow cytometry. A FACS Canto II special order product equipped with a small particle option was used for all our studies (BD Biosciences)[9]. The variables used to detect small particles were close to the optimal version described in Rousseau, *et al*[19]. Polystyrene microspheres of defined dimensions were used to ensure that the instrument settings between each experiment, and the lower and upper gate limits, used 100-nm and 1000-nm diameter polystyrene microspheres, respectively. Prior to the labeling of MP, plasma (5 μl) was incubated with 10 μM of thrombin inhibitor D-phenylalanyl-prolyl-arginyl chloromethyl ketone (Sigma-Aldrich) for 10 min to inhibit coagulation induced by the addition of 95-μl annexin V buffer (BD Biosciences), which contains calcium. The plasma was then incubated with 0.75 μg of Alexa Fluor 647-conjugated $F(ab')_2$ goat anti-human IgG (Jackson ImmunoResearch), 0.5 μg of PE-conjugated mouse anti-human CD41 (M148; Abcam), and 3

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μl of V450-conjugated annexin V (BD Biosciences) in annexin V buffer for 30 min at RT prior to dilution with 400 μl of annexin V buffer. Three different fluorescent probes were excited by 3 distinct lasers to avoid uncertainties associated with fluorescence spillover. Quantification microspheres (15 μm; Polysciences) were added to tubes immediately prior to cytofluorometric analysis and were detected on basis of autofluorescence[9,19]. In these experimental conditions, 97.06% ± 2 of the total CD41+ events (n = 191) were included within the gate (MP gate; Supplementary Figure 1A, available online at jrheum.org). Specificity of the MP labeling was verified in 50% of the samples using isotypic antibodies and by the addition of 0.05% Triton X100 (EMD Millipore), which dissolves membranes of MP, and 20 mM EDTA (Fisher Scientific), which chelates calcium ions necessary for annexin V binding (Supplementary Figure 1B, available online at jrheum.org). Fluorescent-intact platelets that were generated by the addition of 1-μM cell tracker CMFDA (5-chloromethylfluorescein diacetate; Invitrogen) to washed platelets for 15 min following the manufacturer protocols[19] were spiked in SLE plasma to further ensure the distinction, on the basis of size and granularity, of platelets and mpIC (Supplementary Figure 1C, available online at jrheum.org).

Variables collected and measures of outcome. Four clinical outcome variables with proven validity to measure SLE disease characteristics and surrogate atherosclerotic vascular features were collected: (1) disease activity was determined using the SLE Disease Activity Index 2000 (SLEDAI-2K)[20], (2) the Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index (SDI) was used to assess cumulative and irreversible damage because of SLE[21], and US of the carotids

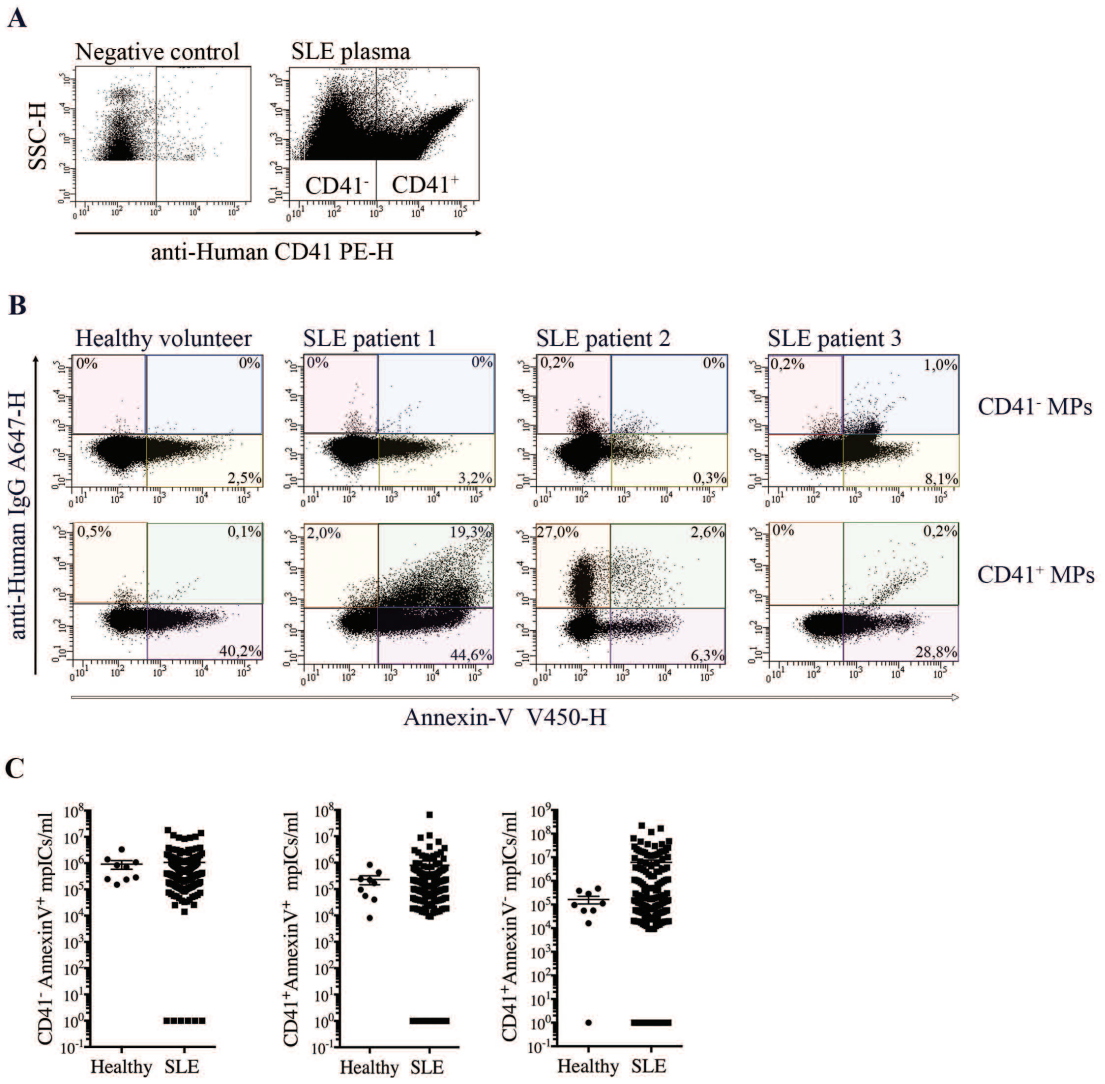

Figure 1. MP in patients with SLE are contained in immune complexes and form mpIC. A. Platelet MP contained in plasma from 1 representative patient with SLE were labeled with PE-conjugated anti-human CD41 and fluorescent-positive signal was compared with background noise of the antibody left in PBS in absence of plasma as negative control. B. MP in plasma from 1 healthy volunteer and 3 representative patients with SLE were labeled with PE-conjugated anti-human CD41, V450-conjugated annexin V, and Alexa Fluor 647-conjugated anti-human IgG, demonstrating the presence of IgG on the surface of MP (mpIC). CD41+ and CD41– populations contained in the MP gate were analyzed. The 4-quadrant gates were positioned according to the isotypic controls and EDTA treatment (for annexin V labelings). The CD41–annexin V+ (blue), CD41+annexin V– (orange), and CD41+annexin V+ (green) mpIC are illustrated in flow cytometric scatter graphs. MP not associated with IgG are presented; CD41–annexin V+ MP are shown in yellow and CD41+annexin V+ MP in purple. IgG+ population not associated with CD41+ or annexin V+ MP is shown in pink. C. Quantification of the CD41–annexin V+ (left), CD41+annexin V+ (middle), and CD41+annexin V– (right) mpIC in plasma of healthy volunteers (n = 9) and patients with SLE (n = 191) are presented on graphs. MP: microparticles; SLE: systemic lupus erythematosus; mpIC: MP-containing immune complexes; PE: phycoerythrin; PBS: phosphate buffered saline; IgG: immunoglobulin G.

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allowed for the characterization of (3) the carotid intima-media thickness (CIMT) and (4) the carotid plaque area (CPA). CIMT was obtained following a standardized, reliable, validated method developed by Lonn, *et al* that is used to assess the global extent of atherosclerosis in patients[22,23,24]. CPA from 2-dimensional US images was measured using a previously described method[25] using a high-resolution duplex US scanner (MyLabTMFive). Intraobserver reliability (intraclass correlation) for this procedure was 0.94 for repeated measurements[26]. US measurements were analyzed using the Image-Pro V4.5.1 software by an independent certified reader blinded to our study.

Statistical analyses. In the descriptive part of our analysis, the median with interquartile range (IQR) were presented for continuous data, and frequency and percentage for categorical data. Normality of continuous data was rejected using Shapiro-Wilks test (not shown). The concentrations of the 3 subtypes of mpIC examined (CD41+ annexin V+ mpIC, CD41+ annexin V– mpIC, and CD41– annexin V+ mpIC) were determined and then correlations with SLEDAI-2K, SDI, CIMT, and CPA were estimated using Spearman correlation coefficients. Partial Spearman correlations were used to obtain the correlation coefficients adjusted for low-density lipoprotein cholesterol (LDL-C), BMI, and antimalarial drug use. Fisher's z transformation was used to estimate 95% CI for Spearman correlation coefficients. SAS 9.3 software was used for all statistical analyses. All the presented data on MP measurements were rounded to 2 digits to comply with the inherent instrument precision.

RESULTS

We verified whether platelet-derived MP, recognized by the expression of platelet antigen CD41 (CD41+), could also harbor IgG in patients with SLE (Table 1), forming CD41+ mpIC. Because an important proportion of the platelet MP in blood does not express surface PS[7,8], the CD41+ annexin V– mpIC were also considered. Using high-sensitivity flow cytometry to distinguish MP subtypes, we found that the median (IQR) concentration in the plasma of patients with SLE for CD41+ annexin V+ mpIC was 130/num/µl (40–400), and 100/num/µl (20–1000) for CD41+ annexin V– mpIC. Significant levels of CD41– annexin V+ mpIC were also measured (400/num/µl, 200–900), pointing to the potential contribution of other cellular lineages to the production of MP in SLE (Figures 1A and 1B). Using a limited number of age- and sex-matched healthy controls for comparison (n = 9), we found that the CD41+ annexin V+ mpIC and CD41+ annexin V– mpIC were essentially absent in healthy individuals, while the induction of CD41– annexin V+ mpIC in SLE appeared more modest (Figure 1C).

Thus, we verified whether the levels of the 3 MP subtypes correlated with disease features. Results show a positive correlation between the SLEDAI and CD41+ annexin V– mpIC ($p = 0.001$), and also with CD41+ annexin V+ mpIC ($p = 0.027$). Further, whereas no correlations were observed between all types of mpIC and CPA, we observed that the concentrations of CD41– annexin V+ mpIC in SLE correlate positively with the SDI ($p = 0.024$) and CIMT ($p = 0.0156$; Table 2). These observations suggest different implications for MP of different cellular origins in the etiology and clinical progression of SLE. Associations were independent of LDL-C level, BMI, and antimalarial drug use (Table 2), and these variables had no effect on calculated disease outcome, including SDI (data not shown).

Table 1. Patient characteristics. Values are n (%) or median (interquartile range).

Characteristics	Women with SLE, n = 191	Patients with Carotid Ultrasound, n = 113
Demographics		
Age, yrs	48.6 (34.8–57.4)	50.6 (39.6–59.5)
Marital status, married/common law	106 (55)	63 (56)
Education, completed college or higher	133 (70)	78 (69)
Employment, working	78 (41)	37 (33)
Race		
White	109 (57)	66 (58)
Asian	39 (20)	25 (22)
Black	33 (17)	18 (16)
Other	10 (6)	4 (4)
Menopausal status, post-menopausal	105 (55)	71 (63)
BMI, kg/m^2		
Obese, BMI ≥ 30	33 (17)	19 (17)
Overweight, BMI ≥ 25 to < 30	47 (25)	27 (24)
Normal, BMI ≥ 18.5 to < 25	100 (52)	60 (53)
Underweight, BMI < 18.5	11 (6)	7 (6)
Smoking		
Current	18 (9)	8 (7)
Past	43 (23)	28 (25)
Never	130 (68)	77 (68)
Hypertension	57 (30)	35 (31)
Diabetes mellitus	6 (3)	6 (5)
LDL-C, mmol/l	2.36 (1.95–2.78)	2.20 (1.89–2.63)
Medication use		
Antimalarial drugs	142 (74)	79 (70)
Antiplatelet or anticoagulant	48 (25)	34 (30)
Lipid-lowering drug	29 (15)	25 (22)
Prednisone	85 (45)	49 (43)
Average dose, mg/day	5 (5–10)	5 (5–10)
Disease characteristics		
Disease duration, yrs	16.0 (9.1–25.2)	16.3 (9.2–26.3)
Disease activity by SLEDAI score	2.0 (0–4.0)	2.0 (0–4.0)
Disease damage by SDI score	1.0 (0–2.0)	1.0 (0–3.0)
Vascular damage characteristics		
CIMT, mm	—	593 (543–678)
Total CPA, mm^2	—	0 (0–0.08)
Concentration of mpIC subtypes		
Total annexin V+ mpIC, num/µl	600 (300–1400)	600 (300–1500)
CD41+ annexin V+ mpIC, num/µl	130 (40–400)	120 (40–400)
CD41+ annexin V– mpIC, num/µl	100 (20–1000)	100 (20–1100)
CD41– annexin V+ mpIC, num/µl	400 (200–900)	400 (200–1000)

SLE: systemic lupus erythematosus; BMI: body mass index; LDL-C: low-density lipoprotein cholesterol; SLEDAI: Systemic Lupus Erythematosus Disease Activity Index; SDI: Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index; CIMT: carotid intima-media thickness; CPA: carotid plaque area; mpIC: microparticle-containing immune complexes.

DISCUSSION

Reduced clearance of autoantigens, mostly DNA, RNA, and nuclear components, is thought to contribute to the formation

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Table 2. Spearman correlation analyses of the microparticle subtype levels and SLE outcomes.

SLE Outcomes	Microparticle Subtype	Crude Spearman Correlation				Partial Spearman Correlation, 3 Partial Variables: LDL-C, BMI, and Antimalarial Drugs			
		n	r	95% CI	p	n	r	95% CI	p
SLEDAI-2K	Total annexin V+ mpIC	191	0.12	–0.02 to 0.26	0.1026	179	**0.15**	0–0.29	**0.0498**
SLEDAI-2K	CD41– annexin V+ mpIC	191	0.00	–0.14 to 0.14	0.9808	179	0.03	–0.12 to 0.17	0.7321
SLEDAI-2K	**CD41+ annexin V+ mpIC**	191	**0.16**	0.02–0.30	**0.0266**	179	**0.21**	0.06–0.34	**0.0058**
SLEDAI-2K	**CD41+ annexin V– mpIC**	191	**0.23**	0.09–0.36	**0.0013**	179	**0.28**	0.14–0.41	**0.0002**
SDI	Total annexin V+ mpIC	191	0.13	–0.01 to 0.27	0.0635	179	0.14	–0.01 to 0.28	0.0625
SDI	**CD41– annexin V+ mpIC**	191	**0.16**	0.02–0.30	**0.0236**	179	**0.18**	0.03–0.32	**0.0181**
SDI	CD41+ annexin V+ mpIC	191	0.08	–0.06 to 0.22	0.2596	179	0.08	–0.07 to 0.22	0.3042
SDI	CD41+ annexin V– mpIC	191	–0.12	–0.26 to 0.02	0.0891	179	–0.14	–0.28 to 0.01	0.0675
CIMT	**Total annexin V+ mpIC**	113	**0.21**	0.03–0.38	**0.0253**	106	**0.21**	0.01–0.38	**0.0364**
CIMT	**CD41– annexin V+ mpIC**	113	**0.23**	0.04–0.39	**0.0156**	106	**0.21**	0.02–0.39	**0.0292**
CIMT	CD41+ annexin V+ mpIC	113	0.14	–0.05 to 0.32	0.1370	106	0.12	–0.08 to 0.31	0.2304
CIMT	CD41+ annexin V– mpIC	113	0.03	–0.15 to 0.22	0.7236	106	0.06	–0.14 to 0.25	0.5739
CPA	Total annexin V+ mpIC	113	0.01	–0.18 to 0.19	0.9384	106	–0.02	–0.22 to 0.17	0.8032
CPA	CD41– annexin V+ mpIC	113	0.04	–0.14 to 0.23	0.6412	106	0.01	–0.19 to 0.2	0.9410
CPA	CD41+ annexin V+ mpIC	113	–0.01	–0.19 to 0.18	0.9191	106	–0.05	–0.24 to 0.14	0.6082
CPA	CD41+ annexin V– mpIC	113	–0.03	–0.22 to 0.15	0.7326	106	–0.07	–0.26 to 0.12	0.4546

Significant data are in bold face. SLE: systemic lupus erythematosus; LDL-C: low-density lipoprotein cholesterol; BMI: body mass index; SLEDAI-2K: Systemic Lupus Erythematosus Disease Activity Index 2000; SDI: Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index; CIMT: carotid intima-media thickness; CPA: carotid plaque area; mpIC: microparticle-containing immune complexes.

of IC in SLE. Because IC appear pathogenic, the identification of the source of autoantigens is clinically important. Because the MP cargo is vast and consists of cytokines, enzymes, immune receptors, and even organelles, mpIC can represent highly potent mediators of autoimmunity. The identification of markers associated with mpIC may unveil cellular sources of autoantigens and molecular mechanisms relevant to SLE pathogenesis.

It was suggested that MP, more particularly those derived from apoptotic bodies and containing nuclear proteins and that may be identified in our CD41– MP subpopulation, could convey autoantigens, thereby providing a platform for the formation of pathogenic IC. Thus we also assessed non-platelet–derived mpIC in our study. Vascular damage, as assessed using CIMT in patients with SLE, correlated with the concentrations of annexin V+ mpIC, which do not express platelet marker CD41. The source of these MP remains to be identified, but it could be the nucleated cells such as leukocytes or endothelial cells, which lack surface CD41 and are reportedly shown to actively produce MP in patients with SLE[27]. Because they express surface PS, this subtype of mpIC may also contribute to thrombosis and thereby increase the risk of CV disease seen in patients with SLE. Intriguingly, no correlations were observed with total plaque area. Although it might be too early at this stage of our research to identify the exact reason for this apparent discrepancy, we suggest that changes to CIMT might be occurring earlier than total plaque area in the development of the SLE disease, and thus might be more efficiently detected and associated with the identified mpIC subtype (annexin V+ CD41– mpIC) in our clinical conditions. In SLE, the mechanism for arterial thickening of the intima-media, rather than a primarily plaque cholesterol-driven deposition, might thus associate with involvement of mpIC. Further studies will certainly provide the complete identification of this MP subtype and could eventually serve as the design of predictive markers of vascular damage in patients, perhaps when used in combination with other markers used in algorithms.

Although exploratory and in need of replication, we reveal in our study that platelets, even though anucleated, represent an important source of MP implicated in the formation of mpIC in SLE. Proteins (e.g., HMGB1)[13,14], and even nucleic acids, are among the different autoantigens potentially conveyed by platelet-derived MP and contributing to the formation of CD41+ mpIC. Recognition of molecules present on platelet MP by autoantibodies may also be because of cross-reactivity. Hence, it was reported that the anti-dsDNA antibodies that prevail in SLE, in fact, recognize platelet glycoprotein IIIa (CD61), thus providing an additional explanation for the high concentrations of platelet mpIC in SLE[28,29].

There is accumulating evidence pointing to MP as a source of antigens in rheumatic diseases. In rheumatoid arthritis, platelet MP in the synovial fluid harbor citrullinated autoantigens (e.g., fibrinogen and vimentin) and form mpIC[9]. In a seminal study that implicated 68 patients with SLE, other investigators had confirmed the presence of IgG on the surface of annexin V+ MP. Consistent with our findings, no correlations were found between annexin V+ mpIC and the SLEDAI[10]. Of importance, our study demonstrates that levels

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of platelet mpIC correlate with disease activity. These results are surprising, considering that it most specifically implied the annexin V– platelet mpIC, and that only more modest correlations were observed between the extensive set of clinical features examined and annexin V+ platelet mpIC. Since PS exposure mediates MP clearance, we propose that PS-negative platelet mpIC, which might be preferentially produced during elevated disease activity and may not be cleared sufficiently rapidly, might act as a source of autoantigens, and could stealthily circulate in blood for a longer period of time. How platelet MP maintain membrane asymmetry is not clear at the moment. Unique activation processes, the hydrolysis of surface PS by secreted phospholipase A_2 present in the plasma, or presence of enzymes packaged within MP and capable of maintaining asymmetry, all could explain the absence of PS on this subset of MP[19]. While these observations reinforce the notion of MP heterogeneity, they also support the notion of platelet contribution to SLE.

It was suggested that the blockade of MP surface autoantigens might represent an attractive treatment strategy in SLE[30]. However, in health, the function(s) of MP as small autoantigen conveyers is unclear. They might serve to the maintenance of homeostatic levels of autoantibodies and to their elimination. Although lower concentrations of mpIC were determined in healthy controls, a finding consistent with studies from other groups[10,11,12], the rather low number of controls examined and potentially the lesser cell activation in the absence of SLE might explain these observations. Examination of larger patient cohorts, ideally with a broader disease activity, and the inclusion of more control volunteers will be required for future investigations.

Although MP have long been identified, the more recent development of improved methodologies permitting their detection and quantification has enhanced the recognition of distinct subtypes of MP in pathologies. Taken together, our observations suggest that potent biomarkers could be unveiled through the appreciation of the heterogeneity of MP. Future studies of microparticle-based biomarkers are therefore necessary to determine whether clinical decisions might be based according to their measurements.

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ONLINE SUPPLEMENT

Supplementary data for this article are available online at jrheum.org.

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