Modulation of interferon-alpha secretion by activated platelets in systemic lupus erythematosus.

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INTRODUCTORY PARAGRAPH

Type I interferons play a key role in systemic lupus erythematosus (SLE) pathogenesis[1,2] as an "IFN signature" is found in the majority of patients with active SLE[3,4]. Immune complexes are internalized by plasmacytoid dendritic cells (DC) via Fc-gamma ReceptorIIA, reach the endosomal compartment and activate IFN-alpha secretion through TLR7/9-dependent pathways[5,6]. Naturally occurring differences in expression of the TLR7/9 gene[7] as well as factors that modulateTLR7/9 expression, including CD154 could therefore contribute to SLE pathogenesis. Although its origin is not elucidated CD154 is hyperexpressed in SLE patients, and is important for the differentiation of autoantibody-secreting cells[8]. We hypothesized that platelets which are an abundant source of CD154, and which can mediate proinflammatory effects[9,10] could be an actor involved in SLE pathogenesis. Platelets from SLE patients are activated in vivo by circulating immune complexes which are abundant in SLE sera, via a CD32-dependent mechanism. Activated platelets formed aggregates with antigen-presenting cells in SLE patients and enhanced interferon-alpha secretion induced by immune-complexes stimulated plasmacytoid DCs. Finally, in vivo depletion of platelets and megakaryocytes in NZBxNZW(F1) lupus prone mice improved all parameters assessing disease activity, whereas transfusion of activated platelets worsened the disease course. Altogether, these data identify platelets as a mediator of SLE pathogenesis and a new therapeutical target.

Platelets isolated from SLE patients with a wide range of disease activity (Supplementary **Table 1**) but not from normal individuals, spontaneously expressed CD154 (CD40 ligand) and CD62P (P-Selectin) at their membrane surface, two markers which are a hallmark of platelet activation [9], [11]. The increased basal levels of platelet CD62P and CD154 surface expression were significantly higher in SLE patients (n=20) than in normal blood donors (n=20) either before (P= 0.023 and P=0.0009 respectively) or after thrombin activation (P=0.0044 and P<0.0001 respectively) (**Fig.1a and b**). Since CD154 on activated platelets is known to be cleaved by a yet unknown mechanism and released in a soluble form (sCD154), we hypothesized that the increased levels of sCD154 observed in the blood of SLE patients [12] (Fig. 1C upper panel) could result from platelet activation. Platelet lysates from SLE patients were assayed for their CD154 content and compared with those from normal donors. SLE lysates contained significantly lower levels of CD154 than healthy individuals (P=0.0478). Furthermore, lysates from active SLE patients contained lower amounts of CD154 than lysates from quiescent SLE patients indicating that disease activity was associated with a depletion of platelet-associated CD154 (P=0.025) (**Fig 1c, lower panels**). This is consistent with the observation that serum levels of sCD154 are correlated with the SLE disease activity (**Fig. 1c, upper panel**). Conversely, in SLE patients a reverse correlation was found between the levels of CD154 platelet lysates and the levels of serum sCD154 in SLE patients (**Fig. 1d**) strongly suggesting that in SLE serum increased sCD154 levels can partly be attributed to an active clipping of the increased CD154 found on SLE platelet membranes. To the best of our knowledge this is the first demonstration that increased sCD154 levels found in SLE sera could be due to an activation of the megakaryocytic lineage.

To understand the mechanisms of platelet activation in SLE patients, we exposed platelets from normal controls to several SLE sera. Contrary to allogeneic normal sera or sera from other autoimmune diseases such as rheumatoid arthritis (data not shown), SLE sera

induced platelet activation in a dose-dependent fashion as assessed by the up-regulation of CD62P and CD154 membrane expression (**Fig. 2a**). Among the soluble factors possibly involved in platelet activation, immune complexes were considered because: 1/ platelets express Fc-gamma Receptor IIA (CD32) [13] which is known to interact with immune complexes 2/ circulating immune complexes are present in large amounts in SLE blood patients [14]. As shown in **Fig 2b upper panel**, platelet activation following incubation with sera from SLE patients was inhibited by preincubation of platelets with a blocking anti-CD32 monoclonal antibody or by depleting the sera of IgG. In contrast, blocking type I interferon or CD154, or heat-inactivating sera did not alter their capacity to induce platelet activation. Moreover, the addition of immune complexes to normal serum was capable of upregulating the membrane expression of CD62P and CD154, mimicking the effects of SLE sera (**Fig 2b lower panel**). To assess whether immune complexes could be found in association with platelets in SLE patients, platelet lysates obtained from SLE patients were analyzed by probing western blots with anti-human IgG. Whereas IgG could be detected in SLE lysates, barely detectable amounts of IgG were found in platelet lysates from RA patients or normal controls (**Fig 2c upper panel**). Antinuclear antibodies were found among the IgG present in platelet lysates from SLE patients but were lacking in those from normal or RA patients even when RA patients had detectable serum anti-nuclear antibodies (**Fig 2c lower panel**). In addition after migration of lysates from purified platelets (>99%) on a 1% agarose gel, cleaved DNA sensitive to DNAse could be detected in platelet lysates from active SLE patients but not in platelet lysates from quiescent diseases (**Fig 2c right panel**). Together, these data support the conclusion that, in SLE patients, platelets are activated by immune complexes in a CD32-dependent fashion.

Once activated, platelets may interact with APCs and form aggregates [15]. Monocytes (known to act as myeloid DCs in SLE [16]) and plasmacytoid DCs could form aggregates with

activated platelets as assessed by flow cytometry and confocal microscopy analysis (**Fig. 3a, b**). The occurrence of monocytes-platelets aggregates was significantly higher in the blood of SLE patients (n=15) than in normal individuals (n=15) (P=0.002) (**Fig.3c**). Aggregates between platelets and circulating plasmacytoid dendritic cells (pDC), were also more numerous than monocyte-platelets aggregates in SLE patients versus healthy controls (P<0.001) (**Fig. 3c**). Incubation of normal platelets activated with SLE sera and APC promoted the generation of aggregates partially blocked by an anti-PSGL1 blocking monoclonal antibody (**Supplementary Fig 1-2**). Since plasmacytoid DCs are known to interact with circulating ICs from SLE patients and secrete type I interferon in a CD32- and TLR9/7-dependent mechanism[17],[5], we examined the effects of the addition of normal activated platelets to purified pDCs from normal donors stimulated by different TLR agonists on the secretion of IFN-alpha. While adding activated platelets or ICs alone triggered IFN-alpha secretion, their simultaneous addition *in vitro* led to a fivefold increase in IFN-alpha secretion (**Fig. 3d**). Addition of L-cells transfected with CD154 could also potentiate ICs-mediated IFN-alpha secretion whereas the increase in IFN-alpha secretion could be severely downregulated by an anti-CD40 blocking monoclonal antibody thus demonstrating that the interaction between CD154 borne by platelets with CD40 borne by pDCs is a prerequisite for up-regulating IFN-alpha secretion (**Fig. 3d**). In addition, we found that activated platelets enhanced IFN-alpha secretion upon TLR7/9 plasmacytoid DC specific stimulation (**Supplementary Fig. 3**). Taken together, these data demonstrate an entirely new and key role for platelets in SLE pathogenesis, through the ability of platelets to interact with pDCs. To demonstrate the relevance of this observation *in vivo*, NZBxNZW(F1) mice with on-going lupus-like disease were depleted of circulating platelets and megakaryocytes. A depleting rat monoclonal antibody (n=6) or the isotypic rat control (n=6) was administered to six month-old mice once a week for 6 weeks. Another group of animals (n=6) was transfused with

platelets previously activated *in vitro*. We found that mice depleted of platelets had 1,5-fold lower level of serum ds-DNA IgG (Fig. 4A) and IgM (**Fig. 4b**) at the completion of the observation period when compared to control mice. No difference between the different groups in total IgG and IgM levels was found (**Supplementary Fig. 4**). The kidney function remained stable in the group of platelet depleted mice whereas mice receiving isotypic control antibody increased their blood urea levels (Fig.4C). This kidney function alteration was more pronounced in the group of mice given activated platelets (**Fig.4c**). After 6 weeks, all mice were sacrificed and their kidneys examined under light microscopy. Inflammatory infiltrates in the interstitial tissue and the glomeruli were reduced in kidneys from platelet-depleted mice than in animals treated with the isotypic control Mab (**Fig.4d**). In contrast, the kidneys of animals transfused with activated platelets displayed dense inflammatory infiltrates, IC deposits and intense damages that correlated with the elevation of blood urea (**Fig 4e**).

Those data unravel a link between haemostasis and the innate arm of the immune system in SLE which is the archetypical human systemic autoimmune disease. Our study demonstrates that platelets in SLE patients are activated by circulating immune complexes through a mechanism requiring CD32. This activation leads to the formation of platelet aggregates with APCs including monocytes and plasmacytoid DCs. This cellular interaction strongly potentiated IFN-alpha secretion by immune-complex-stimulated plasmacytoid DCs. Moreover, in vivo depletion of platelets in NZBxNZW (F1) lupus prone mice improved disease outcome whereas transfusion of activated platelets worsened disease, highlighting platelets as a new and promising therapeutical target for patients undergoing active disease. We suspect as well, that platelets could be involved in the premature atherosclerosis (independent of usual cardiac risk factors) frequently observed in SLE patients, which represents a major comorbid condition [18,19].

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AUTHORS CONTRIBUTIONS

P.D., J.S., did the experiments on human SLE. P.B., J.F.M. wrote the paper. N.C., F.B. and B.W. did the experiment in mice. J.F.V. and J.L.P. conducted the sampling and clinical study of SLE patients. P.D., J.S. and F.B. participated in the interpretation of the results and the editing of the manuscript.

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FIGURE LEGENDS

Figure 1: Platelets are activated in SLE patients and are the source of the elevated level of sCD154 in SLE sera. **(a)** Flow cytometric analysis of CD62P and CD154 expression on freshly isolated platelets from a SLE patient and an healthy volunteer before and after thrombin activation **(b)** Platelet surface CD62P and CD154 expression were evaluated in 20 SLE patients and 20 healthy individuals. The mean fluorescence intensities corresponding to the expression of surface platelet CD62P and CD154 are reported on the vertical axis. Boxes indicate 25th and 75th percentiles; error bars, 10th and 90th percentiles. ○ indicates values outside these ranges. P values are indicated in the boxes (Mann Whitney test). **(c)** sCD154 blood titers were evaluated in 30 SLE sera, 10 control sera and within the platelet lysates for each corresponding patient or control. Boxes indicate 25th and 75th percentiles; error bars, 10th and 90th percentiles. ○ indicates values outside these ranges. P values of the comparisons are indicated in the boxes. **(d)** The levels of sCD154 in SLE sera are inversely correlated to the levels of CD154 assayed in SLE platelet lysates (Spearman test, Pearson coef = 0.86).

Figure 2: Platelets are activated by immune complexes (ICs) via a CD32-dependent mechanism. **(a)** Incubation of normal platelets with a SLE serum from an active patient but not with a control serum or a serum from a quiescent SLE patient activates platelets in a dose dependent fashion. **(b)** Left panel: platelets from normal individuals incubated with 10 different SLE sera were significantly more activated than platelets incubated with normal sera (n=6) (P=0.02). Addition of immune complexes to a normal serum promotes platelet activation to the same extent as SLE sera does (non parametric Mann_Whitney test). Right panel: Pre-incubation of normal platelets with the blocking monoclonal antibody anti-CD32,

or depleting SLE sera of IgGs resulted in an inhibition of the ability of the SLE sera to induce platelet activation. The figure represents the mean values (+/-SD) of the inhibitions obtained in three different experiments using different SLE sera. **(c)** Upper left panel: Platelets from SLE patients interact with ICs. Platelets ($2x10^8$) from SLE patients, RA patients or control individuals were purified, and their corresponding lysates obtained. Western blot analysis on the lysates revealed that binding of IgG by platelets was much higher in SLE patients than in controls. Lower left panel: IgG found within the platelet lysates from SLE patients are directed against nuclear components as assessed by an indirect immunofluorescence assay. Right panel: Platelet lysate from an active SLE patient contained cleaved DNA. Platelet lysate was electrophoresed in a 1% agarose gel before staining with bromide ethydium (one representative experiment from 5 different).

Figure 3: Activated platelets form aggregates with circulating antigen presenting cells which modulate IFN-alpha production. **(a)** Platelet aggregates were counted using flow cytometry. Fresh whole blood was incubated with a mixture of anti-CD62 and anti-CD42b antibodies to stain platelets and an anti-BDCA-2 as a specific marker for pDCs. The proportion of triple positive cell (BDCA2+CD42b+CD62p+) represents the proportion of platelets/pDCs aggregates. **(b)** pDCs obtained by negative selection from SLE patients, were stained on a slide using anti-BDCA2 (green), anti-CD42b (red) monoclonal antibodies and counterstained with DAPI (blue). Analysis by confocal microscopy confirmed that double positive cells represent platelets/pDCs aggregates. **(c)** The proportion of platelets/pDCS and platelets/monocytes aggregates are evaluated in 15 SLE patients and 15 controls by flow cytometry. The proportion of blood platelets/pDCs and platelets/monocytes aggregates were significantly higher in SLE patients when compared to healthy individuals (non parametric Mann Whitney test). P values are indicated in the boxes. **(d)** Purified pDCs ($5x10^5$) were

incubated with ICs in the presence or absence of activated platelets ($5x10^6$) for 24 hours. In one condition blocking monoclonal anti-CD40 antibody (Mab89) was added at culture onset. IFN-alpha levels released in supernatants by pDCs were measured by ELISA. The mean values (+/-SD) of three different independent experiments are shown.

Figure 4: In vivo involvement of platelets in lupus pathogenesis in the mouse NZBxNZW(F1) model. **(a)** Six month old NZB/NZW(F1) (n=6) were injected iv with 3µg/g of anti-CD42b p0p3/4 mAb (Platelet-depleted) or with 3µg/ml of an isotypic control mAb (Isotypic control) on day 0 and once a week for six weeks. A third group was composed of six NZB/NZW(F1) given at day 0 and once a week one milliliter of $2x10^7$ in vitro activated platelets (Platelet transfusion). Serum was harvested on day 0 and six weeks later before assayed for anti-DNA IgG or anti-DNA IgM blood levels **(b)**. Data are expressed as the mean values of six different mice. **(c)** Blood urea levels were measured at day 0 and at six week. **(d)** At week 6, mice were sacrificed and kidneys were analyzed under light microscopy (lower panels). Upper panels depict immune complexes deposit within the glomeruli. **(e)** Immunohistologic and histologic assessment of platelet-dependent lupus nephritis.

Patients, materials, and methods

1/ Patients

SLE patients (n=37) who fulfilled four or more of the 1982 revised ACR criteria for the disease, were enrolled in this study. The group of healthy subjects (controls) comprised our medical staff free of any auto-immune or infectious diseases. Patients with anti-phospholipid antibodies, and/or thrombopenia were excluded from the study. Table S1 summarized demographic and clinical characteristics. An active patient is a patient at diagnosis or a patient with a 3 point-increased of it SLE Disease Activity Index when compared to it previous one.

2/ Platelets

Platelet-rich plasma (PRP)

Preparation of platelet-rich plasma: PRP was prepared by centrifugation of citrated blood samples at 1500 rpm for 10mn. Platelets were counted using a CellDyn 3500 hemocytometer and PRP was adjusted to the desired concentration by addition of Tyrode's buffer (134 mM NaCl, 2,9 mM KCl,0,34 mM Na2PO4, 12mM NaHC03, 20 mM HEPES, 1mM MgCl2, 5mM glucose, [pH 7,2]).

Washed platelets

Preparation of washed platelets: PRP was added 1/10 vol/vol ACD-A (25g/l Tris-sodium citrate, 20g/l D-Glucose, and 15g/l Citric acid), and spun at 1500 rpm for 10mn. The platelet pellet was resuspended in Tyrode's buffer and 1μM PGE1 (final concentrations) and 80 U/ml apyrase were added. Platelets were counted and adjusted to the desired concentration by addition of Tyrode's buffer.

Platelets activation

To activate platelets, 0,5 IU/ml of thrombin (Sigma, L'isle d'abeau, France), final concentration was added to platelets in Tyrode's buffer.

Platelet lysate

Non activated or activated with thrombin platelets ($1x10^8$) were lyzed in Tyrode's buffer supplemented with Triton X-100 1% during 30mn at room temperature (RT) in the presence of leupeptin 20µg/ml (Sigma). The lysate was then centrifuged 10 mn at 10000 rpm at room temperature. Supernatants were harvested and frozen at -20°C until used.

Analysis of DNA fragmentation

Jurkat cells (10^8) have been incubated for 4h with human recombinant Fas-L IgG (Alexis, Paris, France). Platelets ($6x10^8$ per condition) or apoptotic Jurkat cells were lysed and DNA extracted by ethanol precipitation before migration in a 1% agarose gel as described previously (ref)

Western blot analysis

Platelets (10^8) lysates were separated on a 10% SDS-PAGE in reducing conditions and transferred to a polyvinyldifluoride membrane (Amersham, Buckinghamshire, England), as described previously (ref). Ig were revealed by the use of peroxydase labelled human polyclonal anti-Ig and the luminol fluorescent kit (ECL, Amersham).

Determination of anti-nuclear autoantibodies

Antinuclear antibodies were detected by indirect immunofluorescence on Hep-2 cells lines (Institut Jacques Boy, Reims, France).

3/ Immune complexes

Immune complexes were generated as published elsewhere{Bave, 2000 #64}. Briefly, serum samples from SLE patients were obtained and anti-double-stranded-DNA and anti-ribonucleoproteins levels were measured using a commercially available ELISA kits (Fidis,

BMD, France). All samples were filtered through a 0.45-µm polyvinylidene fluoride syringe before purification as described previously. The T cell lines Jurkat were treated at $1x10^6$cells/ml by IgG FasLigand (Alexis,Läufelfingen, Switzerland), 100ng/ml final concentration, for 4h at 37°C. Cells were then washed once before using

4/ Cell preparation

Human PBMC were isolated from buffy coats by Ficoll gradient centrifugation. Primary human plasmacytoid dendritic cells (pDCs) were purified from the PBMCs by immunomagnetic-beads positive selection using BDCA-4 microbeads following manufacturer instructions (Miltenyi Biotec). Briefly, PBMC were stained with anti-BDCA-4 antibody coupled to colloidal paramagnetic microbeads and passed through a magnetic separation column twice (LS and RS column; Miltenyi Biotec). In some experiments, pDCs were then cell sorted using flow cytometry (FACSAria Cell sorting system, Becton-Dickinson, Pont-de-Claix, France) to reach purities > 95%.

All cells were cultured in complete medium (RPMI 1640, 2mM L-Glutamine, 100 U /ml penicillin/streptomycin, and 8% low endotoxin FCS) and maintained at 37° and 5% CO2.

Stimulating conditions

Isolated pDCs were cultured in 96-well round-bottom plates (Nunc, Roskilde, Denmark), ($7.5x10^4$ cells in 200 µl medium/well) with platelets ($5x10^6$) in the presence of the different stimuli.

5/ Flow cytometry

Washed platelets or plasma rich platelets (10^4/µl) were incubated for 15mn at room temperature in the dark with 20µl of FITC-conjugated monoclonal antibodies specific for

CD61 (GP IIIa) or 20µl PE-conjugated monoclonal antibodies specific for CD40L, or 20µl APC-conjugated monoclonal antibodies specific for CD62p (P-selectin), all from Becton Dickinson. All staining were run by using the same instrument settings.

6/ Confocal microscopy

PBMCs were isolated from SLE patients by Ficoll gradient separation and pDCs were enriched by negative selection. Cells (10^5) were let to adhere for 10 mn at room temperature onto poly-L-lysin-coated slides (Kindler, Freiburg, Germany). Then cells have been fixed by dipping slides in PBS containing 4% formaldehyde for 15mn, washed twice in PBS-1% bovine serum albumin and finally stained with a primary mouse anti-human GPIb (Wm23 antibody anti-GPIb, kindly given by M. Berndt, Melbourne, Australia) in PBS-1% bovine serum albumin, during 1h at room temperature. Slides were washed twice with PBS and stained with the secondary Alexa-568-conjugated goat anti-mouse antibody in PBS-1% bovine serum albumin during 1h at room temperature. Slides were washed with PBS and stained with anti-BDCA2-FITC and DAPI during 1h at RT. After washing with PBS, slides were dried and mounted with Dakocytomation mounting medium (Dakocytomation, Denmark). Images were acquired and processed on a confocal microscope (Leica, Germany) with a X63 objective.

7/ ELISA

Commercially available kits were used to quantify IFN-alpha, and sCD154 (all from Bender MedSystems, Vienna, Austria) and were used according to the manufacturer's instructions.

8/ Mice

Six month old NZB/NZW(F1) (n=6) were injected iv with 3µg/g of monoclonal anti-CD42b p0p3/4 (plt-depleted) or not (controls) on day 0 and once a week for six weeks. The platelet-depleting antibody (a mixture of clones p0p3 and p0p4) was generated as previously described {Bergmeier, 2000 #80}. A third group was composed of six NZB/NZW(F1) given at day 0 and once a week one milliliter of $2x10^7$ activated platelets/ml. Serum was harvested on day 0 and once a week and tested for anti-DNA IgG and IgM blood levels. Levels of total anti-dsDNA IgG and IgM Abs were measured using standard ELISA as described previously {Goulvestre, 2005 #81}. Blood urea levels were measured at day 0 and week 6 using standard techniques for laboratory determinations. At week 6, mice were sacrificed and kidneys were analyzed by light microscopy as described previously

9/ Statistical analysis

Pearson's correlation analysis was used to measure correlation between 2 parameters. Comparisons between groups were performed using the nonparametric Mann-Whitney U test with a level of significance at $P \leq 0.05$. The tests were performed with the statistical software Statistica Inc. (Statsoft, Tucson, AZ).

Figure 1A

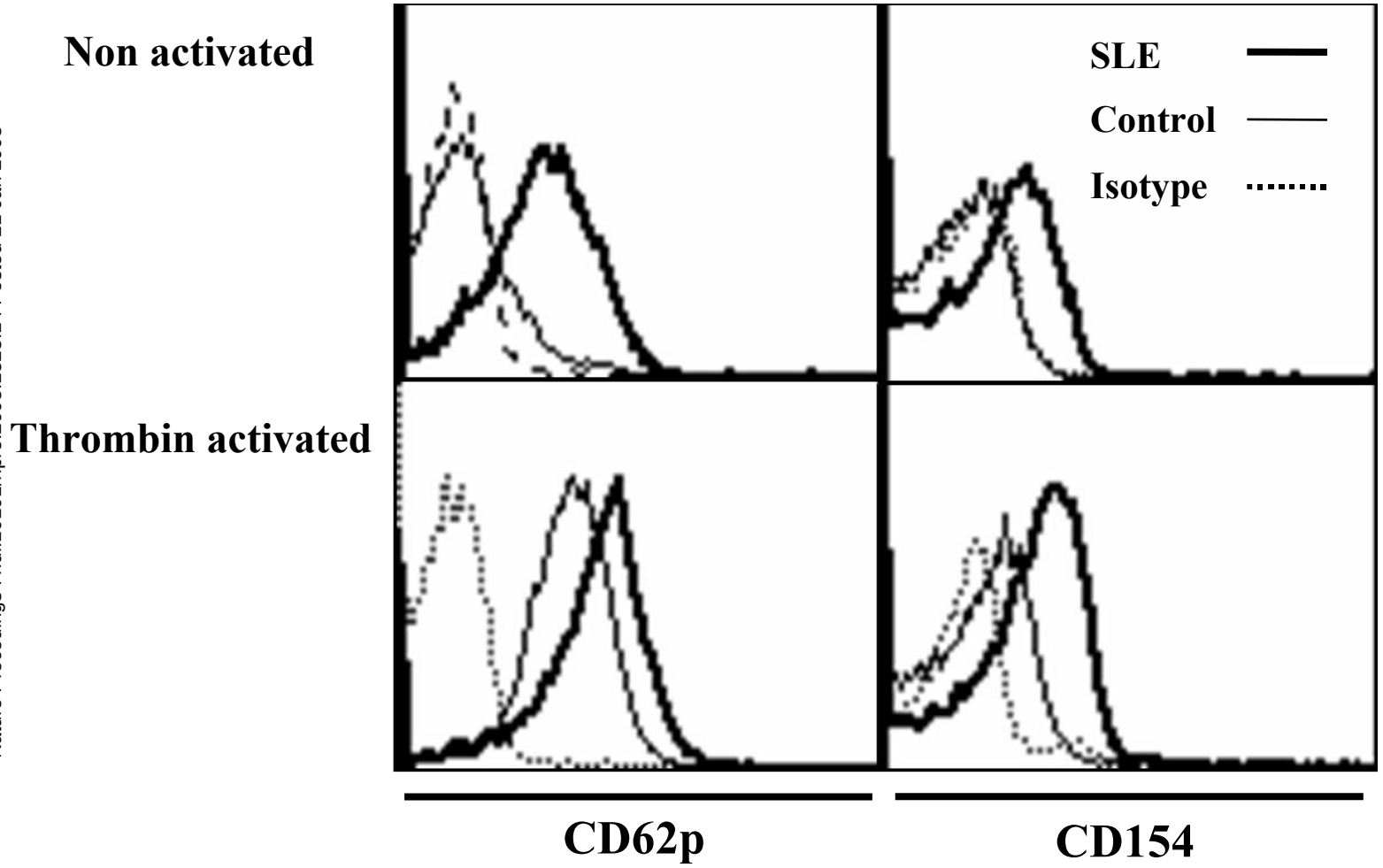


Figure 1B

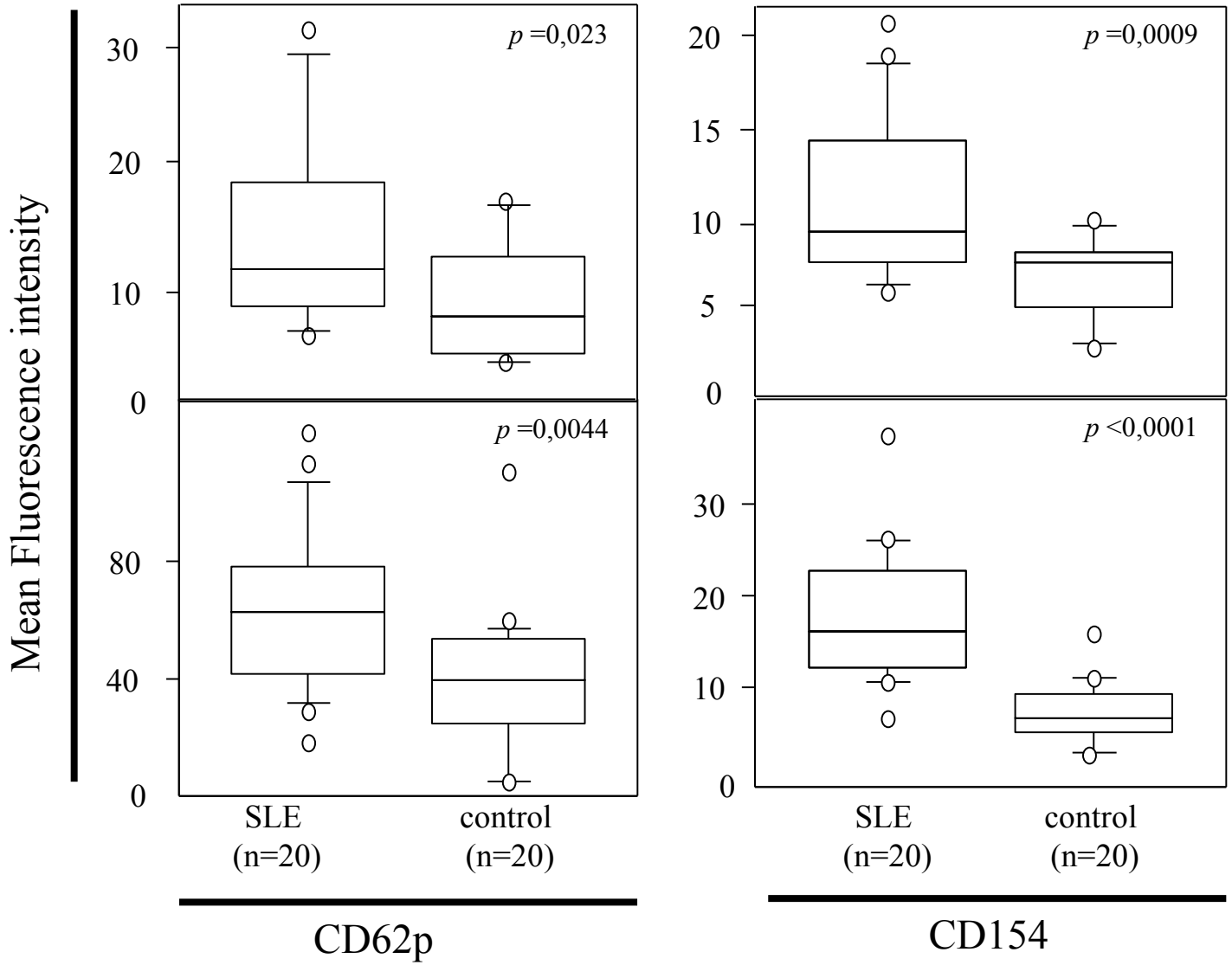
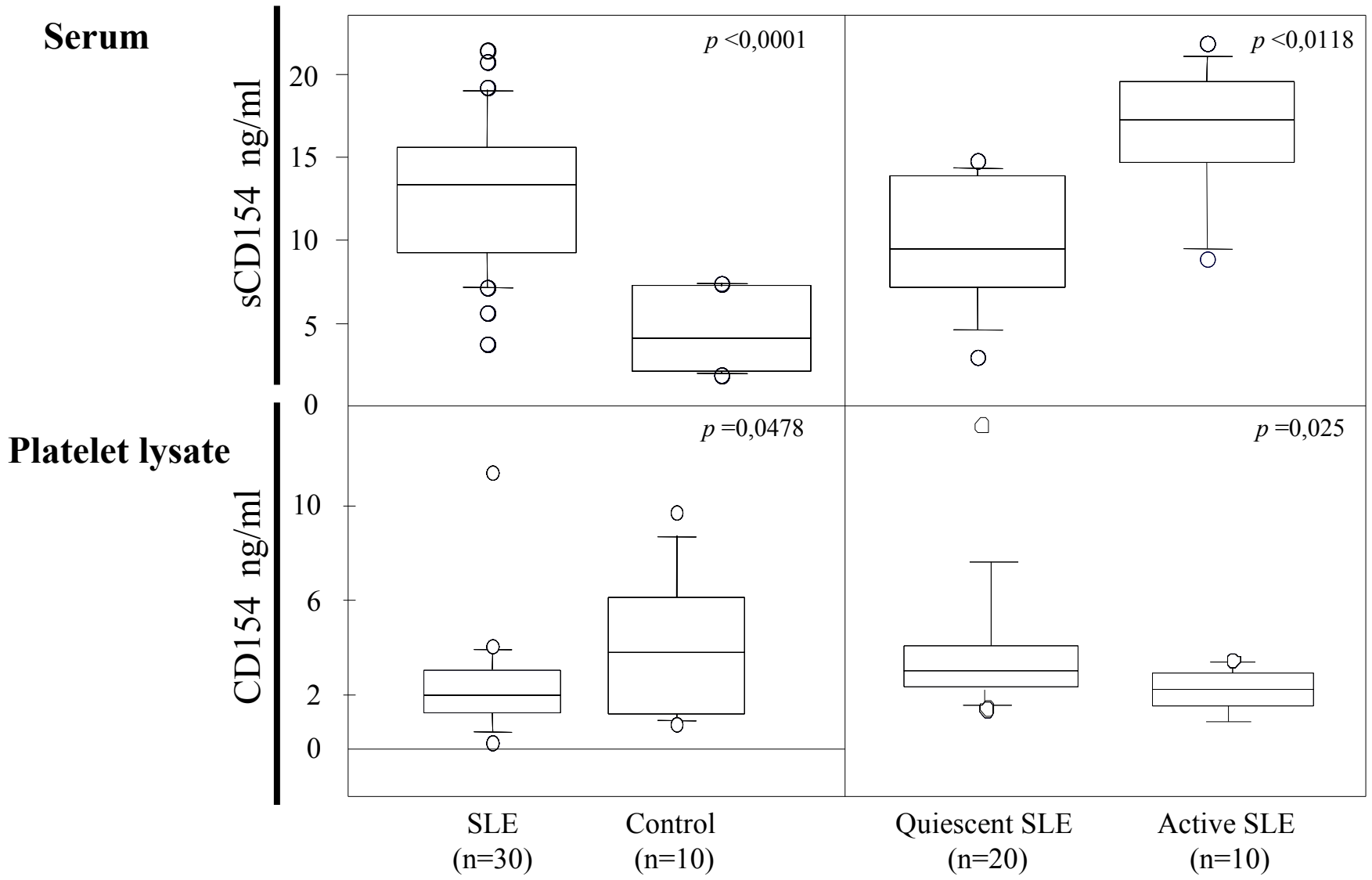


Figure 1C



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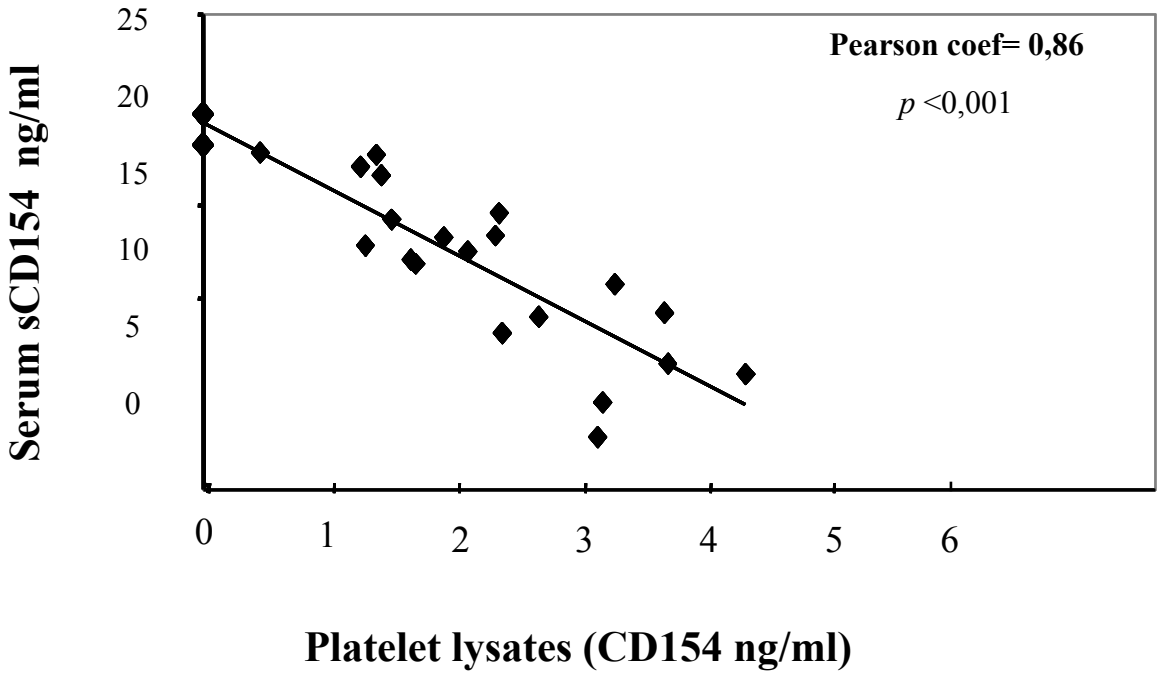

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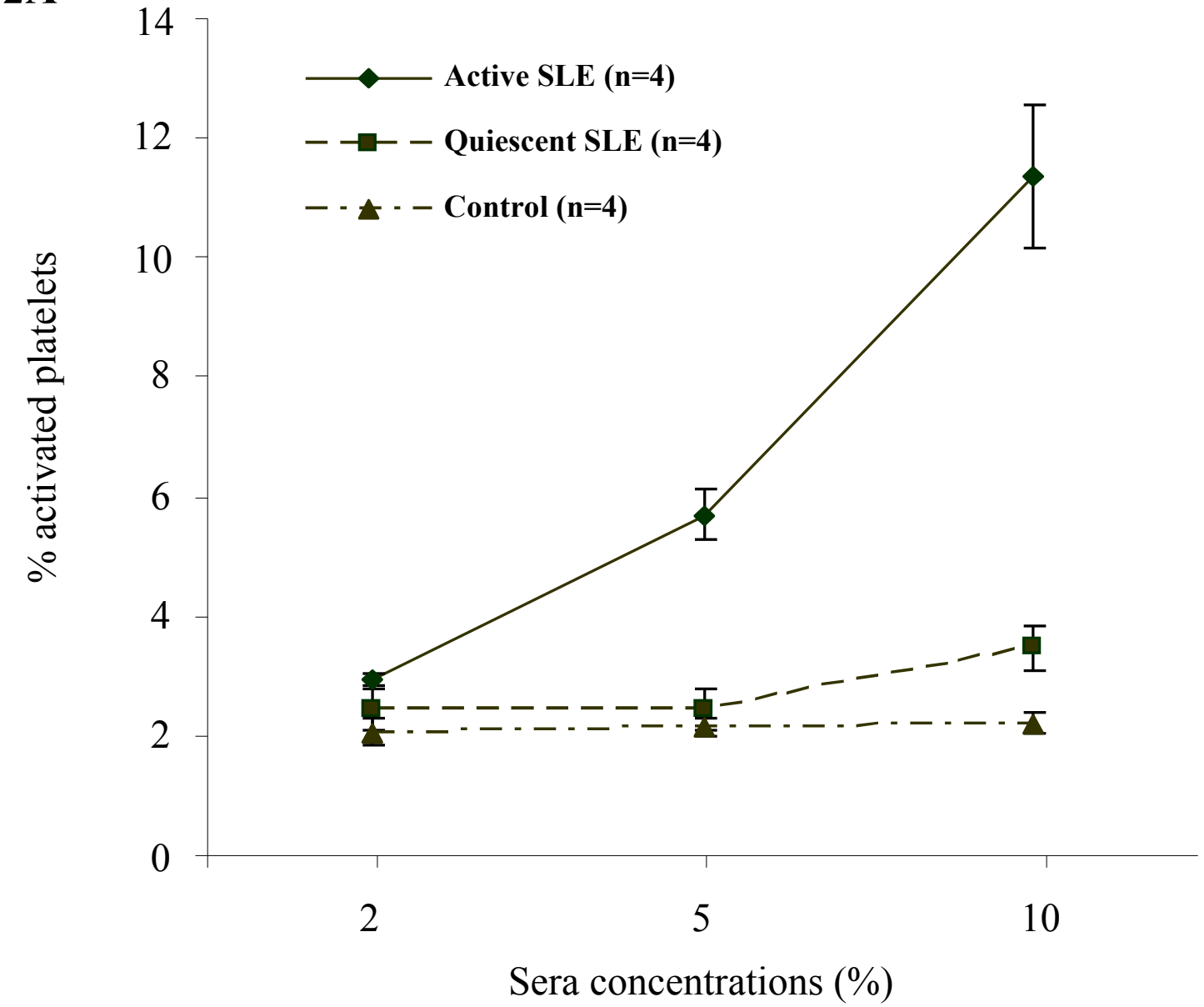

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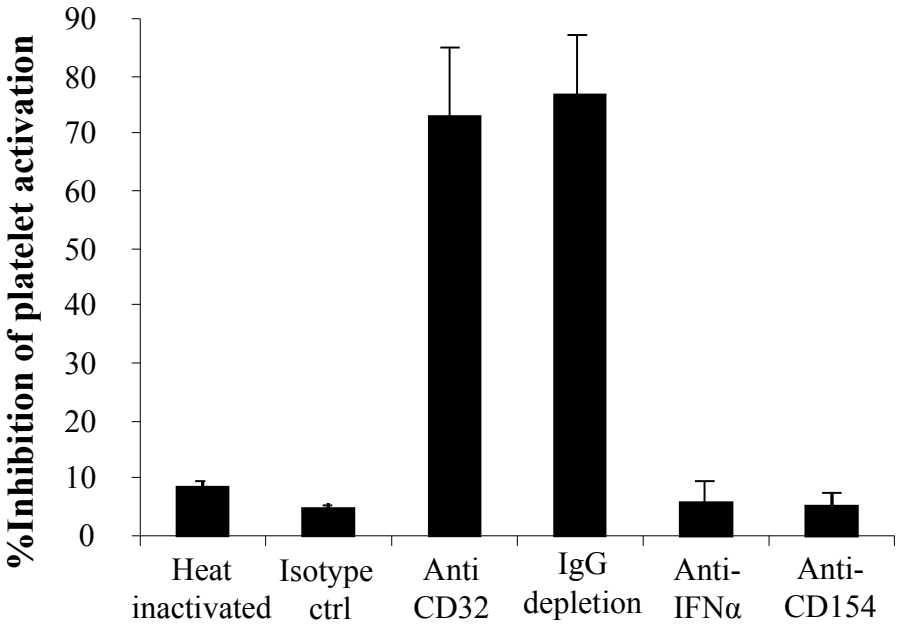
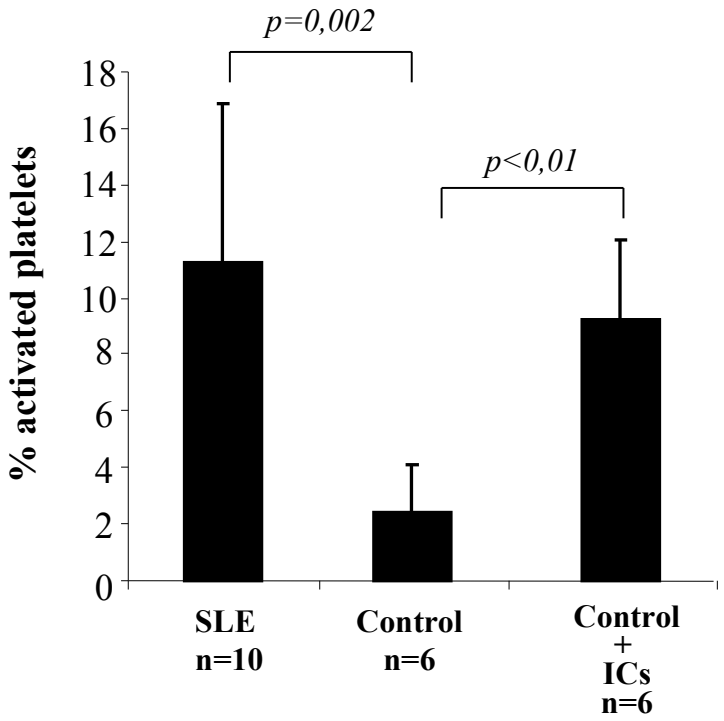


Mean value of 3 different experiments



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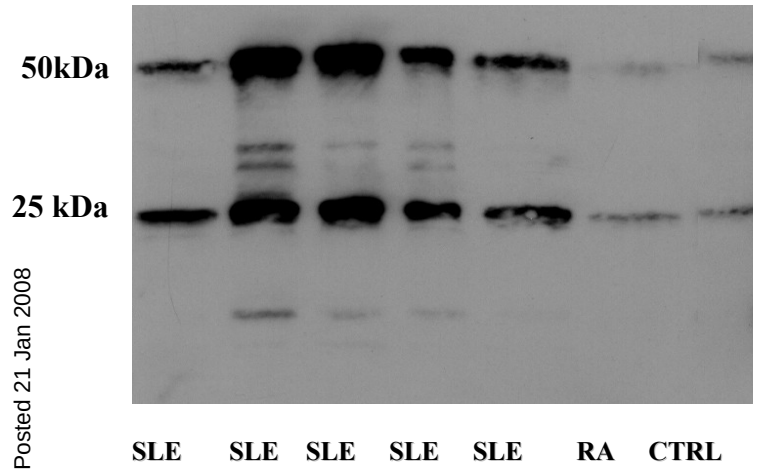
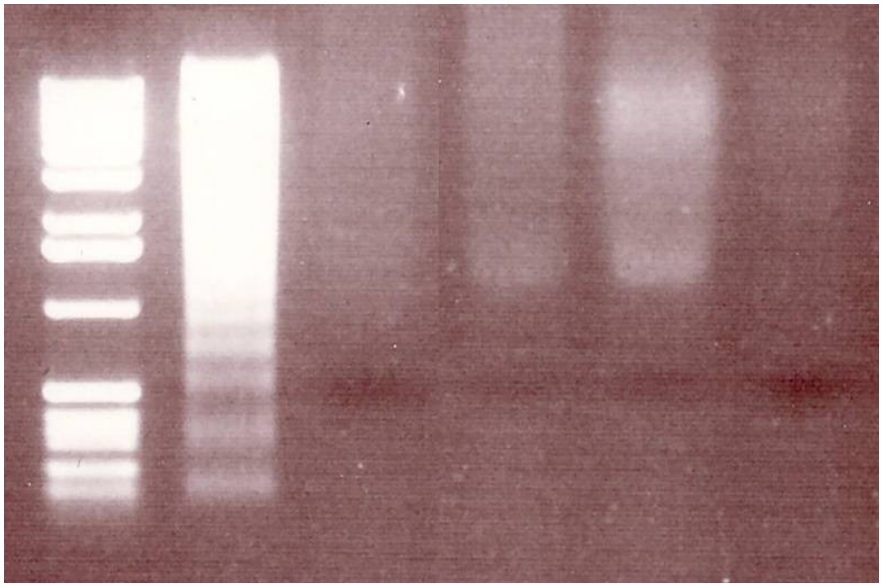




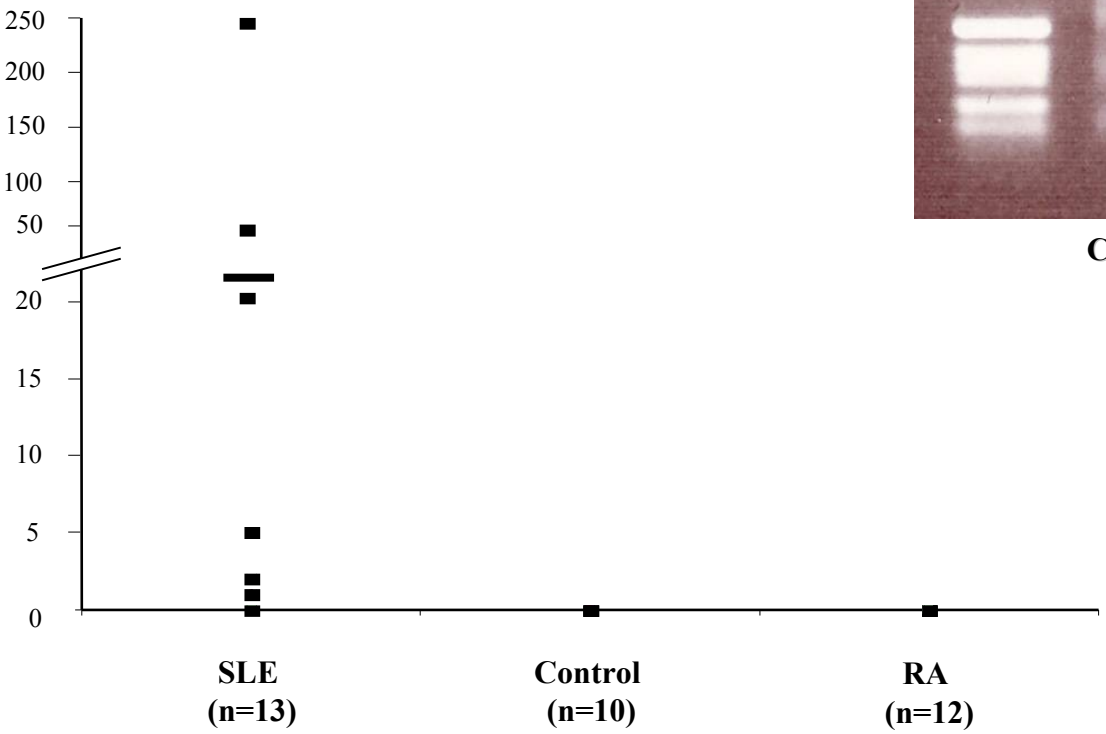


Figure 3A

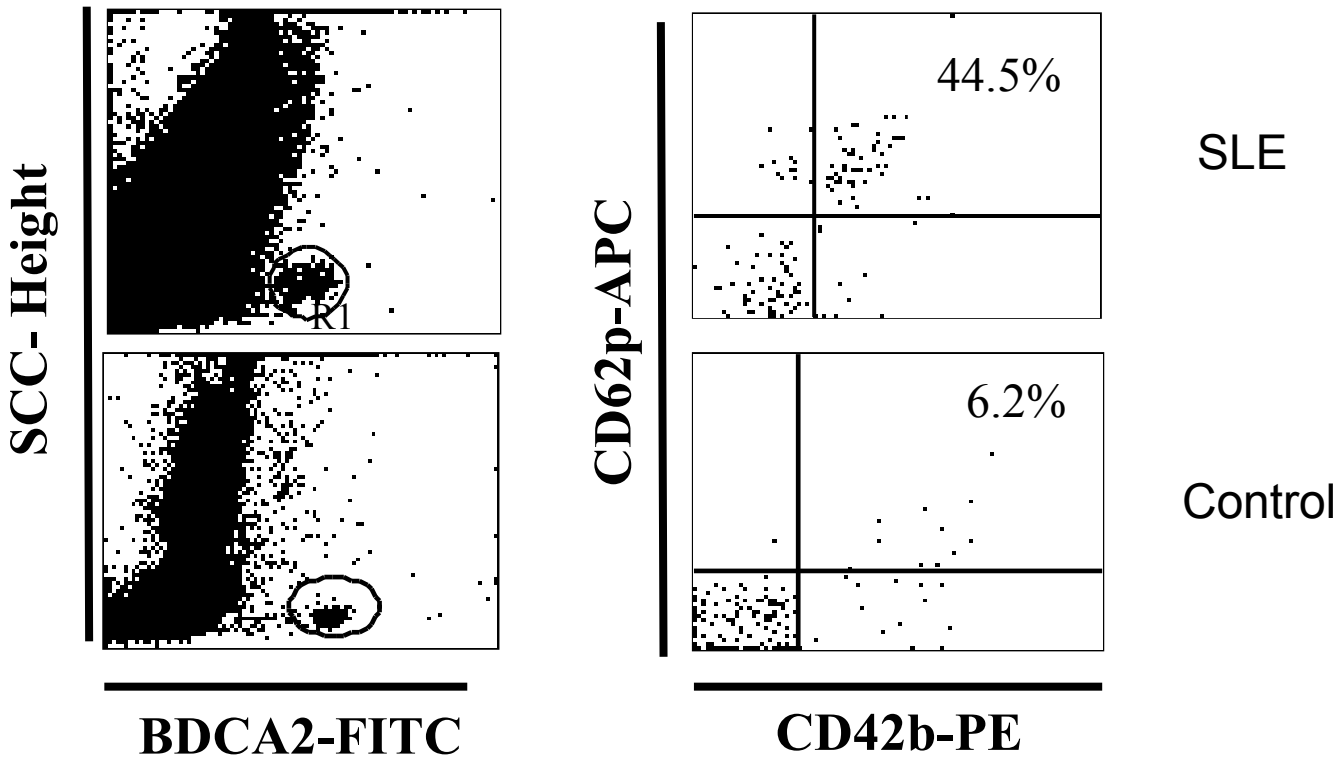


Figure 3B

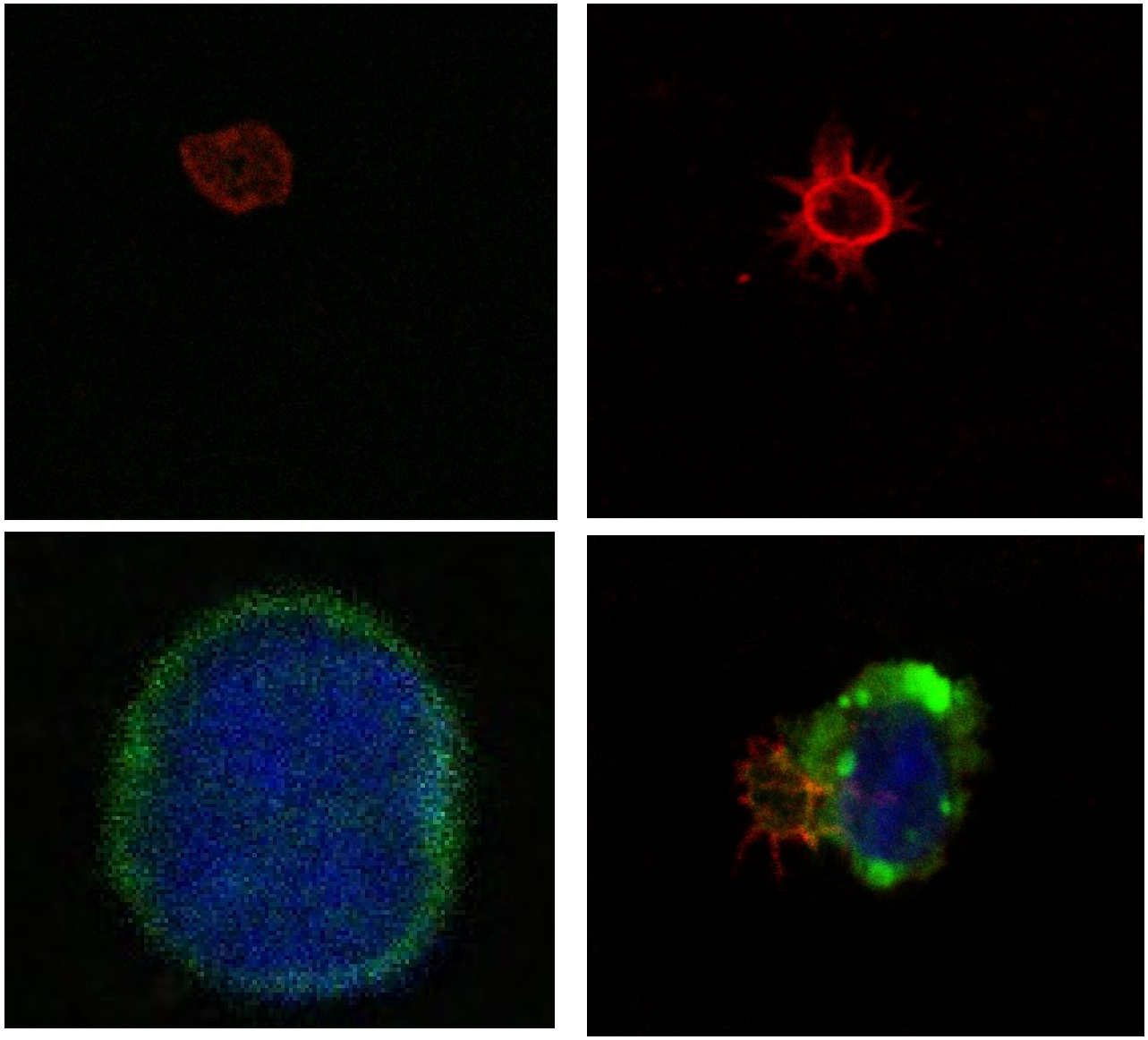


Figure 3C

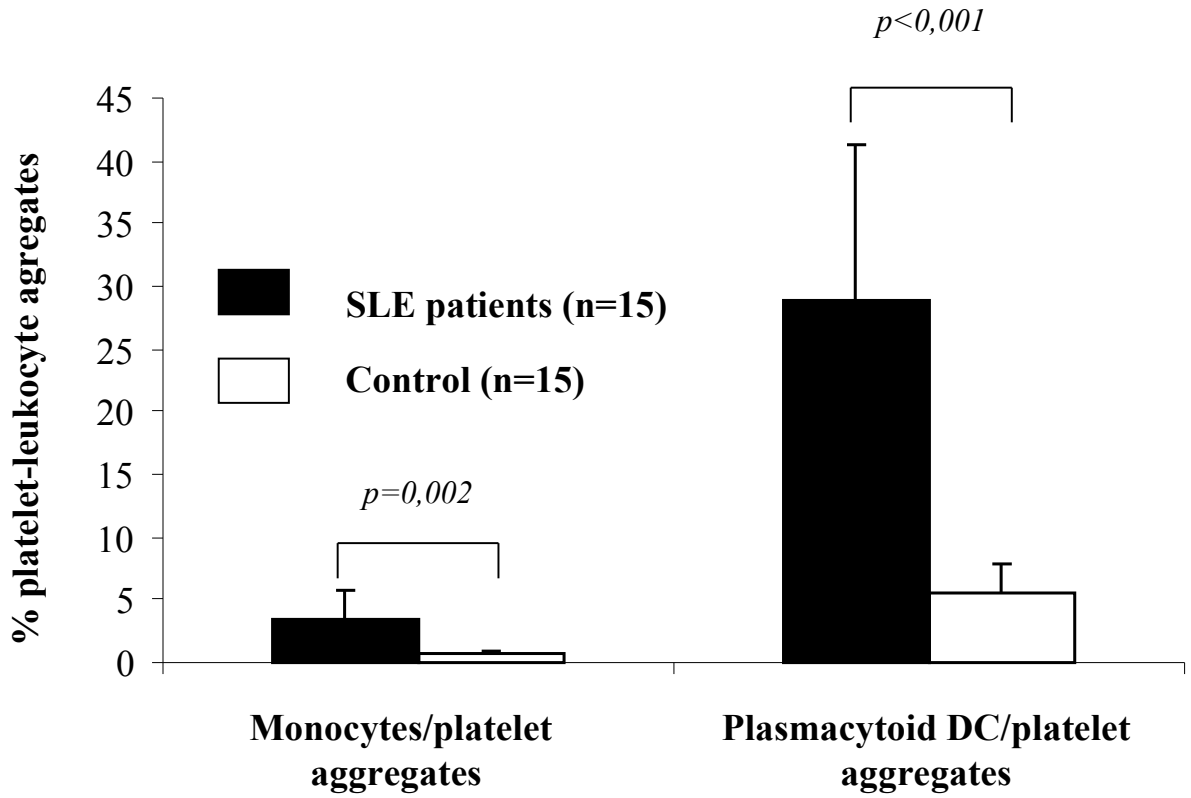
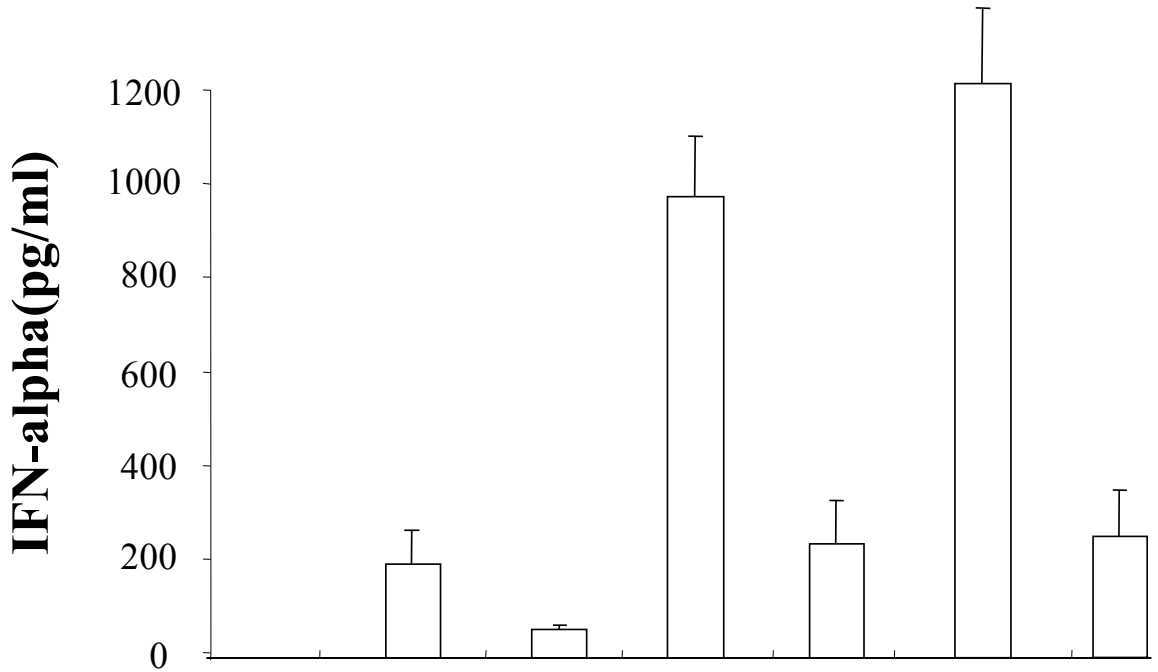


Figure 3D



pDCs	+	+	+	+	+	+	+
Activated platelets	-	-	+	+	+	-	-
Immune complexes	-	+	-	+	+	+	+
Anti-CD40 blocking	-	-	-	-	+	-	-
CD154+ L-cells	-	-	-	-	-	+	-
Control L-cells	-	-	-	-	-	-	+

Figure 4A

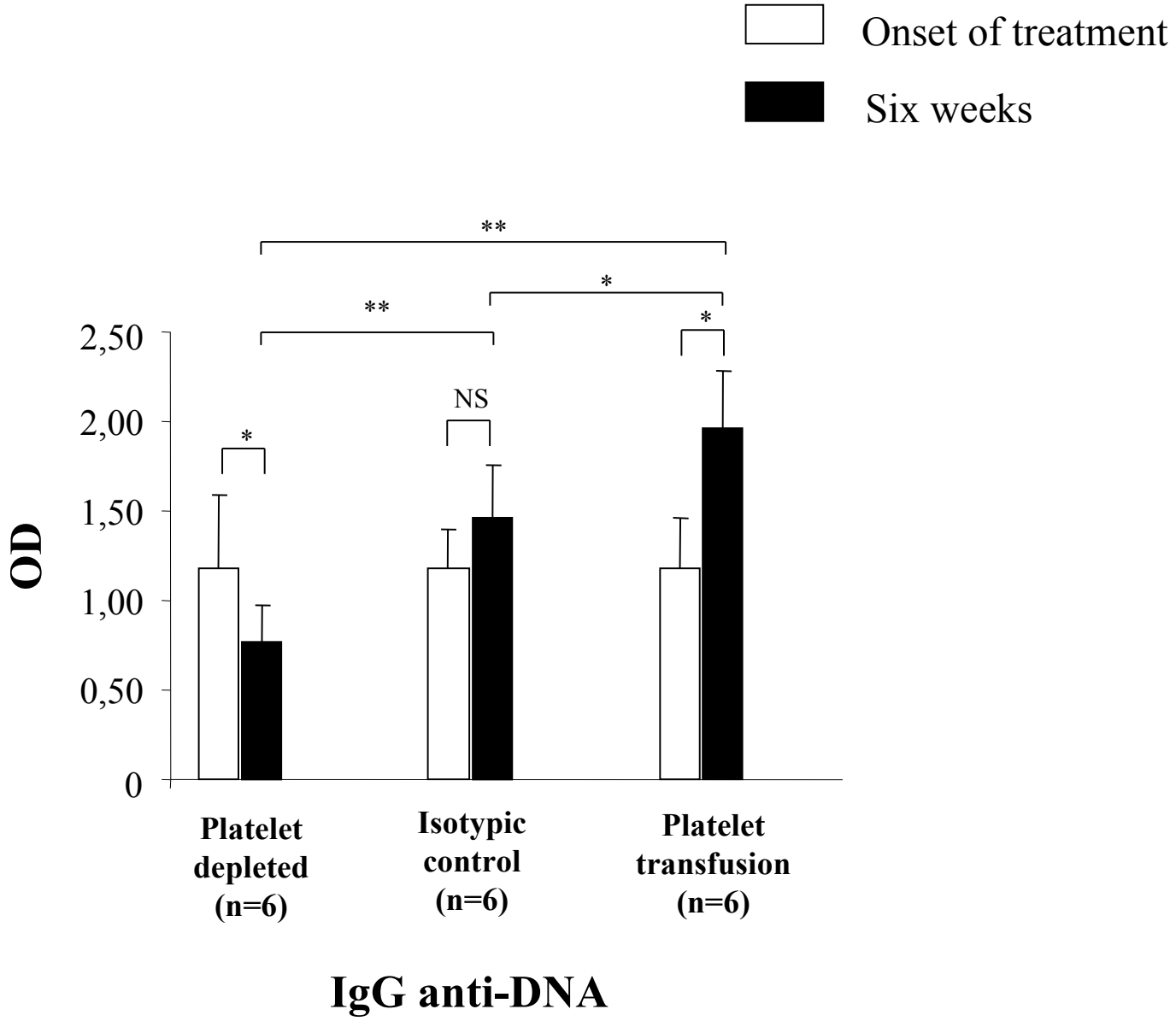


Figure 4B

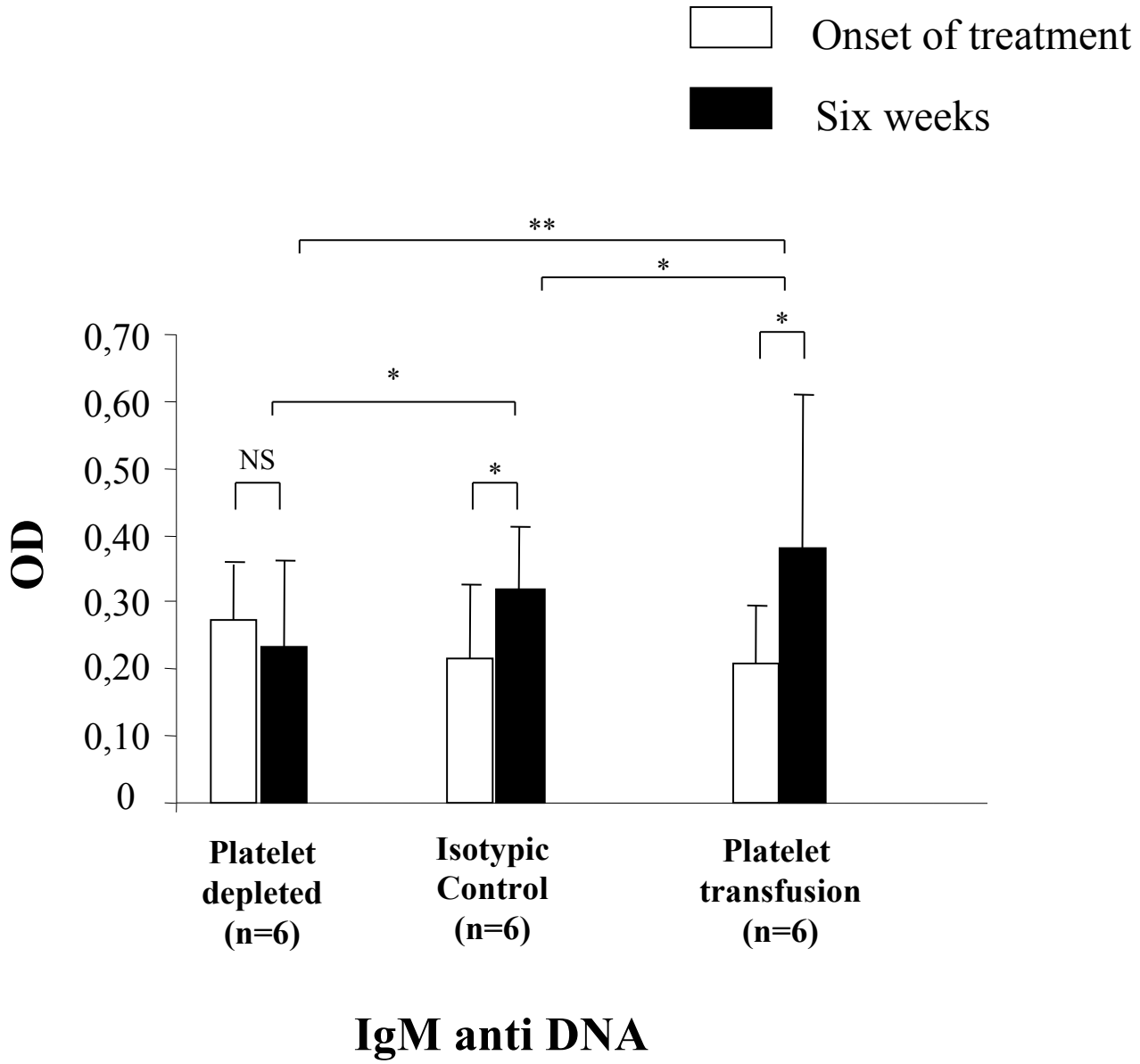


Figure 4C

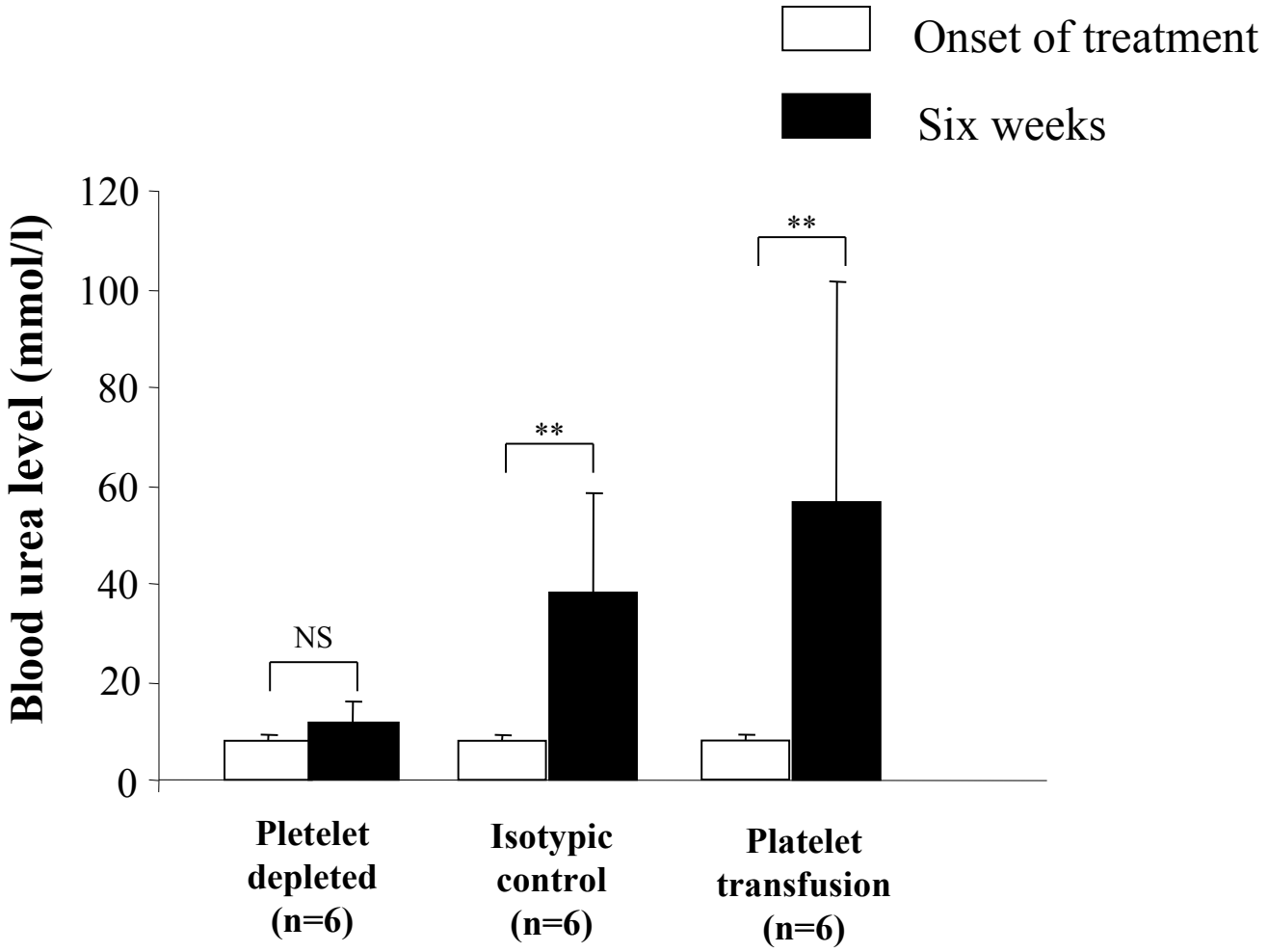
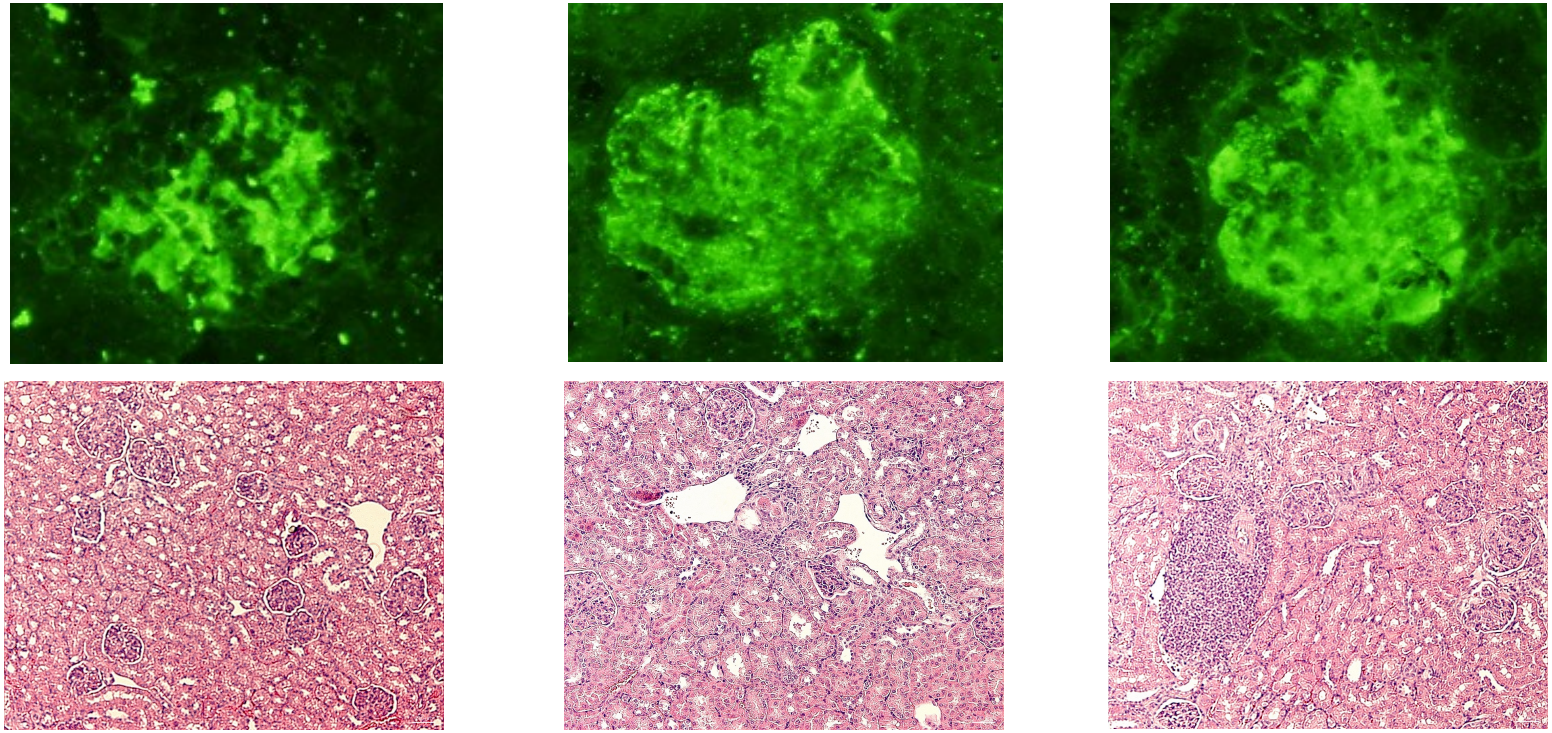


Figure 4D



Platelet depleted | Isotypic control | Platelet transfusion

Figure 4E

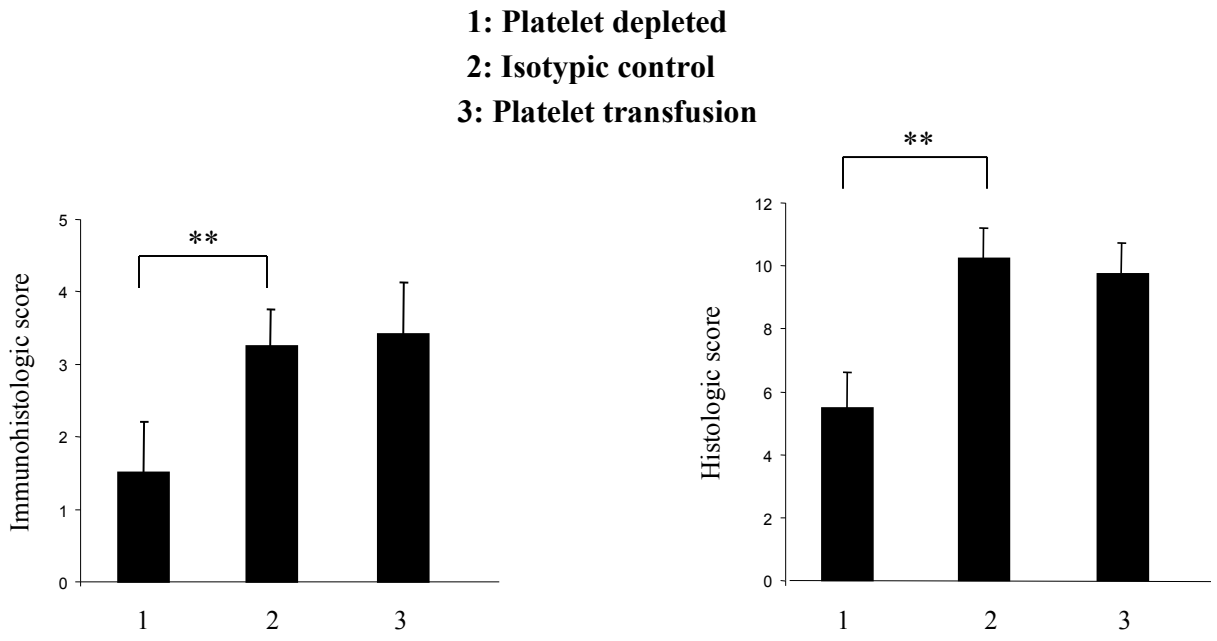


Table 1. Summary of the demographic, clinical, and biological characteristics and treatments of SLE patients in the study

patient/sex/age	disease flare manifestations	SLEDAI score*	treatment	ANA	anti-DNA
1/F/32	Sk,A,Bl	7	Pred	2000	239
2/F/28	Sk,A,R	4	Pred,HCQ,MMF	250	57
3/M/33	A,P,NP	13	Pred,MMF	8000	92
4/F/49	Sk,A,R	6	Pred,MMF	500	29
5/F/47	Sk,A,R	0	Pred,MMF	4000	7
6/F/33	Sk,A,Bl	2	Pred,HCQ	16000	25
7/F/29	Sk,A	0	None	250	1
8/F/35	Sk,A	8	Pred,HCQ	2000	42
9/F/27	Sk,A	10	None	8000	647
10/F/42	Sk,A,Bl	0	Pred,HCQ	250	2
11/F/52	Sk,A	4	HCQ	2000	19
12/F/26	Sk,A	4	Pred	250	24
13/M/46	A,Sk	0	Pred	16000	13
14/F/44	Sk,A	10	HCQ	1000	55
15/F/29	Sk	2	Pred,HCQ	32000	12
16/F/39	Sk,A	8	Pred,Me	2000	52
17/F/28	Sk	0	None	500	18
18/F/30	Sk,R	0	MMF	500	67
19/M/30	A,R	2	None	500	35
20/F/45	A,P	0	None	1000	12
21/F/56	Sk,Bl	4	Pred,HCQ	1000	16
22/F/32	Sk,A	0	None	1000	1
23/F/29	A,R	4	Pred,HCQ,MMF	2000	832
24/F/42	A,R	4	Pred,HCQ	2000	6
25/F/45	Sk,Bl	5	None	2000	136
26/F/32	A,Sk	0	None	2000	30
27/M/38	A,Bl	3	Pred	250	113
28/F/36	Sk,A	2	Pred,HCQ	8000	14
29/F/70	A,Sk	0	HCQ	250	1
30/F/47	A,Bl	0	HCQ,Pred	250	9
31/F/35	Sk,A	4	HCQ,Pred	500	9
32/F/26	Sk,NP	8	Pred,HCQ,MMF	1000	32
33/M/54	Sk,Bl	3	None	8000	130
34/F/34	Sk,Bl	4	Pred,HCQ	500	7
35/M/32	Bl,A	0	Pred	250	9
36/F/24	Sk,A	0	Pred,HCQ	500	32
37/F/28	A,Bl	2	Pred,HCQ	1000	27
38/F/42	R,Sk	10	Pred,HCQ,MMF	1000	47

SLE = Systemic lupus erythematous ; ACR= American College of Rheumatology ; SLEDAI = SLE Disease Activity Index ; A= musculoskeletal system ; Sk= mucocutaneous lesions ; Pred. = Prednisone; HCQ = Hydroxychloroquine; Bl= hematologic abnormality; P= Pericarditis; NP = neuropsychiatric disorders; K = renal disease; MMF = mycophenolate mofetil; Me = Methotrexate
*At the time of blood sampling