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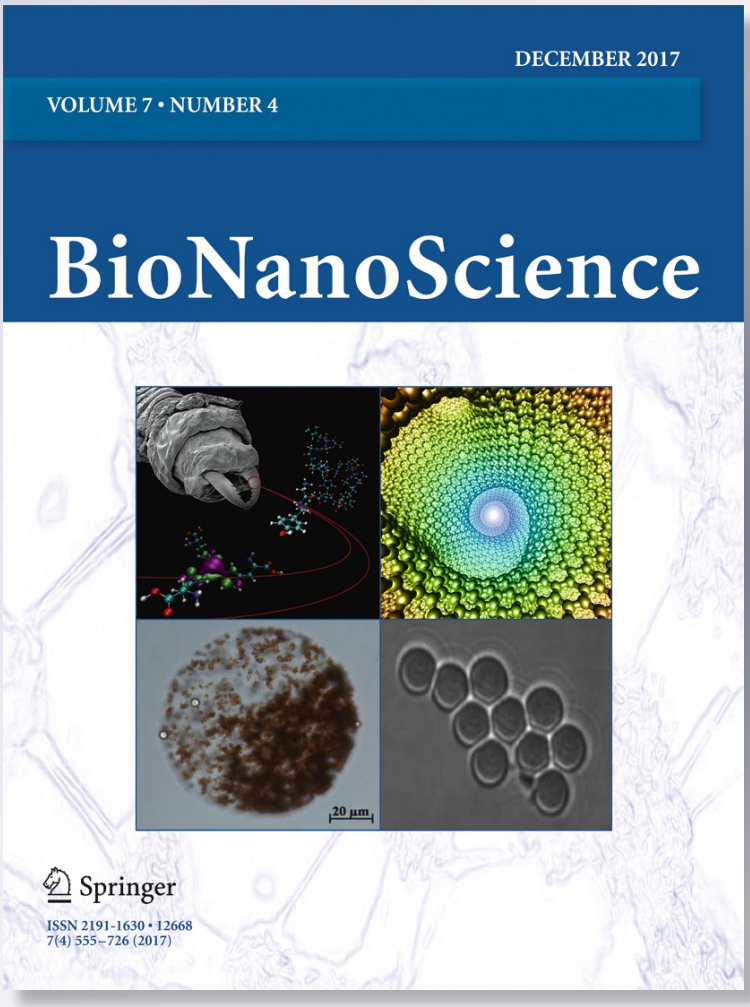

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Autoantibodies Against dsDNA Modulate Contraction of Blood Clots

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Abstract The degree and rate of clot contraction (retraction) in systemic lupus erythematosus (SLE) patients, especially in those with a high level of anti-double stranded DNA (dsDNA) antibodies in the blood, was significantly reduced compared to healthy donors. We hypothesized that this effect was caused by the anti-dsDNA antibodies. To test this assumption, we investigated the kinetics of blood clot contraction in vitro in the absence and presence of anti-dsDNA antibodies purified from the blood serum of SLE patients. The degree of clot contraction was increased immediately after addition of the anti-DNA antibodies in a concentration-dependent manner. This stimulating effect was abrogated by a monoclonal antibody against the platelet Fc-receptor. On the contrary, after prolonged incubation (for hours) of the blood samples with the anti-DNA antibodies, the extent of clot contraction was significantly reduced. These results suggest that anti-dsDNA antibodies in SLE induce Fc-receptor-mediated chronic platelet hyperactivation, resulting in platelet exhaustion and dysfunction, including reduced contractility. The impaired contraction of blood clots and thrombi caused by autoantibodies may be an important pathogenic mechanism that affects the course and outcomes of thrombotic complications in SLE.

Keywords Blood clotting · Clot contraction · Systemic lupus erythematosus · Anti-DNA antibodies

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1 Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease that affects multiple organs and tissues, resulting in diverse symptoms and outcomes. It is characterized by production of antibodies (Abs) against a variety of autoantigens, specifically against double stranded DNA (dsDNA) [1]. Platelets are a well-known target for anti-phospholipid Abs [2] that alter platelet function in SLE [3] and predispose to thrombosis [4]. It is unclear whether anti-dsDNA Abs can also contribute to thrombotic complications in SLE. Blood clots and thrombi are known to undergo volume shrinkage driven by contracting platelets, the process named clot contraction or retraction [5, 6]. Despite its potential clinical importance in modulating vessel obstruction and blood flow, the mechanical remodeling of blood clot and thrombi has been underestimated and understudied [7]. Here we studied the effect of anti-dsDNA Abs isolated form the blood of SLE patients on the ability of platelets to squeeze blood clots.

2 Materials and Methods

Blood from SLE patients and healthy donors was withdrawn using a protocol approved by the Ethical Committee of Kazan State Medical University. Only patients who were not treated with anti-coagulants or anti-platelet drugs were included in the study. Blood samples of 37 SLE patients were analyzed, of which the activity of anti-dsDNA Abs was less than 100 ME/ml in 23 (63%) and > 100 ME/ml in 14 (37%). Blood samples from 60 healthy donors were used as a control. The SLE patients and control subjects were comparable by the age (37 ± 2 vs. 34 ± 2 years, respectively) and sex (women comprised 79 vs. 69%, respectively).

Clot contraction was studied using a novel optical tracking method [6]. Briefly, citrated whole blood was activated by addition of 2 mM $CaCl_2$ and 1 U/ml thrombin (both final concentration) and then immediately transferred to a cuvette, which was pre-coated with 4% Triton X-100 in phosphate-buffered saline to prevent attachment of fibrin to the walls. The cuvette with activated blood sample was transferred to a Thrombodynamics Analyser System (HemaCore, Moscow, Russia) to automatically image a clot based on light scattering. Changes in the clot size were captured every 15 s for 20 min. The collected clot images were analyzed computationally to extract the following parameters of clot contraction: the extent of contraction (percent of the initial clot size) at the end point (20 min) and average contraction velocity (percent per time unit). The kinetics of clot contraction was followed in the absence or presence of anti-dsDNA Abs. Anti-dsDNA antibodies were purified from pooled samples of serum of SLE patients using a two-step affinity chromatography on Protein G- and dsDNA-coated sorbents using a chromatographic system Akta Avant 25. The antibodies were not contaminated with DNA as determined by agarose gel electrophoresis and were highly pure in SDS-PAGE. The high dsDNA-binding activity of the purified antibodies was confirmed by ELISA. The non-dsDNA binding IgG fraction was used as a negative control for anti-dsDNA Abs.

3 Result and Discussion

Anti-dsDNA Abs are one of the most likely modulators of platelet function in SLE because they are known to activate platelets via Fc-receptors [8]. Our measurements showed that the level of these Abs in the blood of SLE patients was increased 22-fold compared to control and comprised on average 115 ± 23 and 5.2 ± 1.1 ME/ml, respectively ($p < 0.001$). Based on the level of anti-dsDNA Abs, the SLE patients were segregated into two groups, namely patients with the level of anti-dsDNA Abs exceeding 100 ME/ml and those below 100 ME/ml. Importantly, the degree and rate of clot contraction were both reduced significantly in patients with the level of anti-dsDNA Abs > 100 ME/ml compared to those with the level < 100 ME/ml. It is noteworthy that irrespective of the level of anti-dsDNA Abs, the extent of clot contraction (37 ± 2%) and average velocity (29 ± 3) × 10^{-3}%/sec in SLE patients were significantly lower than in healthy donors (49 ± 1%), $p < 0.05$, and (41 ± 2) × 10^{-3}%/sec, $p < 0.05$, respectively. These results strongly suggest that anti-dsDNA Abs can modulate clot contraction in SLE, most likely via affecting platelet functionality.

To model the effect of anti-DNA Abs on clot contraction and platelets, we studied the kinetics of clot contraction in the blood of healthy donors in the absence and presence of

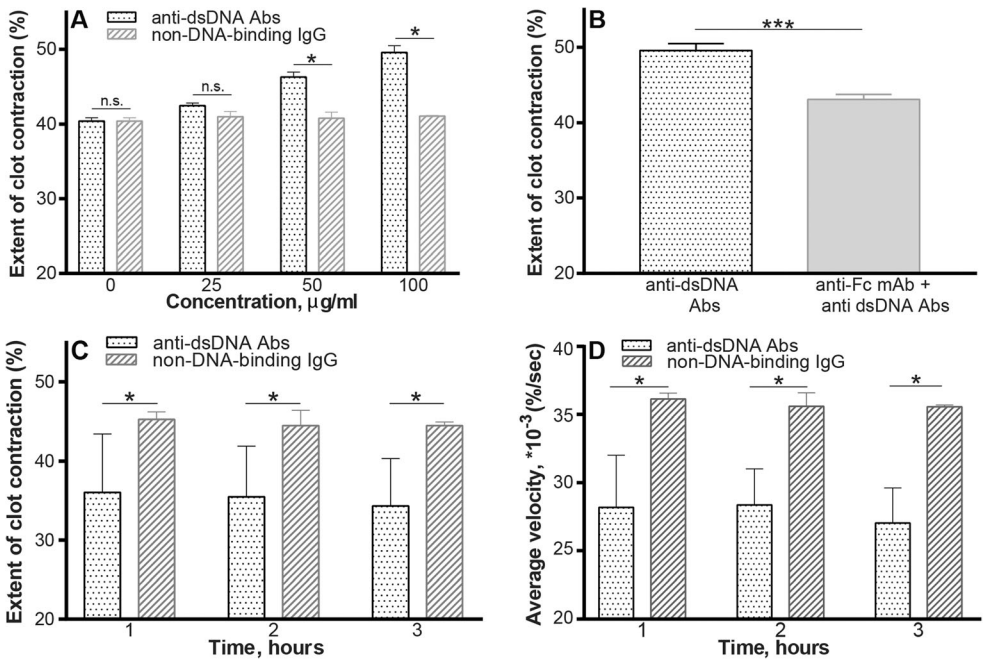

Fig. 1 **a** Clot contraction in the blood of healthy donors immediately after addition of increasing concentrations of purified anti-dsDNA Abs compared to non-DNA binding IgG. **b** Average extent of clot contraction right after addition of 100 µg/ml anti-dsDNA Abs in the absence and presence of mAb against the platelet Fc-receptor. Average extent (**c**) and rate (**d**); concentration of the anti-dsDNA antibodies used to monitor their time-dependent effect on clot contraction was 50 µg/ml and non-DNA-binding IgG. The time-dependent effect of the anti-dsDNA antibodies on clot contraction (at each time point) was measured separately in 3 blood samples from different donors. *$p < 0.05$; ***$p < 0.001$

exogenous anti-DNA Abs purified from the blood of SLE patients. If measured right after addition of anti-DNA Abs to whole blood, the extent of clot contraction and average velocity were significantly higher than those in the presence of non-dsDNA-binding IgG (Fig. 1a). This stimulating effect was dose-dependent and was eliminated after pre-incubation of platelets with an anti-Fc-receptor mAb IV.3 that brought the degree and rate of clot contraction back to the normal values (Fig. 1b). These results indicate direct Fc-receptor-mediated activation of platelets by the anti-dsDNA auto-Abs. On the contrary, if the blood was incubated with the same anti-DNA Abs for 1, 2, and 3 h, the degree of clot contraction was significantly reduced, much further than in control treated with IgG (Fig. 1c, d). These results suggest that platelets become defective over time because of their continuous (chronic) hyperactivation by the anti-DNA Abs, which may also occur in SLE and account for the reduced clot contraction revealed in the blood of SLE patients.

4 Conclusion

Our results show that anti-dsDNA auto-Abs can impair contraction of blood clots by continuous hyperactivation of platelets followed by their exhaustive dysfunction, including the reduced ability to contract. The decreased contraction of clots and thrombi may be a pathogenic mechanism that affects the course and outcomes of thrombotic complications in SLE by modulating the degree of vessel obstruction and abnormal blood flow. Further studies of the mechanisms and pathophysiological significance of the impaired clot contraction in SLE are needed because they may help to improve prophylaxis, diagnosis, prognosis, and treatments of SLE-related thrombotic states.

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