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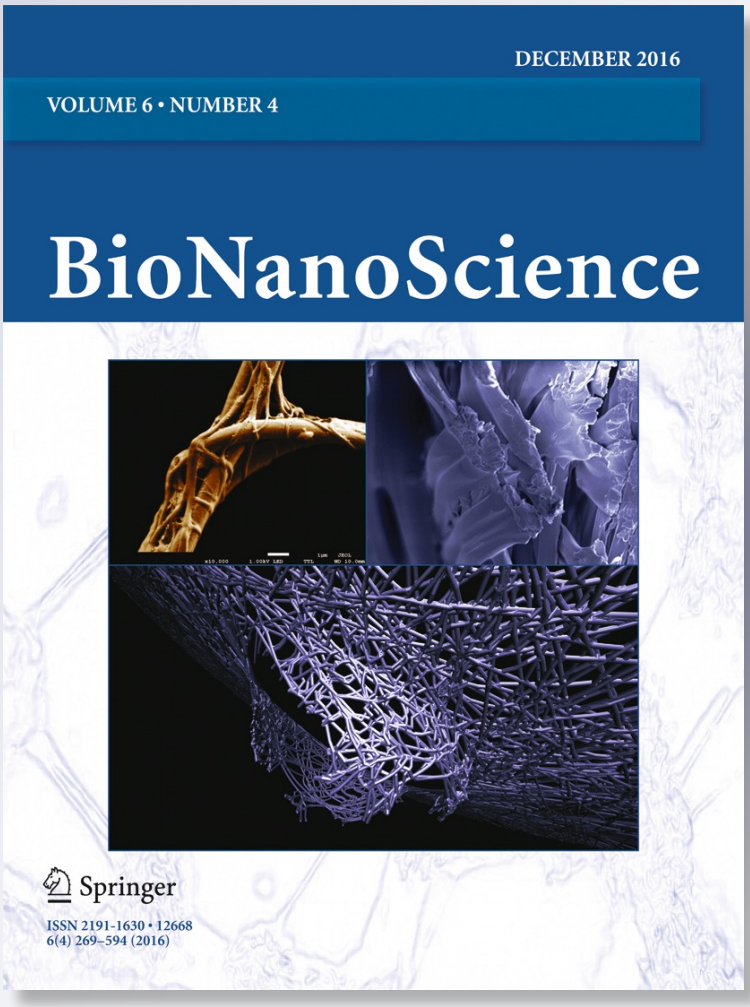

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Fibrin Clot Structure and Properties are Altered in Systemic Lupus Erythematosus

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Abstract Thrombotic complications in systemic lupus erythematosus contribute significantly to the morbidity and mortality rates. Abnormal formation and structure of fibrin clots add substantially to hypercoagulability and thrombosis. We used dynamic turbidimetry to assess in vitro the kinetics of fibrin polymerization and t-PA-induced fibrinolysis in recalcified plasma of patients with systemic lupus erythematosus compared to healthy subjects. Fibrin structure was studied using scanning electron microscopy. Clots from the pathological plasma samples polymerized significantly slower, resulting in formation of fibrin with a higher optical density and less compact fibrin networks with larger pores. These changes were associated with a prolonged clot lysis time and reduced lysis rate. The results show that in the blood of patients with systemic lupus erythematosus fibrin clots have a pro-thrombotic phenotype that comprises an important mechanism underlying lupus-related thrombophilia.

Keywords Systemic lupus erythematosus · Fibrin · Scanning electron microscopy · Fibrinolysis

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1 Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease. A common complication in SLE is thrombosis with the incidence 25- to 50-fold higher than in the general population [1], although the molecular and cellular mechanisms of thrombophilia in SLE are not clear. Fibrin network is a major component of clots and thrombi that forms a mechanical scaffold. Abnormal network structure and increased stability of fibrin clots are directly related to thrombosis [2]. Impaired fibrinolysis in SLE patients may aggravate systemic pro-thrombotic state [3] and induce local pro-inflammatory tissue changes. We studied the formation and properties of fibrin in the blood of SLE patients assuming that the fibrin clots have impaired structure and increased resistance to fibrinolysis that might contribute to predisposition to thrombosis observed in SLE.

2 Material and Methods

The study was approved by the Ethical Committee of Kazan State Medical University. Three SLE patients were included in the study based on the criteria of the American College of Rheumatology (ACR) for active SLE. None of the patients had antiphospholipid antibodies. Ten healthy individuals were studied as a control. Citrated whole blood samples were centrifuged to obtain platelet poor plasma (10 min at 200 g, 15 min at 1500 g, and 5 min at 10,000 g) which was then dispensed into aliquots and stored at −80 °C.

Kinetics of fibrin formation and lysis were studied with dynamics turbidimetry. Clotting of platelet poor plasma was induced by adding $CaCl_2$ (24 mmol/L final concentration) followed by monitoring the optical density at $\lambda = 350$ nm at 37 °C in a Shimadzu UV-1800 spectrophotometer. The following variables were determined: (1) the time needed for

thrombin generation and protofibril formation (*Lag*); (2) the velocity of lateral aggregation of protofibrils and fiber formation (the slope of the curve, *V*); (3) the maximum optical density at a plateau (A_{max}). Fibrinolysis was recorded after simultaneous addition of Ca^{2+} and t-PA (HYPHEN BioMed, France) (50 ng/mL final concentration) to platelet poor plasma and the following parameters were extracted from turbidimetric curves: (1) total lysis time (*TLT*); (2) the rate of fibrin lysis (*V*); and (3) the time needed to reduce the maximum turbidity of the clot to the half-maximal value ($t_{1/2}$). The structure of fibrin clots in recalcified plasma was analyzed with scanning electron microscopy in a MERLIN microscope (Zeiss, Germany). The experimental data were analyzed statistically with a paired Student's *t* test using Microsoft Excel. Data are shown as a mean ± standard deviation, and *P* values are reported as follows: $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

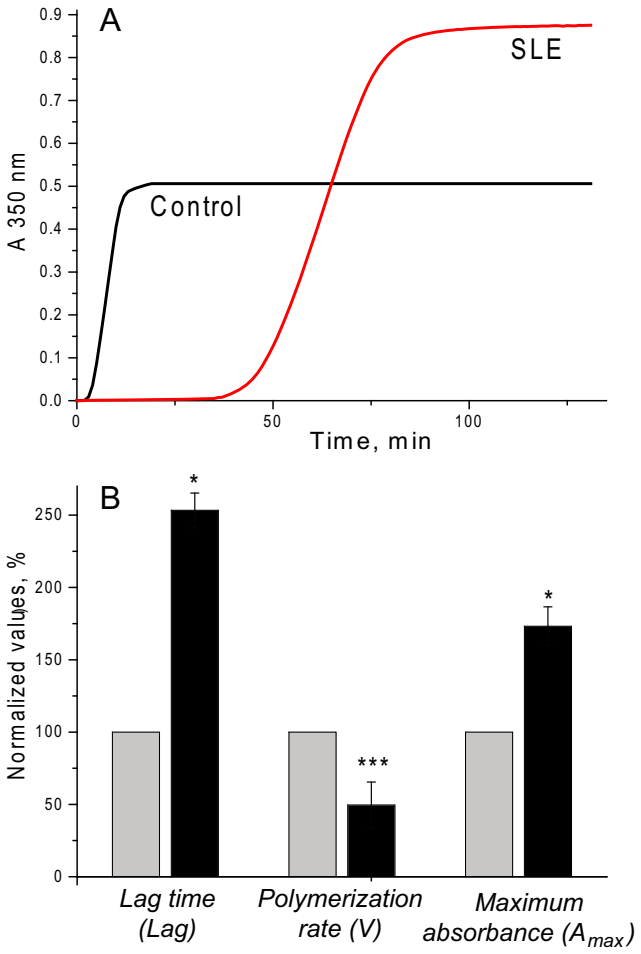

Fig. 1 **a** Representative dynamic turbidity curves obtained by recalcification of plasma samples obtained from the blood of a SLE patient and a healthy subject. **b** Average parameters of the dynamic turbidimetry in SLE normalized by the control (taken as 100 %)

3 Results and Discussion

Results of the turbidimetric clotting assays are presented in Fig. 1. The lag time was prolonged, the polymerization rate was slower, and the maximum optical density was higher in the SLE samples compared to the control. It is noteworthy that the average concentrations of thrombin-clottable fibrinogen in the plasma of SLE patients were higher than in control (4.2 ± 0.9 g/l versus 2.6 ± 0.2 g/l, respectively, $P < 0.05$), so the slower clotting in the SLE plasma samples is due to the decelerated thrombin generation, not a reduced fibrinogen level. The results suggest that the kinetics of fibrin formation and the final clot structure in SLE are different from normal and that these distinctions may exaggerate the pro-thrombotic state observed in SLE.

Because the maximum light absorbance of fibrin clots is affected by both protein density and the network structure, these effects could be distinguished using scanning electron microscopy. The fibrin network in the SLE samples had larger pores and was built of thicker fibers compared to the less porous structures with thinner fibers observed in the control samples (Fig. 2). If the difference in structure was determined by fibrin(ogen) concentration, then the SLE clot would be denser than control, which is not the case. Therefore, the

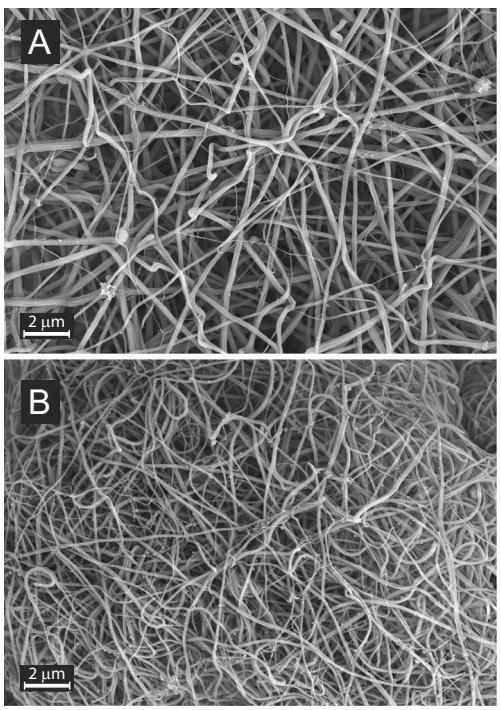

Fig. 2 Representative scanning electron micrographs of fibrin clots formed by recalcification of plasma samples obtained from the blood of a SLE patient (**a**) and a healthy subject (**b**). The fibers in the SLE samples are thicker and the network is more porous, while fibrin structure in the control sample is more compact with smaller intrinsic pores. The images were taken in at least 10 randomly selected areas of each clot in three clots from the plasma of SLE patients and three clots from the plasma of healthy subjects

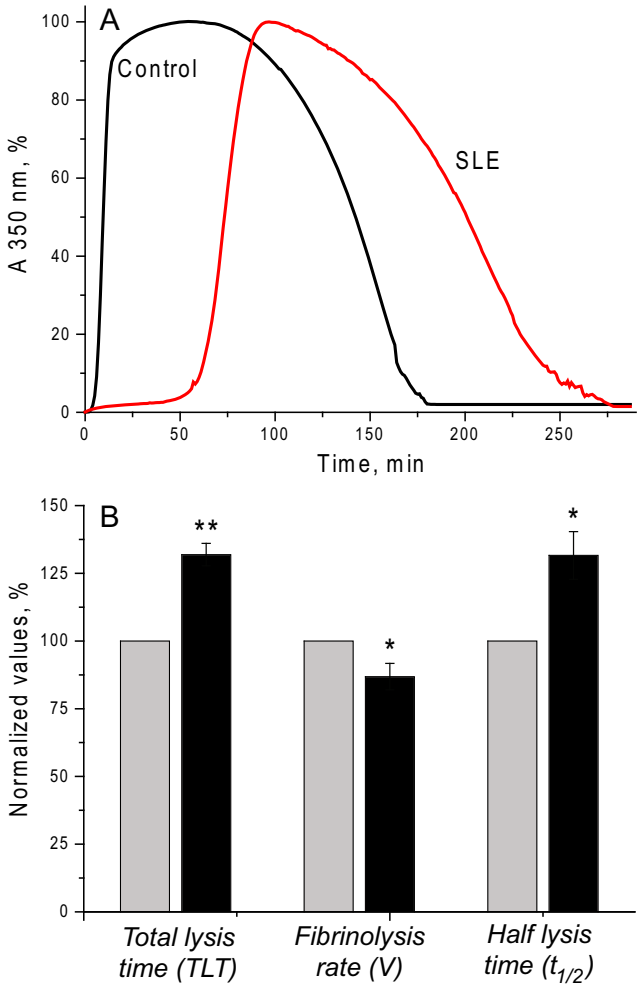

Fig. 3 **a** Representative lysis curves showing dissolution of fibrin clots formed in plasma samples obtained from the blood of a SLE patient (*red*) and a healthy subject (*black*). **b** Comparative parameters of fibrinolysis in the SLE samples normalized by control samples (taken as 100 %). Clots were formed by recalcification of platelet poor plasma in the presence of exogenous t-PA added before the clotting was initiated

distinct structure of the SLE and control clots reflects the much slower kinetics of fibrin polymerization that results in formation of thicker fibers. The ultrastructure of the clots is in agreement with the finding that a lower turbidity is correlated with thinner fibrin fibers [4], while a higher turbidity reflects formation of thicker fibers as observed in the clots made from the SLE plasma samples. Because the clots were formed using recalcification that is followed by activation of the entire blood clotting cascade, the observed alterations in fibrin formation and structure could be attributed to pathological changes of many SLE-related components of plasma, including cellular microparticles previously shown to affect fibrin structure [5].

The relative sensitivity of plasma clots to fibrinolysis is shown in Fig. 3. There was a significant increase in total lysis time (*TLT*) and a decrease in the rate of fibrin lysis (*V*) in the SLE samples compared to controls. This is not consistent with the commonly accepted notion that less compact fibrin clots containing thicker fibers are more susceptible to lysis [6], which means that some factors other than the structure of fibrin clot contribute to the prolonged lysis of the SLE clots. It is possible that the higher fibrin(ogen) amounts revealed in the SLE plasma result in a larger protein mass that physically needs more time to get lysed and therefore can underlie the delayed lysis kinetics. It is also likely that misbalances between pro- and anti-fibrinolytic factors, such as a decrease of plasminogen and plasminogen activator levels and/or an increase of α2-antiplasmin or PAI-1 levels, may explain reduced lysis in the SLE clots. Binding of autoantibodies to fibrin, especially at or near the sites of fibrin cleavage by plasmin, could also interfere with fibrinolysis.

4 Conclusions

Fibrin clots from the blood of SLE patients are different from normal clots in a number of important features that include slower formation, thicker fibers, and increased resistance to fibrinolysis. Irrespective of the underlying mechanism, fibrin clots formed in the blood of SLE patients have a pro-thrombotic phenotype that can contribute to thrombotic complications in SLE.

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