Effect of Fibrinogen on Functional Activity of Blood Leukocytes

B. A. Zhambalova, O. A. Azizova, and Yu. M. Lopukhin

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 133, No. 5, pp. 519-521, May, 2002
Original article submitted January 21, 2002

Fibrinogen intensified luminol-dependent chemiluminescence of blood leukocytes stimulated with opsonized zymosan. It is hypothesized that fibrinogen stimulates and prolongs functional activity of leukocytes during inflammation.

Key Words: *leukocytes; fibrinogen; luminol-dependent chemiluminescence*

Fibrinogen (FG) is a globular plasma glycoprotein. It modulates blood viscosity and thrombocyte aggregation and participates in blood clotting. FG is an acute phase protein and its concentration considerably increases during inflammation. High plasma content of FG is an independent risk factor of complications during inflammation [5,9].

Leukocyte activation plays a central role in inflammatory reactions. It is accompanied by respiratory burst characterized by activation of NADPH-oxidase enzyme complex, enhanced glucose consumption and activation of the hexosomonophosphate shunt, enhanced oxygen consumption, intensive production of reactive oxygen species (ROS), and activation of free radical processes in the organism [1].

It was demonstrated that blood proteins modulate leukocyte function. FG specifically binds leukocytes via α-subunits of CD11b/CD18 [6,7,10], which points to functional interrelations between FG and blood leukocytes. However, processes triggered by FG binding to leukocytes are little studied. An important and poorly studied effect is activation of ROS production in leukocytes after FG binding. Here we studied the effect of FG on luminol-dependent chemiluminescense (LCL) of leukocytes.

Laboratory of Biophysical Basis of Pathology, Institute of Physicochemical Medicine, Ministry of Health of the Russian Federation, Moscow. ***Address for correspondence:*** arsbaya@chat.ru. Zhambalova B. A.

MATERIALS AND METHODS

Human plasma FG (Sigma) was used in the study. Leukocytes were isolated from venous blood [3]. Functional activity of leukocytes stimulated with opsonized zymosan was evaluated by the intensity of LCL [1]. The cells (2×10^6/ml) were preincubated with FG at room temperature, 50 μl 10 mM luminol and 50 μl 5 mM $CaCl_2$ were added, and the sample was adjusted to 1 ml with phosphate buffer (pH 7.4). After recording spontaneous LCL for 3 min, opsonized zymosan (50 μl, 2 mg/ml) was added and stimulated LCL was recorded. All measurements were performed at 37°C and constant stirring.

In series I, the cells were incubated with 300 μg/ml FG for 15-70 min; in series II leukocytes were incubated with FG for 30 min, while FG concentrations varied from 150 to 900 μg/ml.

Functional activity of leukocytes was expressed in relative units (rel. units.) reflecting the difference between maximum stimulated and spontaneous LCL.

RESULTS

FG increased LCL intensity in leukocytes and maximum LCL was observed after 30-min preincubation (Fig. 1, *a*). Prolongation of incubation reduced LCL intensity and the effect of FG was less pronounced.

Analysis of concentration-effect relationships showed maximum LCL intensity after incubation with 600 μg/ml FG (Fig. 1, *b*). In healthy donors, normal

0007-4888/02/1335-0448$27.00

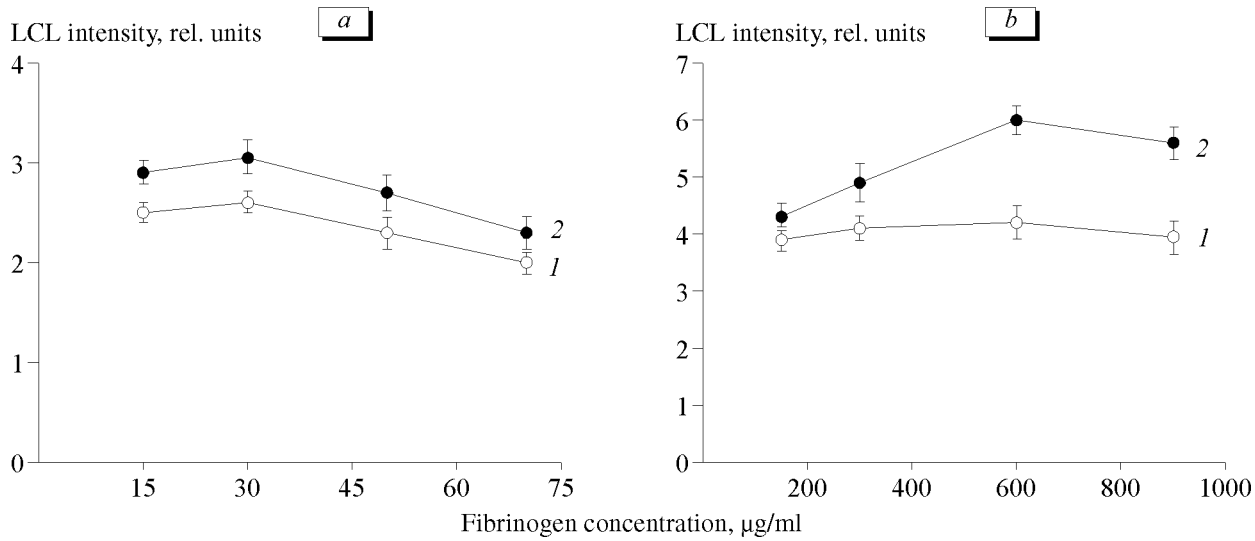

Fig. 1. Intensity of luminol dependent chemiluminescence of blood leukocytes stimulated with opsonized zymosan as a functions of time of preincubation with fibrinogen (*a*) and fibrinogen concentration (30-min incubation, *b*). *1*) without fibrinogen, *2*) with fibrinogen.

plasma content of FG is 1.5-3 g/liter, while the total leukocyte count varies from 4.3 to 10.8×10^9/liter. In our experiments, the ratio between FG and leukocytes was 3-fold lower. Our experiments showed that FG stimulated production of ROS in zymosan-activated leukocytes. It should be noted that we measured luminescence accompanying ROS production by neutrophils [1]. Leukocyte activation can be triggered by various factors. Leukocytes, in particular mature neutrophils, migrating from blood vessels to the inflammatory focus are stimulated by inflammatory and other factors modulating their functions (adhesion, chemotaxis, respiratory burst, absorption, degranulation, bactericidal and cytotoxic activities). The neutrophil response depends on the type and concentration of the factor. This reaction is modulated quantitatively and qualitatively by factors formed in the inflammatory focus and released into the plasma [2,8], in particular, by FG present in the circulation in high concentrations. Moreover, neutrophils express surface receptor for FG [6,7,10]. Therefore, it can be assumed that FG specifically binds to neutrophils, modulates their functional activity, and stimulates ROS generation. In turn, ROS produced in mature neutrophils during respiratory burst determine their bactericidal activity and cytotoxicity [4]. Thus, FG can modulate neutrophil response and enhance their bactericidal and cytotoxic activity. Similar data were reported by C. Rubel, *et al.* [10]. These authors showed that incubation of isolated neutrophils with FG induced rapid increase in intracellular calcium concentration, stimulated phagocytosis, and increased cell cytotoxicity. Hence, FG affects neutrophil functions and increases their activity during inflammatory process.

The study was supported by the Russian Foundation for Basic Research (grant No. 99-04-48255).

REFERENCES

1. Yu. A. Vladimirov and M. P. Sherstnev, *Advances in Science and Technology. Ser. Biophysics*, **24** (1989).
2. *Inflammation*, Eds. V. V. Serov and V. S. Paukov [in Russian], Moscow (1995).
3. A. Kh. Kogan, N. I. Losev, and A. P. Pogromov, *Theoretical and Methodical Basis of Biochemiluminescence* [in Russian], Moscow (1986), pp. 103-107.
4. *Manual on Immunopharmacology*, Eds. M. M. Dale and J. K. Formen, Moscow (1998).
5. J. A. Berliner, M. Navab, A. M. Fogelman, *et al., Circulation*, **91**, 2488-2496 (1995).
6. P. J. Gough, D. R. Greaves, H. Sudzuki, *et al.*, *Arterioscler. Thromb. Vasc. Biol.*, **19**, 461-471 (1999).
7. V. Labrador, S. Legrand, S. Muller, *et al.*, *J. Mal. Vasc.*, **25**, No. 1, 47-52 (2000).
8. G. Muns, I. Rubinstein, and K. C. Bergmann, *Scand. J. Infect. Dis.*, **27**, No. 4, 369-373 (1995).
9. P. Patel, D. Carington, D. P. Strachan, *et al.*, *Lancet*, **343**, 1634-1635 (1994).
10. C. Rubel, G. C. Fernandez, G. Dran, *et al.*, *J. Immunol.*, **166**, No. 3, 2002-2010 (2001).