EFFECT OF THROMBIN ON MACROPHAGE AND LYMPHOCYTE FUNCTION

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UDC 612.112.94.95/017.1-06:
612.115.12]-08(049.32)

KEY WORDS: thrombin; macrophages; phagocytosis; lymphocytes; blast transformation.

Recent investigations have shown that immunologic reactions are accompanied by changes in the hemostasis system and take place against the background of an increased concentration of active blood clotting factors [2]. The latter may be the connecting elements of the two systems. For instance, the writers showed previously that thrombin can potentiate the procoagulant and fibrinolytic activity of macrophages and lymphocytes, by inducing secretion of thromboplastin and fibrinolytic agents [1, 3]. A reaction of this kind must inevitably be accompanied by changes in functional activity of immunocompetent cells.

The aim of this investigation was to study mitotic properties of thrombin in the lymphocyte transformation test (LTT) and also to examine whether thrombin can affect the phagocytic activity of macrophages and the rate of their migration.

EXPERIMENTAL METHOD

Human thrombin, additionally purified by gel filtration on Sephadex G-100, was used in a concentration not inducing a cytolytic effect (1 U/ml). Thrombin inactivated by diisopropylfluorophosphate (DFP-thrombin), which has no clotting activity, was used in the control.

Experiments were carried out on rat peritoneal macrophages [5] and human lymphocytes. The isolated macrophages were suspended in medium 199 at the rate of 10^6 to 1 ml. Thrombin was added to the experimental flasks with macrophages in a dose of 1 U/ml, and an equal volume of physiological saline was added to the control. After incubation for 1 h, 0.1 ml of a 24-h staphylococcal suspension (10^9 cocci/ml) was added to the medium with macrophages, and 30 min later films were prepared by the Romanovsky–Giemes method. The number of phagocytosed microorganisms was counted in 50 phagocytes.

The macrophage migration rate was studied by the method in [4], observing aseptic precautions and using sterile material.

The LTT was studied under cell culture conditions. Lymphocytes ($2.5 \cdot 10^6$/ml) were incubated in medium 199 with homologous serum (10%) and antibiotics (100 U/ml each of penicillin and streptomycin). Lymphocytes

TABLE 1. Effect of Thrombin on Protective Function of Macrophages (n = 10)

Test agent	Ingestive activity of macrophages (number of cocci per macrophage)	Migration capacity (number of phagocytes migrating into incubation med. $\cdot 10^3$)
Thrombin	11,2±0,7	46±8,3
P_1	0,001	0,05
P_2	0,05	0,05
DFP-thrombin	8,8±0,7	95±18
P_1	0,001	0,05
Physiological saline	5,6±0,3	143±10

Legend. Here and in Table 2: P_1) significance of differences compared with physiological saline, P_2) compared with DFP-thrombin.

Department of Normal Physiology, Chita Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR N. V. Vasil'ev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 99, No. 5, pp. 597-598, May, 1985. Original article submitted January 13, 1984.

TABLE 2. Effect of Thrombin on Lymphocyte Transformation (n = 10)

Test agent	Number of cells, %				
	lymphocytes	intermediate cells	blast cells	cells in state of mitosis	total of intermediate and blast cells
Thrombin	84,6±3,3	7,6±1,3	6,5±1,5	0,4±0,2	14,1±1,0
P_1	0,05	0,1	0,1	0,02	0,01
P_2	0,1	—	0,01	—	0,05
DFP-thrombin	92±2,3	6,0±2,0	1,6±0,5	0,4±0,2	7,6±2,2
P_1	—	—	0,1	0,02	—
PHA	57,8±8,3	19,5±2,0	22,3±5	0,8±0,4	41,9±6,4
P_1	0,001	0,001	0,001	—	0,001
P_3	0,001	0,001	0,02	—	0,001
PHA and thrombin	40,6±4,7	21,3±1,3	34,9±5	0,4±0,2	56,1±4,0
P_1	0,001	0,001	0,001	0,02	0,001
P_4	0,1	—	0,1	—	>0,05
PHA and DFP-thrombin	50,6±7,6	22,8±2,6	25,5±3	0,6±0,2	48,3±2,1
P_1	0,001	0,001	0,001	0,2	0,001
P_5	—	—	—	—	—
Control with physiological saline	91,9±1,7	4,9±1,0	0,6±0,3	1,1±0,4	5,5±1,4

Legend. P_3) Significance of differences between action of PHA and thrombin, P_4) significance of differences in effects of PHA and of a combination of PHA with thrombin, P_5) significance of differences between effects of PHA and a combination of PHA with DFP-thrombin.

were stimulated with thrombin (1 U/liter) and phytohemagglutinin P (PHA; from Difco, USA) at the rate of 0.002 ml to 1 ml medium. In a separate series thrombin and PHA were added together. In the control DFP-thrombin was added (final concentration of DFP 10^{-2} M, incubation for 1 h at 20°C).

EXPERIMENTAL RESULTS

After incubation with thrombin the phagocytic activity of the macrophages was considerably increased (Table 1). Inactivated thrombin had a weaker action. The active enzyme also reduced macrophage migration by more than two-thirds. This reaction, in our view, is adaptive in character and helps to maintain a high concentration of macrophages in a focus of injury.

The study of lymphocyte transformation showed that in the presence of thrombin the number of blast cells was more than 10 times higher than in the control. The total number of intermediate forms also increased (Table 2).

The use of the nonspecific lymphocyte stimulator PHA led to an increase in the number of intermediate cells and blast forms in the culture. The combined action of thrombin and PHA led to a tendency for the mitogenic effect to be enhanced.

The results indicate that thrombin is not only an enzyme of the hemostatis system, but it also activates the specific function of immunocompetent cells. The inducing action of thrombin on lymphocytes and macrophages is biologically worthwhile, for whatever injury arises bleeding must be stopped and any infection introduced must be dealt with.

The mechanism of action of thrombin is a particularly interesting question. Since thrombin is a biologically active compound, it must exert its effect on cells through a receptor apparatus. Appropriate receptors have been found on monocytes [6], fibroblasts, and various other cells [1, 3]. There is no doubt that macrophages and lymphocytes have similar structures on their surface. Interaction of thrombin with membrane receptors is a trigger mechanism leading ultimately to changes in the metabolism, morphology, and function of immunocompetent cells.

Considering data obtained previously [1, 3], it can be tentatively suggested that thrombin exerts its action on widely different cells, and that the reaction of lymphocytes and macrophages is simply a special case of its manifestation.

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