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Platelets inhibit in vitro response of lymphocytes to mitogens

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ABSTRACT

Recent studies proposed that besides their role in thrombosis, platelets are involved in modulation of immune response of organisms to foreign bodies through platelet–leukocyte cross-talks at different levels. In the present study, we compared the response of T and B lymphocytes to mitogens in the presence or absence of platelets in cell cultures. Proliferation of T cells in response to lower concentrations of anti-CD3 or ConA stimulation as well as IL2 production of ConA-induced T blasts were inhibited by platelets. Similarly, proliferation and IL6 production of B blasts stimulated with low dose lipopolysaccharide (LPS) or CpG oligodeoxynucleotide 1826 were also dramatically inhibited by platelets. Over-expression of early activation marker CD69 induced by mitogens was blocked by platelets in both T blasts and B blasts. Platelets in culture also blocked production of IgM and IgE in B cells that were induced by anti-CD40/IL4 or LPS/IL4 treatments. These observations provided new evidence for the theory that platelets play more complicated roles in immune compartments. More efforts should be made to address the issue whether such platelet–lymphocyte interactions have any physiological significance in human and animals.

1. Introduction

Platelets are minute anucleated cells that exist in blood circulation system in large number (average $1200 \times 10^6\,mL^{-1}$ in mouse blood, $150–450 \times 10^6\,mL^{-1}$ in human blood). Though originally assigned the main role in blood clotting or prevention of bleeding, they also participate in other biological processes in the body. It has also long been realized that platelets are involved in inflammations caused by infection or metabolic disorder [1,2]. Platelets form aggregate with other cells in blood under physiological or pathological conditions. For example, activated platelets bind lymphocytes in blood circulation and mediate lymphocytes homing to high endothelial venules and entering lymphoid system [3,4]. With the verification of many immune-modulating molecules stored in platelets [5–7], the role of platelets in immune regulations has been well acknowledged in recent years, and scientists are beginning to look at platelets as immune cells [8,9]. Most of the studies proposed that platelets, mainly by breaking into pieces or releasing soluble molecules and microvesicles upon activation, enhance immune response to antigens [9–13]. All of T cells, B cells, natural killer cells and dendritic cells are possible targets of platelets immunomodulation [14–17]. These observations lead to the hypothesis that platelets might play more complicated role in homeostasis of immune compartments. We studied the effects of platelets on lymphocytes response to mitogens in an in vitro model and found that platelets manifested inhibitory effects on T or B cells functions.

2. Materials and methods

2.1. Mice and mitogens

C57Bl/6 mice were used for either splenocytes or platelets preparations. Mitogens concanavalin A (ConA) or anti-CD3 antibody were used for stimulating T lymphocytes, and bacterial lipolysaccharide (LPS) or CpG containing phosphorothioated oligodeoxynucleotide 1826 (CpG-1826, TCCATGACGTTCCTGACGTT) (Coley Pharmaceuticals, Wellesley, MA) for stimulating B lymphocytes.

2.2. Platelets preparation

Platelets were prepared from 8-month-old mice and suspended in Tyrode's buffer as described previously [13]. To obtain fixed platelets, freshly prepared unactivated platelets in Tyrode's buffer was mixed with equal volume of 4% neutral buffered formaldehyde for 10 min before washes with PBS for three times.

2.3. Lymphocytes proliferation

For preparation of splenic cells, spleens were removed from mice (6–8 weeks) and pressed through 70 μm strainer to obtain

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single cell suspensions and erythrocytes were lysed by using Tris–NH_4Cl method. After adhering to flask for 1 h at 37 °C, non-adherent cells were collected and seeded into 96 wells plates. Platelets preparations were added at 3×10^8 mL^{-1} in the final volume of 200 μL/well, reaching a density of platelets comparable in human blood. Different concentrations of mitogens were added at the same time. Forty hours later, 100 μL supernatant was harvested, and 1 μCi ^{3}H-TdR (Amersham Pharmacia Biotec, Piscataway, NJ) in 50 μL fresh medium was added to each well. The incubation continued for 8 more hours before the cells were harvested onto glass fiber filters. Incorporation of isotope was measured using scintillation counter (Wallac Oy, Turku, Finland).

2.4. Immunoglobulin production

B cells were separated by one round of anti-CD4 (clone GK1.5) and anti-CD8 (clone 2.43) plus complement treatment to delete T cells. The resulting B cell preparation contains 83.8% of B cells by FACS staining. B cells were cultured at 10^6 mL^{-1} with LPS (10 μg/mL) or anti-CD40 (1 μg/mL) in combination with IL4 (500 U/mL) for 6 days in the presence or absence of fixed platelets (3×10^8 mL^{-1}) in a total volume of 0.5 mL/well in 24 well plates. Medium was harvested at the end of culture for measurement of IgM and IgE concentration as below.

2.5. ELISA

IL2, IL6 and IgE concentrations in the conditioned medium were measured with OptEIA sets (BD Pharmingen, SanDiego, CA) and that of IgM with IMMUNO-TEK IgM ELISA Kit (ZeptoMetrix, NY) following the protocols recommended by the suppliers. Absolute concentrations were obtained by running standard curves at the same ELISA plates.

2.6. FACS

Cultured cells were harvested and stained with FITC labeled anti-CD4 (BD Pharmingen, San Diego, CA) and anti-CD8 for T blasts or anti-B220 for B blasts in combination with PE labeled anti-CD69. In some experiments, the cocultured cells were harvested and stained directly with 7-AAD Viability Staining Solution (eBioscience, San Diego, CA) for dead cells. All samples were assayed on FACSCalibur using CellQuest program (Becton Dickson Immunocytometry System, San Jose, CA) and data were analyzed using FlowJo software (Tree Star, Inc., San Jose, CA).

3. Results

3.1. Modulation of lymphocytes proliferation by platelets

In appropriate concentrations range, such as between 0.032 and 4 μg/mL, all of the mitogens were found to stimulate splenocytes proliferation efficiently in a dose-dependent manner, and this proliferation was significantly inhibited by freshly prepared resting platelets added to these blast cultures (Fig. 1). At higher concentrations of some mitogens (e.g. 20 μg/mL), effects of both mitogens and platelets were inconsistent and controversial to each other. For example, 20 μg/mL LPS induced profound B lymphocyte proliferation and platelets significantly inhibited this proliferation. But 20 μg/mL CpG ODN induced weaker proliferation of B cells than 0.8 μg/mL CpG ODN, and platelets increased the effect of 20 μg/mL of CpG ODN. Similar two phased effects of mitogens and platelets were observed with T lymphocytes when ConA or anti-CD3 was used (Fig. 1A and B). After overnight culture of the splenocytes, about one-third of the splenocytes in the control medium group were dead (Fig. 2G), and LPS (20 μg/mL) or low concentration ConA (1 μg/mL) decreased cell death to some extent (Fig. 2). But high concentration ConA (20 μg/mL) significantly promoted cells death

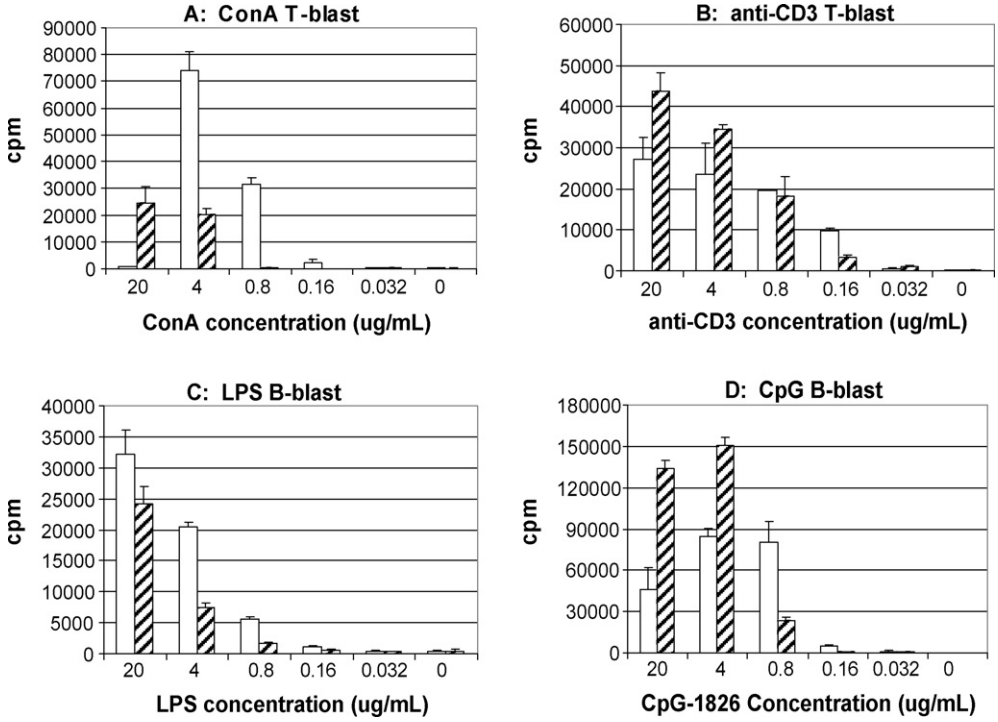

Fig. 1. Platelets inhibit mitogenic effects of ConA (A), anti-CD3 antibody (B), LPS (C) and CpG ODN (D) on splenic cell culture. Blank bars: control culture without platelets; Slashed bars: cultures with platelets. Lymphocytes were seeded into 96 wells plates at density of 5×10^5/well (A and B) or 3×10^5/well (C and D). The proliferation experiments were repeated three (for B-blasts) to four (for T-blasts) times with similar results and representative ones were shown.