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Abstract

The biological specificity of the endotoxin receptor on platelet membranes was examined. The binding indices of platelets in experimental endotoxemia which was induced by intravenous administration of endotoxin (Lipopolysaccharide of E. coli, Difco) to rabbits were found to be 30% of the control at 60 min after the injection. The result suggests that the endotoxin receptor of platelets was already occupied. The binding indices of human platelets were measured after pretreatment with pharmacologically active substances which were assumed to effect platelet activity. The binding of LPS to platelets showed competitive inhibition at pharmacologically effective doses, but other substances merely inhibited platelet activity. One interpretation is that there is a common receptor on platelet cell membranes for lipopolysaccharide of E. coli and endotoxin. The sensitivity to endotoxin in vivo and binding indices of platelets were examined in rabbits and guinea pigs since their response to endotoxin is almost opposite with regard to sensitivity. The binding indices of platelets from rabbits and guinea pigs showed a positive correlation with the endotoxin sensitivity. Those findings indicate that platelets play a key role in vivo in the clinical course of endotoxemia.

KEYWORDS: endotoxin receptor, platelet

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ENDOTOXIN RECEPTOR SITE II. SPECIFICITY OF ENDOTOXIN RECEPTOR OF PLATELETS AND SENSITIVITY TO ENDOTOXIN *IN VIVO*

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Abstract. The biological specificity of the endotoxin receptor on platelet membranes was examined. The binding indices of platelets in experimental endotoxemia which was induced by intravenous administration of endotoxin (Lipopolysaccharide of E. coli, Difco) to rabbits were found to be 30 percent of the control at 60 min after the injection. The result suggests that the endotoxin receptor of platelets was already occupied. The binding indices of human platelets were measured after pretreatment with pharmacologically active substances which were assumed to effect platelet activity. The binding of LPS to platelets showed competitive inhibition at pharmacologically effective doses, but other substances merely inhibited platelet activity. One interpretation is that there is a common receptor on platelet cell membranes for lipopolysaccharide of E. coli and endotoxin. The sensitivity to endotoxin *in vivo* and binding indices of platelets were examined in rabbits and guinea pigs since their response to endotoxin is almost opposite with regard to sensitivity. The binding indices of platelets from rabbits and guinea pigs showed a positive correlation with the endotoxin sensitivity. Those findings indicate that platelets play a key role *in vivo* in the clinical course of endotoxemia.

Key words: endotoxin receptor, platelet

It seems clear that endotoxin binds to platelets preferentially and that it binds by direct adhesion to the endotoxin receptor on the platelet membrane *in vitro* (1). In this report, the specificity and the sensitivity of the endotoxin receptor of platelets was examined both *in vivo* and *in vitro*. Platelets release 5-hydroxytryptamine when the endotoxin binds on the surface of platelet and also release some other substances which have general physiological effects.

The chemical substances which effect the physiological and morphological functions of platelets include adenosine-diphosphate (ADP) (2), epinephrine (3) thrombin (4), chondroitin sulfate (5), arachidonic acid (6), steroid hormone (7), endotoxin (8), ethylenediamine tetracetic acid (EDTA) (9), acetylsalicylic acid (10), concanavalin A (11), carrageenan (12). It is assumed that the effects of

these chemicals are expressed after binding to the endotoxin receptor.

Laboratory animal species differ widely in their sensitivity to endotoxin (13, 14). The author assumed that these differences are due to qualitative and quantitative differences in their respective endotoxin receptors. In this study the binding indices (15) of platelets from rabbits and guinea pigs are compared.

MATERIALS AND METHODS

The diffusion dialysis procedure and the preparation of platelets from humans, rabbits and guinea pigs were carried out as previously described (1).

Induction of experimental endotoxemia. Endotoxin (Lipopolysaccharide of E. coli 0111 B_4 (B) Difco, 2mg/kg) was administered intravenously to mature rabbits (body weight 2.7-3.0kg).

Citrated blood was drawn at 0, 15, 30, 60 and 90min after the administration of LPS. The endotoxin concentration in the blood was assayed by radioimmunoassay (RIA) (16). After the separation of the platelets, the binding of ^{125}I-LPS was measured at 4°C and expressed as the binding index (%d) (15).

Pretreatment of platelets with various reagents. Two ml of a 10^6/ml platelets suspension and 2ml of reagent solution in saline containing 15mM sodium azide (abbreviated NaN_3 saline) were mixed together and spun at 12rpm for 30min at 4°C. After centrifugation at 3000rpm (1600g) for 10min, the pellet was mixed with 2ml of NaN_3 saline to make a suspension of pretreated platelets.

Measurements of binding indices and the fractionation of blood cells were performed aseptically at 4°C, only superfine grade reagents were used.

RESULTS

Changes of binding indices and platelet numbers in experimental endotoxemia. The binding index of platelets in experimental endotoxemia fell to 30 percent of the control value, after 60min as shown in Fig. 1. It is reasonable to assume that the administration of LPS in blood facilitates rapid adsorption to platelets. The platelet number in peripheral blood was measured at the same time. The platelet numbers were found to be 51.4×10^4/mm^3 at 0min, 14.6×10^4/mm^3 at 30min and 19.6×10^4/mm^3 at 60min as shown in Fig. 1. At 30min after the injection, platelet numbers reached their lowest level and then began to recover. Since the endotoxin could still be detected in sera by radioimmunoassay (16), it was presumed that the platelets adsorbed endotoxin *in vivo*.

The effect of pharmacologically active substances on the binding indices of human platelets. Various pharmacologically active substances have been reported to effect platelets *in vivo*. The effects of these substances on the binding indices of endotoxin were examined.

The effect of LPS on the binding indices (%d) of platelet suspensions of 10^6 and 10^7 cells/ml was clear at a concentration of 10^{-5}mg/ml and 10^{-3}mg/ml as shown in Fig. 2. Other active substances and their concentrations were as

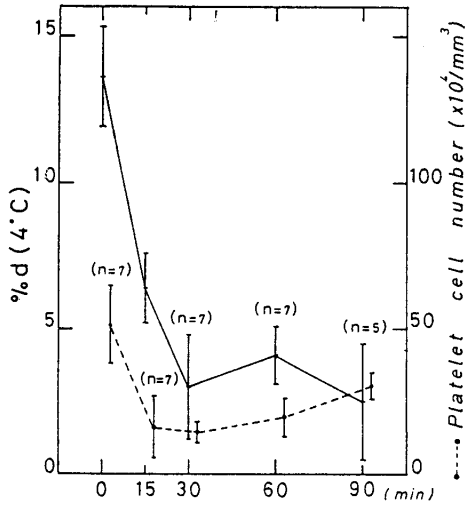

Fig. 1. The relationship of the binding indices (%d 4°C) and platelet number *in vivo* in experimental endotoxemia of rabbits. The experimental endotoxemia was induced by intravenous injection of LPS (2mg/kg) into rabbits (2.7-3.0 kg). The binding indices of platelets after the injection were 13.6±1.7 (n=7, n represents experimental numbers.) at 0 min, 6.4±1.2 (n=7) at 15 min, 3.0±1.8 (n=7) at 30 min, 4.1±1.0 (n=7) at 60 min and 2.5±2.0 (n=5) at 90 min after the administration. Platelet numbers were also measured and they were 51.4±13.6×10^4/mm^3 (n=7) at 0 min, 16.5±10.7×10^4/mm^3 (n=7) at 15 min, 14.6±3.6×10^4/mm^3 (n=7) at 30 min, 19.6±6.7×10^4/mm^3 (n=7) at 60 min and 30.2±4.5×10^4/mm^3 (n=5) at 90 min after the administration. The change of the binding indices correspondes with that of platelets cell numbers. —, %d; - - -, platelet number

follows; carrageenan, at 10^{-4} mg/ml; chondroitin sulfate, concanavalin A and EDTA, at 10^{-3} mg/ml.

It is clear that LPS was the only substance that affected the endotoxin receptor of platelets at a very low concentration. Acetylsalicylic acid, hydrocortisone, epinephrine and arachidonic acid had some effects on the endotoxin receptor of platelets as shown in Fig. 3. Thrombin had some effects at a concentration of 10^{-3} NIH unit/ml as shown in Fig. 4.

Comparative studies of the binding indices (%d) of rabbit and guinea pig platelets at different ages. The L. D.$_{50}$ of endotoxin for newborn rabbits is reported to be 5000 μg/kg, however, it changes with age. L.D.$_{50}$ in adult rabbits is 50 μg/kg, indicating that sensitivity increase almost 100 fold with age. L.D.$_{50}$ in newborn guinea pigs is 50–75 μg kg and that of the adult is 350–500 μg/kg, which indicates that the pattern of the sensitivity with age is the reverse of that of rabbits. The sensitivity is lowered 5–10 fold in the case of guinea pigs (13, 14). The binding indices of platelets from these two different species at different ages were measured at 4°C. A close relationship was found between the sensitivity to endo-

toxin and binding indices in rabbits, while in guinea pigs no clear relationship was evident as shown in Fig. 5.

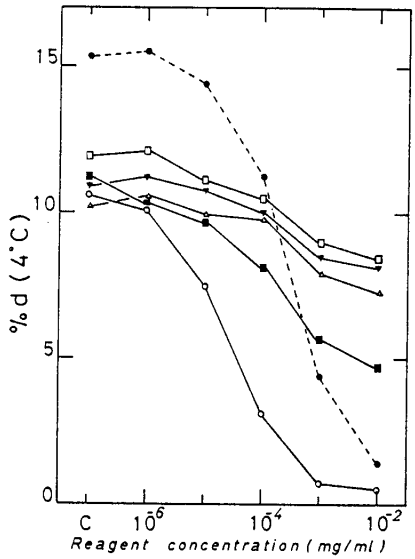

Fig. 2

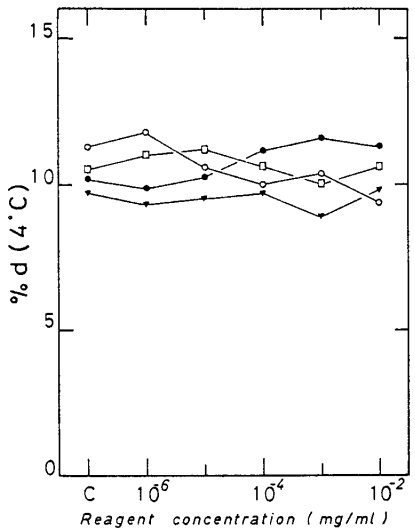

Fig. 3

Fig. 2. The effects of chemicals which are similar to LPS on the binding indices at various concentrations. LPS seems to be the only substance that affects the endotoxin receptor of platelets. (Solid line, 10^6 cells/ml; dotted line, 10^7 cells/ml of platelet number).
○——○>, LPS; □——□, EDTA; ▽——▽, Concanaralin A;
△——△, Chondroitin sulfate; ■——■, Carrageenan

Fig. 3. The effect of some drugs on the binding indices (%d 4°C) of platelets. These drugs had no effect on the binding indices of platelets regardless the consentrations (n=7).
●——●, Acetylsalicylic acid; □——□, Hydrocortisone; ▼——▼, Epinephrine;
○——○, Arachidonic acid

DISCUSSION

This report and the previous report (1) have made clear that endotoxin binds directly to a large number of platelet membranes *in vivo*. The platelets then release biologically active substances and cause aggregation by adhering to endotoxin. The binding of ^{125}I-LPS to platelets in experimental endotoxemia was shown to be almost coincidental with the disapperance of platelets in circulating blood. The decrease of the binding index was caused by the adhesion to endotoxin receptors of platelets. The binding of endotoxin to platelets continues until they are destroyed by the reticuloendothelial system (RES) (17). This process may be the first defense mechanism of the host against endotoxin (18, 19).

Since platelets are easily affected by various substances, the effect of those

substances on the endotoxin receptor were studied by the pretreatment of platelets with them at 4°C for 30 min. LPS affected the binding indices (%d) at concentrations of 10^{-5} mg/10^6 cells and 10^7 cells/ml. EDTA, concanavalin A or chondroitin sulfate affected the binding index at 10^{-3} mg/10^6 cells/ml. Carrageenan worked at 10^{-4} mg/10^6 cells/ml, and has been shown to have endotoxin activity (20). However, acetylsalicylic acid, hydrocortisone, epinephrine or arachidonic acid did not have any significant effect. Thrombin may not affect the endotoxin receptor of platelets. Therefore it seems that LPS is the only substance that affects the endotoxin receptor of platelets.

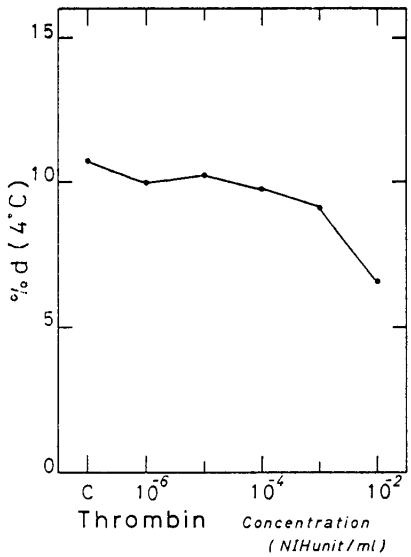

Fig. 4

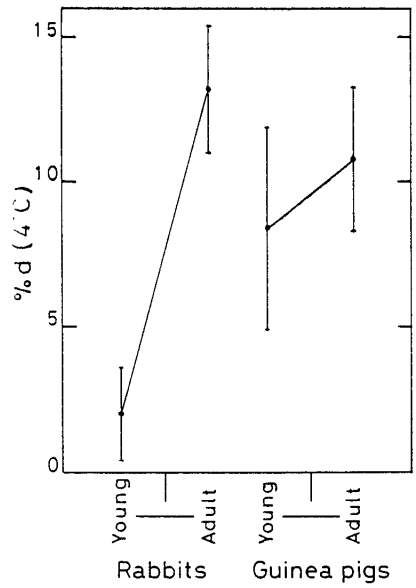

Fig. 5

Fig. 4. The effect of thrombin on the binding indices (%d 4°C) of platelets. The binding indices were 10.7 (control), 10.3 at 10^{-5} NIH unit/ml and 9.1 at 10^{-3} NIH unit/ml (n=7). Thrombin did not effect the binding indices at low concentrations.

Fig. 5. The binding indices (%d 4°C) of platelets in rabbits and guinea pigs at different ages. The binding indices of rabbit platelets were 2.0±1.6 in the second month after birth (body weight 0.85-1.0 kg) and 13.2±2.2 in over the sixth month (2.7-3.0 kg). While, those of guinia pig platelets were 8.4±3.5 in the first month after birth and 10.8±2.5 in the fifth month (n=9).

The relationship between *in vivo* sensitivity to endotoxin and the binding affinity of platelets was examined in rabbits and guinea pigs. Rabbits and guinea pigs were assumed to be opposite in the manner of response with age (13, 14).

The binding index of rabbit platelets was 2.0 in the second month after birth and 13.2 in more than the six month. The binding index of guinea pig platelets

was 8.4 in the first month after birth and 10.8 in the fifth month. It is clear that there is a close relationship between the sensitivity to endotoxin and the binding indices.

The sensitivity of rabbits is reported to be a cross allergic reaction with spontaneous sensitization by intestinal bacterial flora such as E. coli (21). In the case of guinea pigs, however, the sensitivity to endotoxin was found at a very young age when the immune system is still immature, suggesting that the bacterial cross allergic reaction is not the only reason for endotoxin sensitivity. The biological activities of endotoxin are expressed on the cell function of the reticuloendothelial and vascular systems. Particularly, the difference in sensitivity between species is due to the different responsibility of the vascular system and the difference in capacity for vasoactive substance accumulation (22, 23).

Generally, LPS adhere to membranes by lipid-lipid interaction (24). So, there still remain a possibility that low grade binding indices of immature rabbit platelets may indicate a qualitative or quantitative difference of lipids in platelet membranes. The binding of endotoxin to platelets is the initial reaction to endotoxemia to clear circulating free endotoxin (18, 19) and then clinical manifestation follows if there remains an excess of endotoxin in the blood stream (25, 26).

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