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Pulmonary platelet accumulation induced by catecholamines: Its involvement in lipopolysaccharide-induced anaphylaxis-like shock

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ABSTRACT

Intravenously injected lipopolysaccharides (LPS) rapidly induce pulmonary platelet accumulation (PPA) and anaphylaxis-like shock (ALS) in mice. Macrophages reportedly release catecholamines rapidly upon stimulation with LPS. Here, we examined the involvement of macrophage-derived catecholamines in LPS-induced PPA and ALS. A catecholamine or *Klebsiella* O3 (KO3) LPS was intravenously injected into mice, with 5-hydroxytryptamine in the lung being measured as a platelet marker. The tested catecholamines induced PPA, leading to shock. Their minimum shock-inducing doses were at the nmol/kg level. The effects of epinephrine and norepinephrine were inhibited by prazosin (α1 antagonist) and by yohimbine (α2 antagonist), while dopamine's were inhibited only by prazosin. Use of synthetic adrenergic α1- and/or α2-agonists, platelet- or macrophage-depleted mice, a complement C5 inhibitor and C5-deficient mice revealed that (a) α2-receptor-mediated PPA and shock depend on both macrophages and complements, while α1-receptor-mediated PPA and shock depend on neither macrophages nor complements, (b) the PPA and ALS induced by KO3-LPS depend on α1- and α2-receptors, macrophages, and complements, and (c) KO3-LPS-induced PPA is preceded by catecholamines decreasing in serum. Together, these results suggest the following. (i) Catecholamines may stimulate macrophages and release complement C5 via α2-receptors. (ii) Macrophage-derived catecholamines may mediate LPS-induced PPA and ALS. (iii) Moderate PPA may serve as a defense mechanism to remove excess catecholamines from the circulation by promoting their rapid uptake, thus preventing excessive systemic effects. (iv) The present findings might provide an insight into possible future pharmacological strategies against such diseases as shock and acute respiratory distress syndrome.

1. Introduction

Activation of platelets leads to their aggregation within blood vessels and/or adhesion to vascular walls, and has been implicated in various types of pathogenesis [1], including coronary heart disease [2], peripheral arterial disease [3], cerebral infarction [4], Alzheimer disease [5], diabetic vascular disorders [6], tumor metastasis [7], allergic inflammation [8], anaphylaxis [9], and sepsis and/or acute respiratory distress syndrome (ARDS) [10].

Although the direct action of epinephrine on platelets in vitro is generally weak, it has pro-aggregatory effects on platelets [11,12]. Epinephrine and/or norepinephrine are released upon stimulation of adrenergic neurons and adrenal glands by stressful stimuli, and many studies have linked stress to platelet activation (e.g. [13]). Interestingly, recent studies have demonstrated that macrophages also produce and release catecholamines [14–16], with the maximal release of catecholamines occurring *within 15 min* after lipopolysaccharide (LPS) stimulation [14]. It has also been reported that adrenergic agonists stimulate

Abbreviations: ALS, anaphylaxis-like lethal shock; Clo-lip, clodronate-encapsulated liposomes; HPLC, high performance liquid chromatography; 5HT, 5-hydroxytryptamine; KO3, *Klebsiella* O3; LPS, lipopolysaccharide; PBS, phosphate-buffered saline; PPA, pulmonary platelet accumulation; RS, rapid shock.

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macrophages via α2-receptors and so enhance acute inflammatory injury [14]. However, little is known about the effects of adrenergic agonists on the in vivo behavior of platelets.

Interestingly, some types of LPS induce pulmonary platelet accumulation (PPA) *within a few minutes* after their intravenous (i.v.) injection and there is an ensuing degradation of the platelets that have accumulated in the lung, leading rapidly to anaphylaxis-like shock (ALS) [17,18]. These rapid PPA and ALS responses depend both on the structure of the LPS and the strain of mouse [17–20]. Intriguingly, such LPS-induced PPA and ALS responses occur in TLR4-mutant (TLR4-inactive) mice, too [21,22]. Although a complement-C5-dependent degradation of platelets within the lung has been suggested to be involved in LPS-induced ALS [19,20], the molecular mechanisms underlying LPS-induced rapid PPA and ALS remain unclear. PPA also occurs immediately after the delivery of a challenge with an antigen, ovalbumin (OVA), to OVA-sensitized mice, and such PPA is involved in the anaphylactic shock induced by OVA-antigen challenge [9]. In the present study, on mice, we examined the in vivo effects of adrenergic agonists and antagonists on platelets as well as the possible involvement of adrenergic receptors in the rapid PPAs induced by *Klebsiella* O3 (KO3) LPS and by OVA-antigen challenge.

2. Materials and methods

2.1. Animals

Male BALB/c mice (7–9 weeks old) were bred in a mouse room in our laboratory. DBA/2 mice (complement C5-deficient mice) were obtained from SLC Japan (Shizuoka). The mice were kept under a light-dark (19:00–07:00) cycle at a controlled temperature (23 ± 1 °C), and they were allowed standard food pellets and water ad libitum. All studies involving animals were as humane as possible and are reported in accordance with the ARRIVE guidelines for reporting experiments involving animals [23,24]. In total, 726 mice (705 BALB/c; 21 DBA/2) were used. Mice were randomly selected in each experiment. The number of mice used in each experiment is indicated in the relevant figure and/or legend. All experiments complied with the relevant Japanese law (Law no. 105; Notification no. 84) and with the Regulations for Animal Experiments and Related Activities at Tohoku University.

2.2. Materials

Epinephrine [(−)-epinephrine (+)-bitartrate], norepinephrine [(−)-norepinephrine (+)-bitartrate], dopamine hydrochloride, (−)-phenylephrine hydrochloride, oxymetazoline hydrochloride, clonidine hydrochloride, (−)-isoproterenol hydrochloride, prazosin hydrochloride, and haloperidol were from Sigma (St. Louis, MO, USA), fluphenazine was from Wako (Osaka, Japan), and yohimbine was from Tokyo Kasei (Tokyo, Japan). All (except haloperidol) were dissolved in saline and i.v. injected via a tail vein. Haloperidol was dispersed in saline by sonication shortly before i.v. injection. K76, an inhibitor of complement C5 [25], was provided by Otsuka Pharmaceutical Co. Ltd. (Tokushima, Japan). This reagent was dissolved in saline with the addition of sufficient NaOH solution to bring the pH to 7.5, and then injected intraperitoneally (i.p.). Ovalbumin (from chicken; 5× crystallized) was purchased from Seikagaku Corp (Tokyo, Japan). Experiments were carried out at 24 ± 1 °C unless otherwise mentioned. LPS from *Klebsiella* O3 (KO3) strain LEN-1 (S type) [26], which was prepared by the phenol-water method, was dissolved in sterile saline, and then injected (i.v.). The injection of LPS was carried out at a room temperature of 26–28 °C because the rapid ALS induced by LPS is reduced at low room temperature.

2.3. Sampling of the blood

Since we aimed to analyze the very rapid in vivo platelet responses (occurring within a few seconds), as well as rapid changes in catecholamines, we collected the blood flowing from the neck blood vessels after instant decapitation of non-anesthetized mice. It should be noted that the blood obtained by decapitation is largely derived from the carotid arteries (i.e., it has just passed through the pulmonary circulation).

2.4. Platelet count and evaluation of platelet accumulation in tissues

Platelets in the blood were counted as described previously using a cell counter (Sysmex SF-3000; Toa Medical Electronics Co. Ltd., Kobe, Japan) [27]. The 5-hydroxytryptamine (5HT or serotonin) present in the blood and tissues (except intestines and brain) is mostly contained within platelets [28,29]. Armed with this finding, we earlier devised a method for studying the in vivo behaviors of platelets that utilized 5HT as a marker of platelets [9,20]. Our method provides information about *intact* platelets because they are subjected to no form of artificial treatment and it is known that anesthesia, anti-coagulants, and the way platelets are prepared can all affect platelet integrity [30,31]. The 5HT levels in the lung were determined as previously described [9]. Briefly, lungs were rapidly removed and kept in a jar containing dry ice until needed. 5HT was extracted from the lung using 0.4 M $HClO_4$ containing 0.1% *N*-acetylcysteine-HCl and 2 mM EDTA-2Na, and the extract was neutralized by means of 2 M KOH. Then, after the resulting supernatant had been subjected to column chromatography, 5HT was measured fluorometrically as previously described [29]. The amount of 5HT in blood or lung is expressed as nmol/g of blood or lung.

2.5. Platelet depletion and electron microscopy

Platelets were depleted by subcutaneous (s.c.) injection of Pm1 (rat anti-mouse platelet monoclonal antibody) [32,33]. Hybridoma cells producing Pm1 were kindly provided by Dr. T. Nagasawa (Division of Hematology, University of Tsukuba, Japan). The hybridoma cells were inoculated into nude BALB/c mice, and Pm1 (IgG fraction) was prepared from the ascites of each mouse by precipitation with ammonium sulfate, followed by dialysis of the precipitant. Control IgG was similarly prepared from rat serum. Electron microscopic analysis was performed as described previously [9,18].

2.6. Depletion and detection of macrophages

Clodronate-encapsulated liposomes (Clo-lip) have been shown to induce specific depletion of phagocytic macrophages, but not of dendritic cells or neutrophils [34]. A suspension of Clo-lip was prepared by a method similar to that used by [34], as described previously [33]. The diluted suspension of Clo-lip was i.v. injected into mice at 0.2 ml per mouse. Macrophages were detected by immunohistochemical staining with a F4/80 antibody (Serotec, Kidlington, UK), as described previously [33].

2.7. Measurement of epinephrine, norepinephrine, and dopamine in serum and lung

The blood collected by instant decapitation of non-anesthetized mice (see above) was kept on ice and subjected to centrifugation (3000 × *g*, 5 min, at 4 °C) within 20 min of its collection. The supernatant (plasma) was stored at −80 °C until use. The lung, which was removed rapidly after such blood collection, was also stored at −80 °C until use. Catecholamines in the plasma and lung were determined by high performance liquid chromatography, as described previously [35].

2.8. Scoring of the toxicity or shock signs

The severity of the toxicity or shock signs seen in each mouse was scored as follows: 0 (no abnormal signs), 1 (staggering and/or prostration), 2 (convulsion), 3 (death). A note was made of the maximum score