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Accumulation of platelets in the lung and liver and their degranulation following antigen-challenge in sensitized mice

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1 Mast cells and basophils are believed to trigger allergic reactions and anaphylaxis. They rapidly release histamine (H), a typical mediator of inflammation, in response to antigens. In the mouse, platelets contain much 5-hydroxytryptamine (5HT), an additional inflammatory mediator, while human platelets contain both H and 5HT. Here, we examined the response of platelets in sensitized mice to antigen challenge.

2 Platelets accumulated in the lung and liver almost immediately after intravenous injection of ovalbumin (OVA), in mice sensitized to it, and platelet degranulation occurred during these reactions.

3 These responses of platelets preceded H release from mast cells and/or basophils, occurred at doses of OVA lower than those inducing H release, and contributed to the signs of shock.

4 We reported previously that intravenous injection into mice of LPS (a membrane constituent of gram-negative bacteria) induces a similar platelet response (accumulation of platelets in the lung and liver) and shock.

5 Blood that has passed through the body (other than the digestive tract) passes first to the lungs before being recirculated by the heart, and blood that has passed through the digestive tract passes next to the liver. Thus, our findings suggest that in addition to their role in haemostasis, platelets, tiny anuclear cytoplasts, may be important in both innate and acquired immunity, and that the lung and liver may be the fronts at which platelets wage war on pathogens.
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Keywords: Platelets; anaphylaxis; allergy; 5-hydroxytryptamine (serotonin); histamine; lung; liver

Abbreviations: H, histamine; 5HT, 5-hydroxytryptamine; LPS, lipopolysaccharide; OVA, ovalbumin; PAF, platelet-activating factor; PRP, platelet-rich plasma

Introduction

Allergic reactions, which are induced by antigens in individuals sensitized to them, include anaphylaxis, a systemic allergic reaction occurring almost immediately after exposure to an antigen. Mast cells and basophils contain large amounts of histamine (H), a well-known shock-inducing substance. Antigen-mediated cross-linkage of the Fc receptors for IgE that are present on their cell membrane (FcεRI) triggers them to release a variety of mediators, including histamine, and it is recognized that this induces allergic reactions and anaphylaxis. However, anaphylaxis occurs even in mast cell-deficient mice (Jacoby *et al*., 1984; Ha *et al*., 1986; Martin *et al*., 1993; Choi *et al*., 1998). Although it used to be thought that basophils are very scarce or even non-existent in mice, recent studies have demonstrated that the number of FcεR-positive basophils in the circulation, spleen, and bone marrow can be increased by immunizing mice with foreign proteins (Uribina *et al*., 1981; Dvorak *et al*., 1993). However, their contribution to the development of anaphylaxis in mice has not been clarified, and anaphylaxis has actually been found to occur in mice deficient in IgE (Oettgen *et al*., 1994). Nevertheless, mast cells, basophils, and IgE have long been believed to play central roles in mediating allergic reactions and anaphylaxis in most mammals.

Platelets in the mouse contain much 5-hydroxytryptamine (5HT). Using 5HT as a marker, we previously found that lipopolysaccharide (LPS), a component of the outer cell membrane of gram-negative bacteria, induces in mice a rapid accumulation of platelets in the lung and liver, and that their destruction in these organs results in an anaphylaxis-like shock (Shibazaki *et al*., 1996; 1999; Endo *et al*., 1997). Although release of H cannot be detected, the signs of shock present in this reaction (such as the sluggish gait, dyspnea, prostration, and convulsions seen within 3–10 min of an injection of LPS) are very similar to those seen in IgE-dependent anaphylactic shock (Choi *et al*., 1998). Consequently, we decided to examine whether an accumulation of platelets like that seen in response to LPS might occur in response to the antigen ovalbumin (OVA) in mice.

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Methods

Animals and materials

Female BALB/c mice obtained from the animal facility of our university were used in most of the present experiments. WBB6F1 (B-W/+ × C57BL/6-W^v/+) − W/W^v mice (mast cell-deficient mice) were obtained from Japan SLC, Inc. (Shizuoka, Japan). All experiments were carried out at 27–28°C. The anti-platelet mouse monoclonal antibody, Pm1, was provided by Dr T. Nagasawa (Division of Hematology, University of Tsukuba, Japan) (Nagata *et al*., 1995). OVA (from chicken, five times crystallized) was purchased from Seikagku Corp (Tokyo, Japan). All experiments complied with the Guidelines for Care and Use of Laboratory Animals in Tohoku University.

Sensitization of mice to antigen

A suspension (0.5 ml) containing OVA (50 μg) and alum (3 mg) was injected intraperitoneally on day 0 and day 10. The first injection was given to BALB/c and W/W^v mice at 6 weeks of age (19–21 g and 20–22 g, respectively). Experiments involving the induction of anaphylaxis were performed on days 20–30. The antigen challenge was delivered by intravenous injection (*via* a tail vein) of OVA dissolved in saline at 4–200 μg ml^{-1} (0.25 ml per mouse).

Platelet count and electron microscopy

Two or three drops of blood from each decapitated mouse were directly collected into a pre-weighed test tube containing 1.0 ml of 4 mM EDTA in 0.01 M phosphate-buffered saline (pH 7.0). The tube plus blood was weighed, and the volume of blood was estimated from the weight of the blood. The number of platelets was then ascertained using a cell counter, Sysmex SF-3000 (Toa Medical Electronics Co. Ltd., Kobe, Japan). Electron microscopic analysis was performed as described previously (Shibazaki *et al*., 1996; Endo *et al*., 1997; Endo & Nakamura, 1992; 1993; Nakamura *et al*., 1998).

Determination of 5HT and H

After collecting the blood for measuring platelet count, the next two or three drops of blood from the same mouse were collected into another pre-weighed tube containing 3 ml of 0.4 M $HClO_4$, 0.2% *N*-acetylcysteine-HCl, and 4 mM EDTA-2Na. After reweighing, the platelets were destroyed by sonication, and each tube was cooled in an ice bath. Lung and liver were rapidly removed and kept in a jar containing dry ice until needed. The determination of the 5HT level in blood was carried out soon after the blood was collected. The 5HT levels in the tissues were determined within 2 days of collection. After 5HT had been separated by column chromatography, it was measured fluorometrically as previously described (Endo & Nakamura, 1992). A portion of the extracts of blood or tissues obtained as described above was used for the determination of H, as described previously (Endo, 1983).

Statistical analysis

Experimental values for 5HT, H, and platelet counts are given as mean ± standard deviation (s.d.). The statistical significance of differences was assessed using a Student's unpaired *t*-test after ANOVA: *P* values less than 0.05 were considered to indicate significance. The difference between two experimental groups in terms of the scores allocated for shock was analysed using a Ridit (relative to an identified distribution) test (Bross, 1958), a non-parametric test.

Results and Discussion

5HT injected intravenously is largely and rapidly removed by the lung and rapidly metabolized in this organ

Before describing our results on platelets, we should emphasize the following points. The lung of dogs can remove 90% or more of the 5HT injected into the blood within a single circulation time (Thomas & Vane, 1967) and 5HT infused into the lung of rats is also rapidly removed by the lung and metabolized there (Alabaster & Bakhle, 1970). We confirmed these findings in intact mice, which were injected with 5HT *via* a tail vein. The weight of the total blood is around 1.75 g in our mice, which was estimated as 7% of the body weight (Wish *et al*., 1950). Thus, a 5HT dose of 250 nmol per mouse may result in 143 nmol g^{-1} blood, when 5HT is not removed from the circulation. This level is more than 10 times the normal level of 5HT in the blood. However, as shown in Figure 1, at 10 s the 5HT in the blood had increased from about 10 to 35 nmol g^{-1}, corresponding to only 44 nmol per whole blood. This increase disappeared completely within the next 10 s. On the other hand, at this time the 5HT injection produced a huge increase in 5HT in the lung, from about 10 to 140 nmol g^{-1}. However, this value corresponds to only a 13 nmol increase per whole lung, because the weight of the murine lung is about 0.1 g. The elevated level of 5HT in the lung declined by about 40% over the next 20 s. In the liver, the increase in 5HT was negligible compared with those seen in the lung and blood. When we injected 5HT at a dose of 25 nmol per mouse, we could not detect any increase in 5HT in the blood, although 5HT increased markedly in the lung (data not shown). These results indicate that the 5HT injected intravenously is largely and rapidly removed by the lung, in which it is rapidly metabolized, and that the half-life of the injected 5HT in the blood is $<<10$ s, although we cannot determine this time accurately. Thus, the values given in the following sections for the amount of 5HT in the blood (and

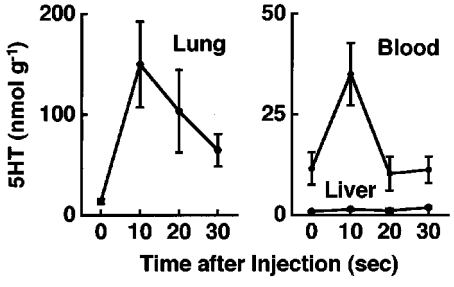

Figure 1 5HT levels after injection of 5HT itself. 5HT (250 nmol per mouse) was injected into a tail vein and animals were killed at the times indicated. Each value is the mean ± s.d. from four mice. *$P<0.05$ *vs* time 0.

possibly in the lung and liver) may represent almost entirely the amount of 5HT present within the platelets themselves.

The following points should also be noted. Mast cells in mice contain a large amount of H, but no detectable 5HT (Endo & Nakamura, 1992). In contrast, the blood of mice contains a large amount of 5HT, but a negligible amount of H (Endo & Nakamura, 1992). In addition, most of the 5HT in the blood is present within platelets (Yamamoto *et al*., 1986; Endo & Nakamura, 1992; 1993).

Platelet response precedes histamine release from mast cells and/or basophils

When 12.5 μg per mouse of OVA was given to sensitized mice, we observed that at 30 s after the injection there was around a 30% decrease in the platelet count and around a 70% decrease in the 5HT content of the blood (Figure 2a). These results indicate that 40% of the 5HT present in platelets was liberated within 30 s of the OVA-challenge. At 120 s, the platelet count was even lower, but there was no further decrease in 5HT. Although we cannot explain this result, we speculate that as they circulate around the body, platelets may take back 5HT from the lung or other sources (such as the intestine). Indeed, it is recognized that 5HT released from enterochromaffin cells in the gastrointestinal tract is taken up and stored in platelets (Erspamer & Testini, 1959; Fanburg & Lee, 1997), and release of 5HT from enterochromaffin cells has been shown to occur during anaphylaxis (Gershon & Ross, 1962). In contrast, no elevation in the blood content of H was detected at 30 s, although an increase was detected at 120 s, indicating that platelet degranulation preceded the degranulation of mast cells and/or basophils.

When a larger dose of OVA (50 μg per mouse) was given to sensitized mice, profound decreases in both platelets and 5HT were detected in the blood at 30 s (Figure 2c). In this case, H too was raised significantly in the blood at 30 s, and the level was even higher at 120 s (Figure 2c).

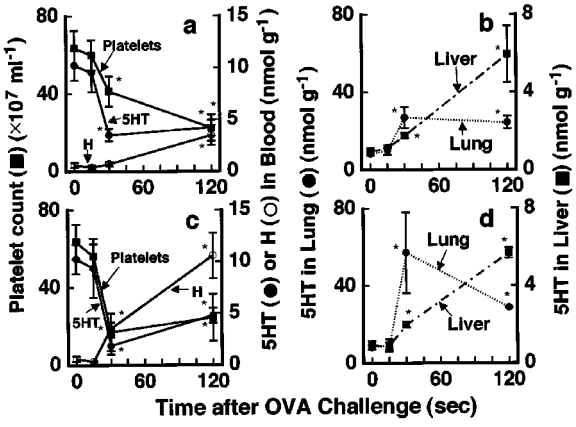

Figure 2 Changes in platelet count and the levels of 5HT and H in the blood, lung, and liver following an OVA-challenge to sensitized mice. Measurements were made at the indicated times after a challenge with 12.5 μg per mouse of OVA (in a and b) or 50 μg per mouse of OVA (c and d). Each value is the mean $\pm$ s.d. from four mice. $^*P<0.05$ *vs* time 0. There was no significant difference in platelet count between sensitized and non-sensitized mice, and in non-sensitized mice, 50 μg per mouse of OVA induced neither detectable change in 5HT levels in the lung and liver nor in platelet count in the blood (data not shown).

Platelets accumulate in the lung in response to OVA-challenge

In the lung and liver, however, there were no significant changes in the levels of H following an OVA-challenge (data not shown), suggesting that the mast cells in these organs do not represent a major source of the H found in the blood – indeed, skin, skeletal muscle, and the intestines have larger stores of H (Endo & Nakamura, 1992). In contrast, over the same time-scale as the decrease in blood platelet ($\sim$120 s), 5HT increased sharply in the lung (initially, at least) and slowly in the liver (Figure 2b,d), indicating that platelets were translocated to these organs. In the lung capillaries of mice killed at 30 s after a challenge with 50 μg per mouse of OVA, many platelets were detected by electron microscopy (Figure 3), and three types of platelet aggregations, with distinct ultrastructural features, could be identified. In type (1), each platelet had an intact structure and contained many granules; in type (2), each platelet had an electron-lucent cytoplasm and a reduced number of granules, and in type (3), each platelet a displayed a paucity of granules. All three types were observed in the lung of all sensitized mice given OVA ($n=3$). In contrast, we could scarcely detect platelets at all in the lung of non-sensitized mice

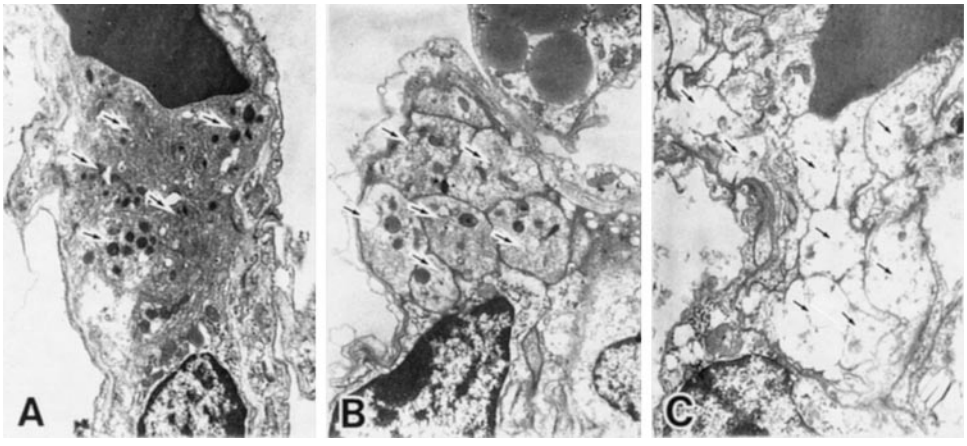

Figure 3 Electron microscopy of the lung at 30 s after an OVA challenge (50 μg per mouse). Immediately after decapitation, the lung was rapidly removed and processed as described previously. Original magnification $\times$7500. Arrows indicate platelets and their remnants.

given OVA. These results indicate that platelets themselves, retaining 5HT, accumulate in the lung in response to OVA and that some are degranulated there, and/or that platelets that are degranulated in the blood accumulate in the lung.

Platelets may accumulate in the lung and then in the liver

As shown in Figure 2b, 12.5 μg per mouse of OVA induced a progressive increase in 5HT in the liver, which roughly mirrored the decrease in the platelet count (Figure 2a), suggesting that some platelets from the general circulation accumulate slowly in the liver. However, in the period from 30 to 120 s after the injection of 50 μg per mouse of OVA (Figure 2c,d), despite the lack of a significant change in platelet count, 5HT increased in the liver and decreased in the lung. One possible explanation for these results is that some of the platelets that had accumulated in the lung may have been returned to the circulation only to be trapped again, this time by the liver. Although this idea is speculative at present, we have in the past observed just such a return of platelets from the lung to the circulation in LPS-injected mice (Shibazaki *et al*., 1999).

Platelet response is more sensitive to OVA-challenge than histamine release from mast cells or basophils

Next, changes in platelet count and the amounts of 5HT and H in the blood, lung, and liver were examined at 4 min after challenges with various doses of OVA (Figure 4), because at this time 5HT and H levels have reached a plateau (Figure 5). As shown in Figure 4, platelets and 5HT showed a dose-dependent decrease in the blood at this time-point, and the increase in 5HT in the liver was significant at a dose of OVA as low as 1 μg per mouse. An increase in 5HT in the lung was not detected until a dose of 25 μg per mouse OVA was given, suggesting that an accumulation of platelets occurs more readily in the liver than in the lung. On the other hand, an increase in H in the blood was detected only at the highest dose given, 25 μg per mouse of OVA. These results indicate that platelets respond to lower doses of OVA than those needed to make mast cells or basophils release H.

Platelets contribute to the signs of shock

Within 10 min of an OVA-challenge at 25 μg per mouse, a number of signs can be observed. Scratching, increased activity, and increased excitability were the initial signs seen during the first 0.5–3 min after the antigen-challenge. However, this was soon followed by various signs of shock (piloerection, dyspnea, sluggish gait, paresis, prostration, and convulsions, but rarely death, which occurred in only one out of 50 or more mice). We therefore tested the effects of several drugs on the signs of shock induced by an OVA-challenge (Table 1). Cyproheptadine (an antagonist of both 5HT and H) almost abolished the shock signs. Pyrilamine (an antagonist of histamine type-1 receptors) also suppressed

Table 1 Effects of drugs, platelet depletion and mast cell-deficiency on signs seen following OVA-challenge (50 μg per mouse)

Experiment	*Scores of shock signs*[e]
OVA-challenge alone	5.7±1.5
Cyproheptadine[a] + OVA-challenge	1.8±1.3*
Pyrilamine[a] + OVA-challenge	3.0±0.7*
K-76[a] + OVA-challenge	6.5±2.3
Aspirin[b] + OVA-challenge	6.8±0.4
Pm1 alone (200 μg per mouse)[c]	5.4±0.5
Pm1 (100 μg per mouse)[d] + OVA-challenge	2.2±0.03*
In W/W^v mice	
OVA-challenge alone	4.2±0.2
Pm1 (100 μg per mouse)[d] + OVA-challenge	1.5±1.8*

[a]Cycloheptadine (10 mg kg^{-1}), pyrilamine (10 mg kg^{-1}) or K-76 (10 mg kg^{-1}) was injected intraperitoneally 30 min before the OVA-challenge. [b]Aspirin (10 mg kg^{-1}) was injected intraperitoneally 1 h before the OVA challenge. [c]Pm1 was injected intravenously. [d]Pm1 was injected intravenously 3 h before the OVA-challenge. [e]Scores were allocated at 3, 4, 5, and 10 min for each of the later signs of shock (piloerection, dyspnea, sluggish gait, paresis, prostration, and convulsions). Scoring was as follows: none (0), weak (0.5), medium (1), and strong (2). The values are the mean±s.d. of the total score given to each mouse (four to five mice per group). *$P<0.05$ *vs* the group of OVA-challenge alone.

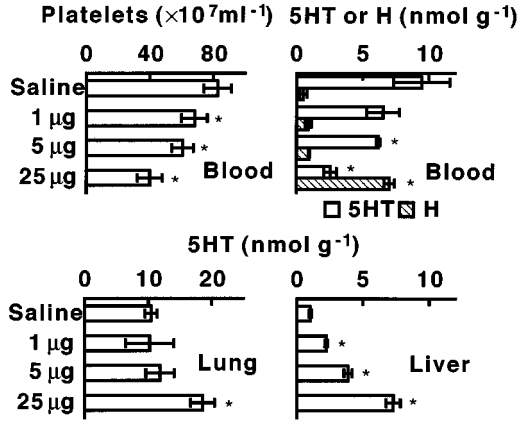

Figure 4 Dose-dependent changes in platelet count and in the levels of 5HT and H in the blood, lung, and liver following an OVA-challenge. Measurements were made at 4 min after either saline injection or an OVA-challenge at the indicated doses. Each value is the mean±s.d. from four mice. *$P<0.05$ *vs* saline.

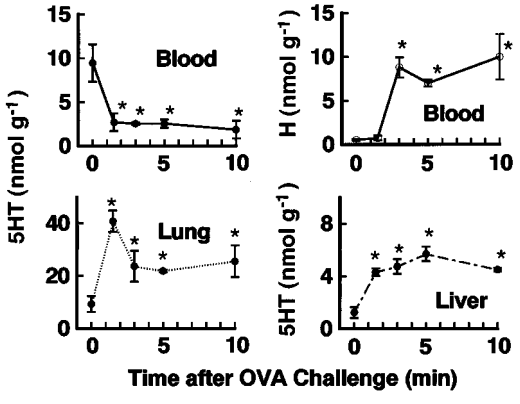

Figure 5 Time course of the changes in platelet count and the levels of 5HT and H over the first 10 min after an OVA-challenge. Measurements were made at the indicated times after either saline injection or an OVA-challenge (25 μg per mouse). Each value is the mean±s.d. from four mice. *$P<0.05$ *vs* time 0.

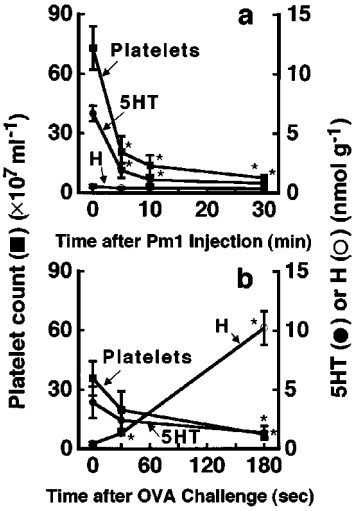

Figure 6 Effects of platelet depletion on the changes induced by an OVA-challenge in platelet count and the blood levels of 5HT and H. (a) Effects of Pm1, a monoclonal antibody to platelets, on platelet count and blood 5HT. Measurements were made at the indicated times after intravenous injection of the antibody (200 μg per mouse). (b) Effects of an OVA-challenge on platelet count and the blood levels of 5HT and H in platelet-depleted mice. Pm1 (100 μg per mouse) was injected intravenously into mice sensitized to OVA, and 3 h later an OVA-challenge (50 μg per mouse) was delivered. Each value is the mean $\pm$ s.d. from four mice. $^*P<0.05$ *vs* time 0.

the shock signs, although less powerfully than cyproheptadine. K-76 monocarboxylic acid is an inhibitor of complement C5 (Hong *et al*., 1979), while aspirin is a typical antiplatelet agent. The LPS-induced destruction of platelets and the ensuing anaphylactoid shock can both be prevented by either of these two agents (Shibazaki *et al*., 1999, and our unpublished data). These agents, however, were not effective at reducing the OVA-induced shock, suggesting that mechanisms underlying the OVA-induced degranulation of platelets may be different from that induced by LPS.

Pm1 is a monoclonal antibody against murine platelets (Nagata *et al*., 1995). Its intravenous injection, at 200 μg per mouse, into control BALB/c mice produced parallel decreases in the platelets and 5HT in the blood (Figure 6a). Moreover, shock signs similar to those seen after an OVA-challenge occurred in Pm1-injected control (non-sensitized) mice (Table 1). We then examined the effects of an OVA-challenge (50 μg per mouse) in OVA-sensitized, platelet-depleted mice. In this experiment (to prevent shock being induced by Pm1, its dose was reduced to 100 μg per mouse), the initial platelet count was about 40% of that seen in control mice. After OVA administration, it was further depleted, reaching a value equivalent to 10% of the initial level seen in the control mice within 3 min of the OVA-challenge. In these mice, too, H increased in the blood as it had in OVA-sensitized, non-platelet-depleted mice (compare Figure 6b with Figure 2). However, in the platelet-depleted mice the score allocated for the signs of shock was reduced significantly (Table 1).

W/W^v mice are deficient in mast cells (Kitamura *et al*., 1978; Suda *et al*., 1985). As shown in Table 1, these mice, too, produced anaphylactic shock in response to OVA (as shown by other investigators: Jacoby *et al*., 1984; Ha *et al*., 1986; Martin *et al*., 1993; Choi *et al*., 1998). Platelet depletion in these OVA-sensitized mice significantly reduced the signs of shock induced by the OVA-challenge (Table 1).

Previously, we reported that the platelet degradation induced by LPS produces similar signs of shock in BALB/c mice those seen here in response to an OVA-challenge (Shibazaki *et al*., 1996; 1999; Endo *et al*., 1997): however, in the case of LPS, there is no increase in H in the blood (unpublished data).

The above results strongly suggest that in addition to mast cells and basophils, platelets are also involved in the anaphylactic shock induced by OVA in mice sensitized to it.

General discussion

Joseph *et al*. (1986) found a receptor for IgE on human platelets. On the basis of other findings, they proposed the concept of a role for platelets in various immune reactions (Capron *et al*., 1987). Recently, to add to the low-affinity IgE receptor (FcεRII/CD23), a high-affinity IgE receptor (FcεRI) was also identified on human platelets (Joseph *et al*., 1997; Hasegawa *et al*., 1999). Since, at present, these receptors have not been demonstrated on platelets in mice, there is no basis for asserting that these IgE receptors are responsible for the anaphylaxis seen in sensitized mice. However, neither IgE (Oettgen *et al*., 1994) nor FcεRI (Miyajima *et al*., 1997) is a prerequisite for inducing anaphylaxis in mice. Indeed, Miyajima *et al*. (1997) reported that systemic anaphylaxis in mice may be mediated largely through IgG1 and FcγRIII. Thus, it is of particular interest to know whether this type of receptor is present on platelets in mice. In fact, murine platelets, have been reported to lack FcγRII (CD32) (Kato *et al*., 1998), and Nieswandt *et al*. (1999) reported that mouse platelets were not stained by an anti-FcγRII/III monoclonal antibody. Although antiplatelet antibodies can activate human platelets *in vitro*, causing platelet aggregation and degranulation (Horsewood *et al*., 1991), we have failed to detect such aggregation (by means of aggregometry) using platelet-rich plasma (PRP) exposed to various concentrations of OVA (data not shown). A similar nonresponsiveness of mouse PRP to a monoclonal antibody to fibrinogen receptors (gpIIb/IIIa) has also been reported by Nieswandt *et al*. (1999). Although we have no available data explaining the mechanism underlying the platelet response to OVA, it is of particular interest that platelet α-granules contain IgG, IgA, IgM and IgE and release them in response to stimulators of platelets (Harrison & Cramer, 1993; Klouche *et al*., 1997).

As early as 1962, Gershon and Ross (1962), discussing the identity of the effector molecule inducing anaphylactic shock, doubted that H plays a significant role in bringing about anaphylactic shock, because while mice are extremely insensitive to H, much less than a toxic amount of H is present in this species. In addition, they found that antihistamine drugs, which completely protect guinea-pigs, do not protect the mouse from anaphylactic shock, a result also obtained in our study (Table 1). At that time (1962), it had already been reported that giving 5HT to mice mimics anaphylaxis, although there are no significant changes in 5HT content in the blood and tissues of mice dying of anaphylactic shock (Waalkes & Coburn, 1960). Gershon & Ross (1962) suggested that 5HT might be released from enterochromaffin cells during anaphylaxis. However, we cannot directly compare our results with those obtained by these pioneers

because they determined 5HT in dying mice or after anaphylactic shock, and they collected the blood by direct aspiration from the heart. In contrast, we measured 5HT before and during shock, and we collected the blood from the neck by decapitation.

Pharmacologic studies by Halpern *et al*. (1963) suggest that the anaphylactic reaction seen in mice results from the release of both H and 5HT. Indeed, relatively low doses of mixtures of H and 5HT produce severe or lethal symptoms similar to those of anaphylaxis (Iff & Vaz, 1966). Thus, taking these results together with ours strongly suggest that H and 5HT are both important mediators of anaphylactic shock in mice. In this regard, it should be emphasized here that platelets in humans contain both 5HT and H (Mannaioni *et al*., 1997). However, recent studies have shown that platelet-activating factor (PAF) is also involved in this reaction, because (1) anaphylaxis is markedly reduced in PAF receptor-deficient mice (Ishii *et al*., 1998), and (2) BN50739, a PAF antagonist, can prevent fatal reactions during anaphylaxis (Choi *et al*., 1998). Interestingly, in the study by the latter authors, the time course of the increase in H in plasma was very similar to that of PAF, thus release of PAF being slower than platelet response shown in the present study.

A few years ago, Leir *et al*. (1995) reported that although some protein antigens induce rapid thrombocytopenia in sensitized mice, they could neither find degranulation of platelets (or release of their contents) nor link the phenomenon to anaphylaxis or allergic reactions. In fact, they suggested that the rapid thrombocytopenia involved the complement system, but not IgE. However, the anaphylaxis induced in IgE-deficient mice by OVA has been shown to be complement-independent (Oettgen *et al*., 1994). In our study, an inhibitor of complement C5 was not effective at reducing shock scores. Hence, the mechanisms underlying the platelet response to antigen await complete clarification. Incidentally, it should be emphasized that as early as 1977, Pinckard *et al*. (1977) found that pulmonary sequestration of platelets occurs in the rabbit during IgE-induced systemic anaphylaxis, and that platelet depletion abrogates lethal anaphylactic shock.

Finally, it should be remembered that blood that has passed through the body (other than the digestive tract) passes first to the lungs before being recirculated by the heart, whereas blood that has passed through the digestive tract passes next to the liver. Thus, when pathogens have entered blood vessels, they soon reach either the lungs or the liver, and these organs must destroy the intruders if they are not to invade the rest of the host. In addition to their role in the responses induced by OVA, platelets accumulate rapidly in the lung and liver in response to an intravenous injection of LPS (Shibazaki *et al*., 1996; 1999; Endo *et al*., 1997). Moreover, platelets accumulate slowly and selectively in the liver in response to an intraperitoneal injection of a very low dose of LPS, IL-1 and TNF (Endo & Nakamura, 1992; 1993; Nakamura *et al*., 1998). Platelets are tiny cell-remnants without a nucleus, and the traditional view is that haemostasis is their major role. However, we now believe that they may play an important role (as aggressive foot-soldiers?) in both innate and acquired immunity, and that the lung and liver may be the fronts at which they wage war on pathogens. Studies designed to clarify the mechanisms by which platelets accumulate in the lung and liver are now in progress in our laboratory.

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