REVIEW *Pharmacology*

Platelet Dysfunction in Chediak-Higashi Syndrome-Affected Cattle

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ABSTRACT. A serious symptom of cattle affected with Chediak-Higashi syndrome (CHS) is a bleeding tendency. This diathesis is characterized by insufficient platelet aggregation as a result of depressed response to collagen. One possible cause for the depression is a decrease in contribution of endogenous agonists such as ADP or thromboxane A_2, which are released following collagen stimulation. However, these endogenous agonists play only a minor role in collagen-induced aggregation of bovine platelets. More importantly , activation of phospholipase C as a result of a direct action of collagen is depressed, leading to a depression of Ca^{2+} mobilization, in platelets from CHS-affected cattle. Several types of collagen receptor are proposed to work in concert to induce aggregation. Among them, glycoprotein VI (GPVI) and GPIa/IIa (integrin $\alpha 2\beta 1$) have been supposed to play dominant roles in collagen-induced aggregation. However, there are arguments about the role of each receptor, especially the role of GPIa/IIa, and the crosstalk between receptors. Recently, we reported that the Ca^{2+} signaling produced by rhodocytin, which had been first reported to be an agonist for the collagen receptor GPIa/IIa, produced much less Ca^{2+} signaling in CHS platelets than in normal ones, whereas that produced by GPVI activators was normal. These suggest that GPIa/IIa or the rhodocytin-associated pathway is impaired in CHS platelets. CHS platelets are valuable to reassess the mechanism of collagen-dependent signal transduction system and to delineate the inter-relationship among collagen receptors.
KEY WORDS: Ca^{2+} signaling, cattle, Chediak-Higashi syndrome, collagen receptor, platelet.

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Chediak-Higashi syndrome (CHS) is an autosomal recessive genetic disease, which is manifested of partial oculocutaneous albinism, increased susceptibility to infections, and a bleeding tendency, and is found in humans, cattle, minks, cats, mice, rats and fox [27, 29, 31, 44, 48, 49, 63]. Most human and animal patients affected with CHS show bleeding diathesis. The bleeding diathesis of CHS results from impairment of platelet functions. In fact, *in vitro* aggregation studies using platelets from CHS patients revealed that aggregation induced by collagen, which is an important platelet activator to form a thrombus when a vessel wall is injured, was remarkably impaired in platelets from almost all patients. In this review, we first describe abnormality in CHS platelets and possible causes for insufficient aggregation to collagen in CHS platelets.

We have recently demonstrated that handling of cytosolic Ca^{2+} due to a direct action of collagen was abnormal in platelets from Japanese Black cattle affected with CHS [59, 61]. This observation suggests that a collagen receptor-Ca^{2+} signaling system is impaired in CHS-affected platelets. There are several subtypes of collagen receptor on platelets and their interrelationship seems to be very complicated, so that the mechanism of collagen-induced aggregation has only partially been elucidated. On this standpoint, the study to explore a cause for insufficient aggregation of CHS platelets will give a new insight into a collagen receptor-associated signaling pathway. Mainly based on our data, therefore, we next introduce a current idea about a receptor responsible for the impaired response to collagen in platelets from CHS-affected cattle and inter-relationship among collagen receptors.

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CHARACTERISTICS OF CHEDIAK-HIGASHI SYNDROME

CHS was initially found as the case in humans [4] and named as CHS after the works by Chediak, who described hematological characteristics of the disorder [14], and Higashi, who found giant peroxidase-containing granules within cells from the disease-affected patients [19]. In most human patients affected with CHS, death often occurs in the first decade of life due to infection, bleeding, or development of the 'accelerated phase', which is characterized by a lymphoproliferative syndrome with lymphohistiocytic infiltration. CHS in cattle is found in Hereford [49], Japanese Black [69] and Brangus breeds [2]. In Japan, Japanese Black cattle affected with CHS are frequently seen in South Kyusyu area (Miyazaki and Kagoshima Prefectures) [46] and infrequently in other places. A simple autosomal recessive condition was documented in Hereford and Japanese Black cattle [48, 68].

Symptoms of CHS seen in humans and cattle are listed in Table 1. Patients affected with CHS show a variable degree of partial oculocutaneous albinism, increased susceptibility to infections, and a bleeding tendency. In cattle affected with CHS, the most important clinical manifestations are bleeding diathesis. In 1976, Bell *et al.* reported that, during past 7 years, 20 of 56 deaths among the Hereford cattle maintained at Washington State University could be directly associated with hemorrhage [5]. Ogawa *et al.* reported that 56 animals of more than 200 bleeding Japanese Black cattle

Table 1. Clinical signs of Chediak-Higashi syndrome in humans and cattle

	Human	Hereford	Japanese Black
Infection	Dermatitis Rhinopharyngitis Otitis Oral ulcer Abscess ‡ Pneumonia Nephritis Hepatitis Peritonitis	Abscess ‡ Pneumonia Endocarditis Nephritis Dermatitis Diarrhea Upper respiratory infection Peritonitis	Abscess ‡ Pneumonia Endocarditis Nephritis Dermatitis
Bleeding	Easy bruisability Mucosal bleeding Epitaxis	Bleeding following surgical procedures Hematoma Mucosal bleeding	Bleeding following surgical procedures Hematoma Epitaxis Melena Umbilical bleeding
Ocular findings	Pale iris Photophobia Nystagmus Abnormal fundic reflection	Pale iris Photophobia Abnormal fundic reflection	Pale iris Abnormal fundic reflection
Hypopigmentation of skin, and hair	Present	Present	Present
Neurologic findings*	Present	Absent	Absent
Accelerated phase†	Present	Absent	Absent

* Peripheral and cranial neuropathy, autonomic dysfunction, weakness, sensory deficits, hyporeflexia, clumsiness, and seizures. † Fever, anemia, neutropenia, thrombocytopenia, hepatosplenomegaly, lymphadenopathy and jaundice. ‡Found in muscle, lung, skin and liver.

examined were diagnosed as CHS, and a typical sign of the disease among the affected cattle was bleeding disorder [46].

Human CHS patients most commonly exhibit easy bruisability, mucosal bleeding and epistaxis. The bleeding manifestations are mild to moderate [12, 13]. Severe gastrointestinal hemorrhage was observed in the late stage of the disease in child CHS patients, which may be caused by thrombocytopenia in the accelerated phase. The bleeding tendency in Hereford cattle with CHS is often observed as fibrosis in resolution of large subcutaneous accumulations of blood and as production of numerous, firm subcutaneous nodules at sites of previous trauma [5]. Bleeding manifestations of Japanese Black cattle affected with CHS are observed as excess bleeding during or after castration, development of superficial or intrapubic (or abdominal) hematoma [46].

Prolonged bleeding time has been reported in most of affected animal species [2, 5, 6, 12, 16, 35, 46, 63]. On the other hand, coagulation system is normal in humans, Japanese Black cattle and cats affected with CHS [12, 35, 46]. Platelet count is normal in most cases of humans, Japanese Black, Hereford cattle and minks [5, 6, 46]. These findings imply that the bleeding diathesis of CHS may result from impairment of platelet functions but not from thrombocytopenia or coagulopathy.

STRUCTURAL OR FUNCTIONAL ABNORMALITIES IN CHS PLATELETS

Platelets are small discoid cell fragments (approximately 0.5×3.0 μm long) produced from megakaryocytes in bone marrow. Under a normal condition, platelets are not adhesive to a vessel wall and do not adhere each other. When a blood vessel is damaged at the luminal side, however, platelets adhere to subendothelial extracellular matrix (ECM) and become activated (Fig. 1). Collagen is rich in the ECM within vessel wall, and provides an important site when platelets adhere to the exposed ECM. The adhesion is mediated through indirect and direct interaction. Under high shear stress, glycoprotein Ib (GPIb) interacts indirectly with collagen via von Willebrand factor (vWf) [56]. Several surface glycoproteins on platelet have been identified as collagen receptors including most importantly glycoprotein VI (GPVI) and GPIa/IIa (also termed $\alpha 2\beta 1$) [43, 65]. Activated platelets change the shape from a discoid to a spheroid form, accompanied by formation of filopodia-like structures. This is followed by secretion of granule contents, pro-

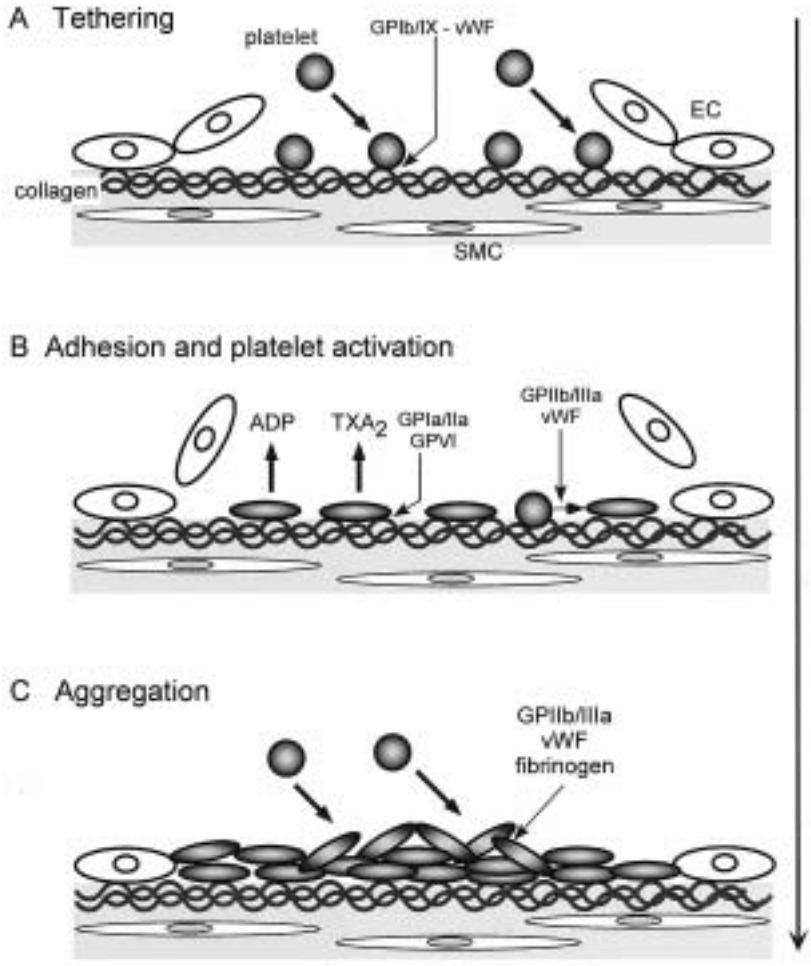

Fig. 1. Sequential events from injury to a vessel wall to platelet aggregation. When a blood vessel is damaged, the subendothelial layer is exposed to platelets. Platelets are tethered to the layer through interaction of von Willebrand factor (vWf) and glycoprotein Ib/IX (GPIb/IX) (A). Then, platelets firmly adhere to collagen, mediated by GPIa/IIa and GPVI. Activated platelets change the shape from a discoid to a spheroid form, accompanied by formation of filopodia-like structure and spread out on ECM. GPIIb/IIIa and vWF are involved in the spreading. This is followed by secretion of granule contents (ADP) and production of thromboxane A_2 (TXA_2), which augment aggregation (B). Then, platelets aggregate to form a thrombus. The binding of fibrinogen and vWF to GPIIb/IIIa (αIIbβ3) is involved in this stage. This scheme is based on the conventional idea that GPIa/IIa and GPVI play major roles. The significance of GPIa/IIa is now challenged (see text and Fig. 3). EC; endothelial cell, SMC; smooth muscle cell.

duction of thromboxane A_2 (TXA_2) and finally platelet aggregation, in the stage of which GPIIb/IIIa activated on platelet surface binds to fibrinogen with high affinity. Platelet activation includes these whole processes and it is vital to the formation of a haemostatic plug under physiological or pathophysiological conditions.

Abnormal platelet aggregation to collagen: Table 2 lists data from *in vitro* aggregation experiments using platelets from CHS patients. Decreased aggregative response to collagen is a common feature in almost all patients affected with CHS (Fig. 2A). Besides, in human patients with CHS, impairment of epinephrine-induced aggregation was also observed [1, 5, 12]. Platelet aggregation by ADP is normal or sometimes slightly decreased with a tendency to disaggregate in CHS platelets. Phorbol-12-myristate 13-acetate (PMA)-induced aggregation was normal in Japanese Black cattle with CHS [66], while it was depressed in mice with CHS [53]. It is agreed that the bleeding tendency in CHS is attributable to abnormal platelet activation in response to collagen. In the following sections, we describe possible

Table 2. Platelet aggregation induced by various agonists in animals affected with Chediak-Higashi syndrome

Animal	Coll	ADP	Thr	Epi	A23187	PMA	5-HT	AA	Author
Human	A	N	-	A	-	-	-	-	Buchanan and Handin [12]
	A	A	-	A	-	-	-	-	Bell *et al.* [5]
	V	V	-	V	-	-	-	-	Boxer *et al.* [13]
	N	N	-	-	-	-	A	-	Parmley *et al.* [50]
	A	A*	-	-	-	-	-	A	Weening *et al.* [73]
	-	N	A	-	-	-	-	N	Rendu *et al.* [55]
	A	N	-	-	-	-	-	-	Legrand & Nurden [30]
	A*	A*	-	A	V	-	-	A	Apitz-Castro *et al.* [1]
Cattle/Her	A	A	-	0	-	-	-	-	Bell *et al.* [5]
Cattle/JB	A	A*	N	-	-	N	-	-	Suzuki *et al.* [66]
	A	A*	-	-	-	-	-	-	Ogawa *et al.* [46]
	A	A*	-	-	-	-	-	-	Shiraishi *et al.* [59]
Mink	A	N	-	-	-	-	-	-	Bell *et al.* [6]
Cat	A	A	-	0	-	-	A	-	Meyers *et al.* [35]
Fox	A	A*	-	0	-	-	V	A	Sjaastad, *et al.* [62]
Mice	A†	-	A†	-	A†	A	-	-	Pratt *et al.* [53]
Rat	A	A*	-	-	-	-	-	-	Ozaki *et al.* [47]

Her, Hereford; JB, Japanese Black. Coll, collagen; Thr, thrombin; Epi, epinephrine; 5-HT, serotonin; AA, arachidonic acid. A, abnormal; N, normal; 0, no aggregation; V, variable. *, tend to disaggregate; †, only at low concentration.

causes underlying the depressed responsiveness to collagen in CHS platelets.

Storage pool deficiency: Ultrastructural examination of platelets revealed that a shape and size of platelets from CHS-affected Hereford [54], Japanese Black cattle [46] and humans [55] was normal. At least three types of secretary granule exist in platelets; lysosomes, α-granules, and dense granules (otherwise called δ-granules). Interestingly, an abnormality in lysosomes, which is typical for CHS leukocytes, was not observed in platelets from cattle [34, 37, 54], cats [34], minks [34] and most humans affected with CHS [13, 55]. α-Granules in CHS platelets, which contain vWf, platelet factor 4, and thrombospondin, were similar in structure and quantity to those in normal platelets [33, 34, 55]. Dense granules contain ADP, ATP, serotonin and Ca^{2+}. ADP is released from dense granules to extracellular space when platelets are activated by various kinds of stimulation, and acts as a secondary agonist, thus playing an important role in aggregation of human platelets. A dense body in human CHS platelets is irregular [32] and a number of dense granules in platelets from Hereford and Japanese Black cattle [33, 34, 36, 46, 54], humans [32, 55], and rats [47] was less in patients with CHS than in normal controls. Dense granule precursors in megakaryocytes were absent in CHS-affected Hereford cattle [37]. In accord with these observations, contents in dense granules (ADP, ATP, serotonin and Ca^{2+}) were greatly decreased in humans and animals affected with CHS [6, 13, 33, 35, 46, 47, 53, 63]. These observations lead to the hypothesis that a decrease in release of ADP from a dense body (δ-storage pool deficiency, δ-SPD) is a major cause for defective aggregation in CHS platelets [5, 13, 33, 55].

Since ADP plays an important role in the collagen-induced aggregation in human platelets [24, 59], δ-SPD could probably be responsible for decreased responsiveness to collagen in human CHS platelets. However, Nieuwenhuis *et al.* reported that 23% of patients having δ-SPD showed normal aggregation responses to collagen [44]. Furthermore, it has been reported that ADP content in bovine platelets (normal Hereford cattle) is only about 30% of human platelets [5, 34]. Therefore, it is not clear whether a decreased release of endogenous ADP is involved in the blunted collagen-induced aggregation of CHS platelets. In platelets from normal Japanese Black cattle, aggregation induced by collagen was only partially inhibited by AR-C66096, an antagonist to ADP $P2Y_{12}$ purinoceptor, which mediates the ADP-induced aggregation in human and bovine platelets [18, 59]. The extent of ADP-dependent aggregation seems to be 20 to 30% of the total aggregation response to collagen in platelets from normal cattle, whereas it was negligible in the response of platelets from CHS-affected cattle [24, 59]. This suggests that endogenous ADP is only partially involved in the collagen-induced aggregation of bovine platelets and that a decrease in release of ADP is partially responsible for the blunted response to collagen in CHS platelets. However, a decreased release of endogenous ADP is not the sole mechanism for impaired aggregation to collagen in platelets from CHS-affected cattle, because the aggregation response to collagen in normal platelets after blockade of $P2Y_{12}$ purinoceptors was still much greater than that in CHS platelets [59].

Impairment of thromboxane A_2 production: TXA_2 is released from activated platelets as a result of sequential activation of phospholipase A_2, cyclooxygenase-1 and thromboxane synthase and acts as a secondary agonist as well as ADP on platelets [41, 52, 62]. A decrease in TXA_2 production could be a case in human CHS platelets because aggregation to exogenous arachidonic acid, which is converted to TXA_2, was reported to decline in two human CHS cases [1, 73]. Suzuki *et al.* suggested that impairment of

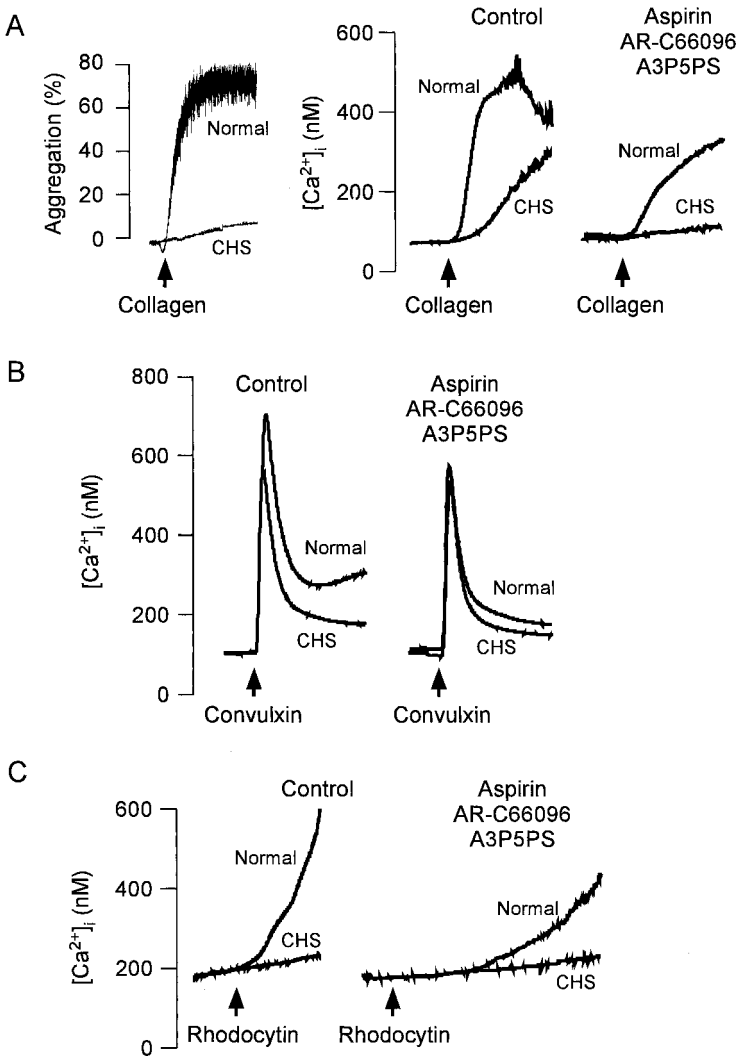

Fig. 2. Characteristics of aggregation and $[Ca^{2+}]_i$ responses in bovine CHS platelets. A, aggregation and $[Ca^{2+}]_i$ responses to collagen. When influences of endogenous agonists (TXA_2 and ADP) were excluded, platelets were pretreated with 1 mM aspirin (a cyclooxygenase inhibitor), 100 nM AR-C66096 (a $P2Y_{12}$ receptor antagonist) and 100 μM adenosine 3'-phosphate 5'-phosphosulfate (A3P5PS, a $P2Y_1$ receptor antagonist). B, $[Ca^{2+}]_i$ response to 3 *n*g/m*l* convulxin, a GPVI activator. C, $[Ca^{2+}]_i$ response to 10 nM rhodocytin, a putative GPIa/IIa activator. Reproduced from [58–60].

production of TXA_2 is a main cause for a depression of aggregation to collagen in CHS platelets since indomethacin, a cyclooxygenase inhibitor, inhibited the collagen-induced aggregation in normal platelets, while it exerted a smaller effect on the aggregation in CHS platelets from Japanese Black cattle [66]. Unlike human platelets, however, arachidonic acid metabolites seem to play a negligible role in bovine platelets, because cyclooxygenase inhibitors showed no effect on aggregation response to collagen, ADP or platelet-activating factor in platelets from healthy cattle [11, 17, 24]. Consistent with these reports, our study revealed that aggregation or an increase in cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) in response to collagen was scarcely affected by cyclooxygenase inhibitors and U446619, a TXA_2 mimetic, caused no aggregation response and only a slight increase in $[Ca^{2+}]_i$ in bovine platelets [59]. In contrast to Suzuki *et al.*'s paper [66], the $[Ca^{2+}]_i$ response to collagen in bovine CHS platelets was slightly decreased by pretreatment with cyclooxygenase inhibitors, whereas it was not affected in normal platelets [59–61]. Probably, contribution of TXA_2 increased in the collagen-induced response of CHS platelets as a compensation for a decrease in release of ADP,

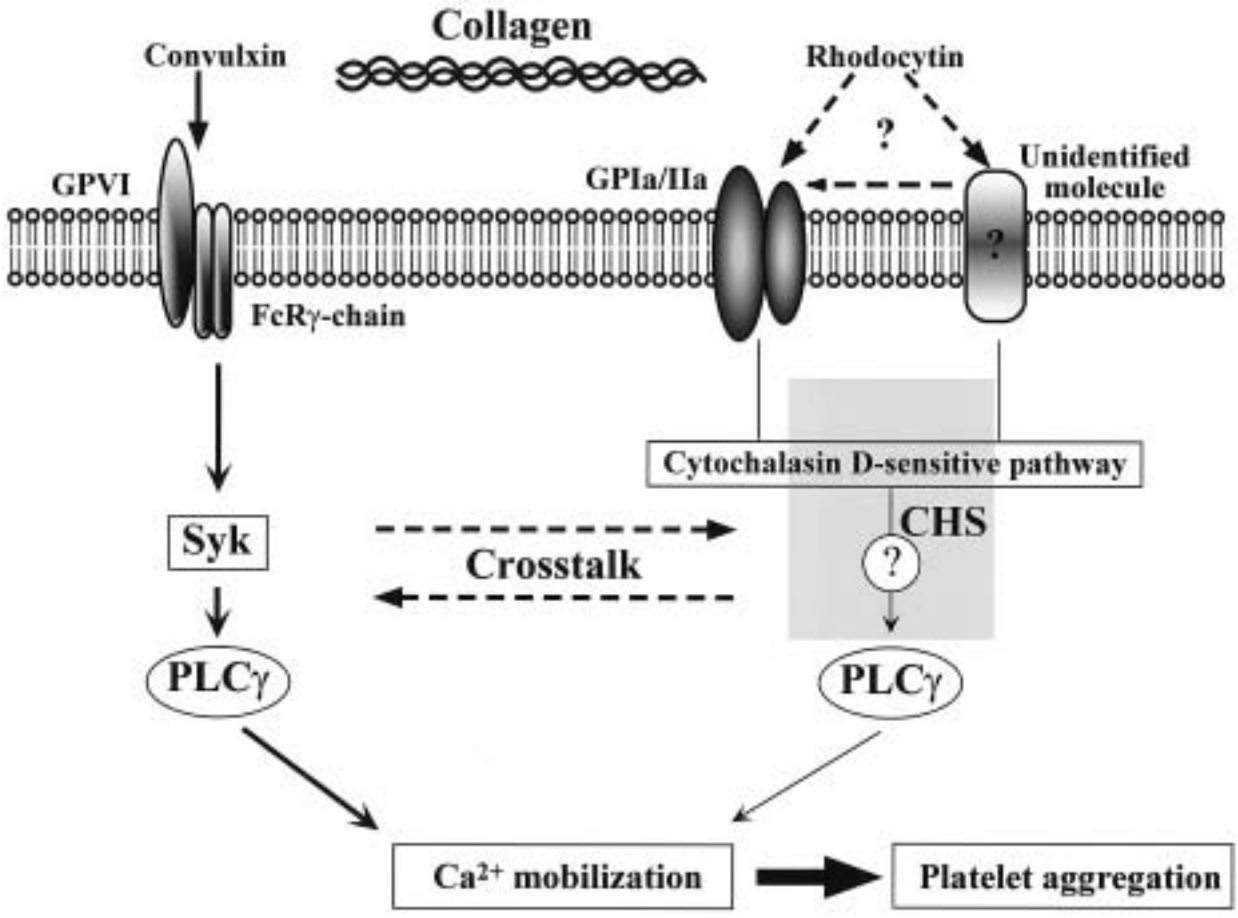

Fig. 3. Speculative model for platelet collagen receptors. Until recently, GPVI and GPIa/IIa are supposed to be predominant receptors for collagen in platelets. However, recent works from Germany and Switzerland [7, 42] proposed that GPIa/IIa is not essential for collagen-induced activation, at least in mouse platelets. It has not been determined whether rhodocytin binds to GPIa/IIa in bovine platelets. If rhodocytin does not bind to GPIa/IIa, our study [61] suggests that another receptor, which is sensitive to rhodocytin, participates in the collagen-induced activation. This receptor probably delivers a signal for full activation of GPVI when collagen acts on platelets. It is likely that CHS platelets lack a receptor that binds rhodocytin or a signal existing downstream of the receptor. This pathway is sensitive to cytochalasin D [61]. GPVI is coupled to Fc receptor γ-chain (FcRγ) and a signal from GPVI is probably transmitted to several tyrosine kinases and adaptor proteins (not shown in the figure), then activates the tyrosine kinase Syk to phosphorylate PLCγ. It is possible that GPIa/IIa or the rhodocytin-sensitive receptor also activates PLCγ, since it has been shown that rhodocytin induces tyrosine phosphorylation of Syk and PLCγ in human platelets [67]. Signaling system downstream of GPVI, which leads to Ca^{2+} mobilization, is nearly intact in CHS platelets, when GPVI is activated by convulxin.

although we do not know the mechanism for the compensation. Taken together, we think that the primary cause for the impaired response to collagen in platelets from cattle affected with CHS is not a decreased release of endogenous agonists.

Ca^{2+} mobilization and phospholipase C activation: Ca^{2+} is an important intracellular messenger within many cells [8]. In platelets, it is agreed that an increase in $[Ca^{2+}]_i$ is an initial event required for activation by almost all agonists [57, 64]. Collagen gradually increased $[Ca^{2+}]_i$ in bovine CHS platelets, while it more rapidly increased $[Ca^{2+}]_i$ in normal platelets. In CHS platelets, the maximum increase in $[Ca^{2+}]_i$ induced by collagen at each concentration was significantly lower than in normal platelets [59]. The fraction of collagen-induced Ca^{2+} mobilization that was independent of endogenous agonists in CHS platelets was only about 15% of that in normal platelets [61] (Fig. 2A). This result suggests that a Ca^{2+} signaling mechanism as a consequence of the direct action of collagen is defective in bovine CHS platelets and that this defect is important in the etiology of insufficient aggregation.

A rise in $[Ca^{2+}]_i$ caused by receptor agonists usually consists of two components: Ca^{2+} release from intracellular Ca^{2+} stores and Ca^{2+} entry across the plasma membrane [57]. Ca^{2+} release from intracellular Ca^{2+} stores is mediated by inositol 1,4,5-trisphosphate ($Ins(1,4,5)P_3$) produced following activation of phospholipase C (PLC) [9]. In our study with bovine platelets [60], collagen increased cytoso-

lic Ins(1,4,5)P_3, and the collagen-induced Ca^{2+} mobilization was inhibited by treatment with U73122, a PLC inhibitor [10, 70]. Thus, the collagen-induced Ca^{2+} mobilization depends on the PLC activity. The type of PLC involved in the collagen-induced Ca^{2+} mobilization is PLCγ, which is different from the type (PLCβ) associated with a receptor coupled to heterotrimeric GTP-binding protein (e.g. ADP or thrombin) [72]. In contrast to PLCβ, PLCγ is activated through tyrosine phosphorylation [26]. In platelets from cattle with CHS, the collagen-induced increase in Ins(1,4,5)P_3 was greatly depressed compared with that in normal platelets [61]. Therefore, a cause for the insufficient Ca^{2+} mobilization in CHS platelets is present in the pathway between the binding of collagen to receptors and the activation of PLCγ.

Abnormality in signal transduction system in CHS platelets—a clue to understanding signal transduction system of collagen receptor: Although a number of candidates have been proposed as collagen receptors to date, several lines of evidence have indicated that glycoprotein (GP) VI and GPIa/IIa (also termed integrin $\alpha2\beta1$) are predominant receptors for collagen in platelets, since a defect in expression of GPIa/IIa or GPVI in human platelets brought about insufficient platelet aggregation response to collagen, while responses to other agonists were normal [38, 43, 65, 71]. GPVI, which belongs to an immunoglobulin superfamily [15], is accepted as a major collagen receptor that mediates platelet activation due to collagen, whereas GPIa/IIa has been suggested to be responsible for the platelet adhesion to collagen [71]. Recently, several tools have been introduced as specific activators of GPVI or GPIa/IIa (Fig. 3). Convulxin, isolated from the snake *Crotalus durissus terrificus* venom, and collagen-related peptide (CRP), which consists of a glycine-proline-hydroxyproline repeat and is cross-linked via cysteine residues at its C- and N-terminals, have been shown to activate platelets by interacting with GPVI [25, 39, 51]. On the other hand, rhodocytin, isolated from the other snake *Calloselasma rhodostoma venom*, was first reported to activate GPIa/IIa [21, 58, 67]. In our study, the GPVI-specific activator convulxin- or CRP-induced increase in $[Ca^{2+}]_i$ was normal or only slightly inhibited in bovine CHS platelets [61] (Fig. 2B). On the other hand, an increase in $[Ca^{2+}]_i$ induced by rhodocytin was greatly depressed in CHS platelets when the influence of endogenous agonists was excluded (Fig. 2C). GPIa/IIa-dependent adhesion of CHS platelets to type I collagen was not different from that of normal platelets, suggesting that the GPIa/IIa receptor is expressed normally on CHS platelets and collagen can bind to it in a way similar to normal platelets [61]. These data led us to deduce a tentative idea that GPIa/IIa-dependent signaling system is impaired in CHS platelets. However, Bergmeier *et al.* [7] recently reported that rhodocytin induced platelet activation in platelets from $\beta1$ integrin (GPIIa)-null mice. Moreover, mouse platelets lacking both $\alpha2\beta1$ integrin (GPIa/IIa) and GPVI responded normally to rhodocytin, suggesting that rhodocytin does not bind to GPIa/IIa at least in mice. This is contradictory to the Suzuki-Inoue group's finding that rhodocytin bound to recombinant human GPIa/IIa [67]. Hence, the site of action of rhodocytin has been thrown into argument. As for bovine platelets, there are some common features between the collagen- and rhodocytin-induced responses; the response to collagen or rhodocytin was similarly sensitive to cytochalasin D, an actin polymerization inhibitor, but that to convulxin was insensitive to cytochalasin D, and CHS platelets showed a similar decrease of sensitivity to collagen and rhodocytin [61]. From these, it is very conceivable that the rhodocytin-sensitive mechanism is involved in the collagen-induced activation of platelets and a defect in the rhodocytin-sensitive mechanism is related to dysfunction of CHS platelets.

The understanding that GPIa/IIa functions as a receptor for the adhesion of platelets to collagen and GPVI for the platelet activation has to be reassessed [71]. Recently the study by Nieswandt *et al.* [42] using $\beta1$ knock out mice showed that GPIa/IIa is not essential for the collagen-induced aggregation. Thus, it should be determined whether GPIa/IIa is truly involved in the collagen-induced activation in bovine platelets. If not, what receptor other than GPVI is important for the activation? Figure 3 shows a speculative model for platelet collagen receptors. It has been reported that GPIa/IIa and GPVI are not independent of each other, but are likely to exchange signals, because upon stimulation of platelets by CRP, GPIa/IIa can be converted to a form with high affinity for soluble collagen [22], and a topographic association between GPIa/IIa and GPVI has been suggested [23]. From the following data, we think that GPVI may not be activated by collagen without help from another receptor system. When the influence of endogenous agonists was excluded, collagen scarcely increased $[Ca^{2+}]_i$ in CHS platelets [61]. Since the GPVI-mediated signaling (response to convulxin or CRP) was normal in CHS platelets and the binding of collagen to GPIa/IIa was normal [61], collagen should have been able to increase $[Ca^{2+}]_i$ via a GPVI-mediated pathway in these platelets. Nevertheless, the response to collagen was very weak in CHS platelets. This means that a signal from other receptor system is required for full activation of GPVI when collagen acts on platelets. Probably cooperation between collagen receptors, such as GPVI and GPIa/IIa, or in other words GPVI and the rhodocytin-sensitive mechanism, determines the adhesion and the platelet activation in the collagen-induced response. It is possible that a signal from the rhodocytin-sensitive mechanism plays an auxiliary role for the GPVI-dependent pathway. The extremely depressed Ca^{2+} signaling as a result of direct action of collagen in CHS platelets suggests that collagen-induced Ca^{2+} mobilization requires both signals from GPVI and other collagen receptor(s).

PERSPECTIVES

Recently, the gene responsible for CHS has been cloned in humans, mice and Japanese Black cattle [3, 28, 40]. The gene associated with CHS in these species encodes a novel

400-kDa protein, LYST, which stands for lysosome trafficking regulator. So far, biological functions of the LYST protein are not completely understood. At least it is possible that a defect in this gene is related to an abnormality of dense body function (δ-SPD) [20]. It is an important issue to clarify how alteration in LYST gene produces a defect in a protein responsible for the collagen receptor - Ca^{2+} signaling system in CHS platelets.

The available data at this moment demonstrate that CHS platelets have a defect in a signal transduction system associated with a type of collagen receptor other than GPVI. Now, the scheme that GPIa/IIa plays a role for adhesion and GPVI for platelet activation has become more complicated than ever and has to be reconstructed. Considering that the mechanism for platelet aggregation to collagen is very different among animal species [24], the role of each collagen receptor could be different depending on species. To reassess the mechanism of collagen-dependent signal transduction system, rhodocytin is a good tool and CHS platelets are a good model. Identification of target of rhodocytin is important at this moment. The rhodocytin binding protein could be GPIa/IIa itself, another receptor that interacts with GPIa/IIa or a molecule that is independent of GPIa/IIa and transmits a signal to GPVI (Fig. 3). Further study to explore the etiology of insufficient aggregation of CHS platelets will provide the information about the crosstalk among collagen receptors and the mechanism of hemostasis.

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