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Increased aggregation response of platelets in patients with inflammatory bowel disease

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Background. Platelets play an important role in hemostatic and inflammatory responses. To evaluate any potential enhancement of platelet activity in patients with inflammatory bowel disease (IBD), we measured the platelet aggregation responses to various stimuli. ***Methods.*** Twenty-two healthy controls, 24 patients with ulcerative colitis (UC) and 25 patients with Crohn's Disease (CD) were studied. The aggregation responses induced by three agonists (epinephrine, collagen, and ADP) were measured by an 8-channel aggregometer. The platelet-derived microparticles (PDMP) levels were measured by an enzyme-linked immunosorbent assay. ***Results.*** Twenty-one out of the 22 healthy controls did not respond to epinephrine (0.1 μg/ml), collagen (0.2 μg/ml), or ADP (1.0 μM). Eight out of the 12 active UC patients were sensitive to all agonists, and 4 patients showed increased sensitivity to epinephrine/collagen or epinephrine/ADP. Three out of the 12 inactive UC patients were normal, but 9 of these patients showed increased sensitivity, mainly to epinephrine. Ten out of the 12 active CD patients were sensitive to all agonists, and 2 active CD patients were sensitive to epinephrine/collagen or epinephrine/ADP. Eight out of the 13 inactive CD patients were sensitive to two or all agonists. Even after remission, almost all of the UC and CD patients showed some increased sensitivity to the agonists. The platelet number and the plasma PDMP levels were significantly higher in the active IBD patients than in the control group. ***Conclusions.*** Platelet aggregation responses are enhanced in IBD, even in inactive-phase patients. This increased sensitivity of the platelets may play an important role in the pathophysiology of IBD.

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Introduction

The precise etiologies of ulcerative colitis (UC) and Crohn's disease (CD), the two major forms of inflammatory bowel disease (IBD), remain unclear. Recent studies have suggested that the mucosal inflammation observed in IBD is not exclusively dependent on dysregulated immune responses but also involves the active participation of other cellular systems. Platelets are anuclear, nonimmune cells derived from the cytoplasm of bone marrow megakaryocytes, and play a key role in blood hemostasis and inflammation.[1,2] Recently, an increasing number of reports have described the contribution of platelets to the pathogenesis of IBD. For example, an increase in the number of circulating platelets is a common feature in active IBD patients.[3,4] A lower mean corpuscular volume of platelets is considered to be a potential marker of clinical activity in IBD.[5] Histopathological studies have revealed the presence of mucosal capillary thrombi in biopsy specimens from patients with IBD,[6,7] and thromboembolic complications are substantially increased in active IBD patients.[7,8] Furthermore, strong activation of the platelets in IBD patients is supported by the expression of platelet activation markers such as P-selectin (CD62P) and by serum measurements of β-thromboglobulin (β-TG).[9,10] Enhanced platelet activation in IBD is also supported by reports showing an elevation in various products such as platelet factor 4 (PF4) and CD40 ligand (CD40L).[9,11,12]

Platelets respond to a number of stimuli by changing their shape from discoid cells to spherical spiny cells, and by aggregating into large clumps. Experimentally, these dynamic processes can be measured by turbidometric aggregometry.[13] As the platelet suspen-

Table 1. Baseline clinical characteristics

	Control ($n = 22$)	UC ($n = 24$)	CD ($n = 25$)
Sex (M/F)	12/10	13/11	12/13
Smokers	2	4	4
Age (years)	33.4 ± 7.5	35.6 ± 15.5	32.4 ± 7.5
Disease duration (years)	—	8.7 ± 7.5	8.5 ± 6.8
Body mass index (kg/m^2)	23.8 ± 2.8	22.3 ± 1.8	21.3 ± 2.8
Platelet count (×10^4/μl)	19.2 ± 3.3	33.3 ± 15.0*	28.7 ± 9.6*
5-ASA treatment	—	24	25
Corticosteroid use	—	15	18
Type of CD			
Ileal	—	—	3
Ileocolonic	—	—	20
Colonic	—	—	5
ED over 1 year	—	—	17
CDAI > 150	—	—	12
Average CDAI			204 ± 63
Type of UC			
Proctitis	—	4	—
Left-side colitis	—	8	—
Pancolitis	—	12	—
CAI > 5	—	12	—

Values are means ± SD
CD, Crohn's disease; UC, ulcerative colitis; CDAI, Crohn's disease activity index reported by Best et al.;[17] CAI, clinical activity index of UC reported by Rachmilewitz;[15] ED, elemental diet
* $P < 0.05$ compared to control value

sion is stirred, agonists (e.g., collagen, ADP, and epinephrine) are added, and the coalescence of the individual platelets into aggregates is measured by the changes in light transmission.[13] This is a typical tool for the functional analysis of platelets, although various other markers such as sP-selectin, PF4, and β-TG are not functional but represent a consequence of platelet activation. The most characteristic feature of platelet aggregometry is its ability to serve as a functional evaluation tool of platelet samples. However, in IBD patients, there are few reports of platelet functions evaluated by aggregometry. Van Wersch et al.[4] reported that ADP (a low concentration of 0.2 or 2 μM)-induced aggregation was increased in active IBD patients. However, they failed to detect any abnormality for the other types of agonist (collagen, ristocetin, or epinephrine)-induced platelet aggregation, since they used high concentrations (10 μM) of agonists, which easily induce aggregation even in normal platelets. Webberly et al.[14] showed that platelets of IBD patients had a marked sensitivity to low concentrations of aggregating agents (ADP, collagen, and arachidonic acid). They did not find any differences between UC and CD patients, and did not perform any analysis in association with disease activity.

In this study, we evaluated the platelet functions of IBD patients by means of a detailed protocol of platelet aggregometry. The key points of this study are follows: (1) we focused on the platelet responses to low to high concentrations of agonists (epinephrine, 0.1, 0.2, and 2.0 μg/ml; collagen, 0.2, 0.5, and 2.0 μg/ml; and ADP, 1.0 and 3.0 μM); (2) we evaluated platelet function in association with disease activity and clinical course; and (3) we compared these findings with other platelet activation markers. We found that platelets are functionally activated in IBD patients. An increased sensitivity of the platelets to agonists was observed even in inactive UC and CD patients, although other platelet activation markers were not elevated. These findings suggest that platelet aggregometry is useful for the evaluation of the activation status of platelets in patients with IBD.

Methods

Patients

The backgrounds of the patients enrolled in this study are presented in Table 1. Twenty-two healthy controls (10 women and 12 men), 24 in- and outpatients with UC (11 women and 13 men), and 25 in- and outpatients with CD (13 women and 12 men) were studied (Table 1). All patients were managed at the Division of Gastroenterology at the Hospital of the Shiga University of Medical Science. The baseline characteristics of the healthy controls were matched to those of the patients with UC and CD. All patients had their diagnosis established by the usual radiological, histological, and clinical criteria.

Twelve UC patients had a high clinical activity index (CAI ≥ 5) as described by Rachmilewitz,[15] and these patients were regarded as active-phase patients.

Twelve patients with a CAI ≤ 4 were regarded as remission patients.

Twelve CD patients had a high disease activity [Crohn's disease activity index (CDAI) ≥ 150[16]], and 13 patients had a lower CDAI (CDAI < 150). In this study, those patients whose CDAI was above 150 points were regarded as active phase cases, and those patients whose CDAI was lower than 150 were defined as remission cases.

Sample collection and platelet aggregation test

To minimize any platelet activation during sample collection, blood was drawn from an antecubital vein through a 20-G needle and mixed with a one-tenth volume of ACD (2.2 g trisodium citrate, 0.807 g citric acid, 2.2 g dextrose in 100 ml of distilled water). Platelet-rich plasma (PRP) was prepared by centrifugation at 150 *g* for 5 min at room temperature. The PRP was added to one-half volume of 0.1% ethylene diamine tetra acetate/saline, and centrifuged at 1500 *g* for 20 min (platelet-poor plasma; PPP).

The number of platelets in the PRP was adjusted to 30×10^4/μl using PPP. A 200-μl aliquot of the PRP and a magnetic stir bar were added to each channel of the platelet aggregometer. Next, freshly prepared agonist solution (epinephrine, 0.1, 0.2, or 2.0 μg/ml; collagen, 0.2, 0.5, or 2.0 μg/ml; or ADP, 1.0 or 3.0 μM) was added. Platelet aggregation was then measured by a turbidometric method. The light transmission of the PPP was defined as the 100% aggregation value, and the light transmission before the addition of the agonist was regarded as 0% aggregation.

The ethics committee of Shiga University of Medical Science approved this project, and the blood was sampled with the patients' informed consent.

Measurement of platelet-derived microparticles

The platelet-derived microparticle (PDMP) levels were determined by an enzyme-linked immunosorbent assay (ELISA) system, as was recently reported by Osumi et al.[17] This system is commercially available from Japan Immunoresearch Laboratories (Takasaki, Japan). One unit/ml of PDMPs was determined to represent 24 000 solubilized platelets/ml. An ELISA system for sP-selectin was purchased from BioSource (Sunnyvale, CA, USA).

Statistical analysis

Data are expressed as means ± SD. Comparisons between the means were performed using the Mann-Whitney *U* test. Differences resulting in *P* values less than 0.05 were considered to be statistically significant.

Results

Aggregation patterns

In normal subjects, platelet aggregation was induced by a high concentration of agonist (2.0 μg/ml epinephrine, 2.0 μg/ml collagen, or 3.0 μM ADP), but the platelets did not respond to low concentrations of agonists (0.1 μg/ml epinephrine, 0.2 μg/ml collagen, or 1.0 μM ADP) (Fig. 1, pattern 1). Platelet aggregation caused by low concentrations of agonists therefore represents an increased sensitivity of the platelets.

In IBD patients, five patterns of increased platelet sensitivity were observed. In all patterns, an increased sensitivity to epinephrine was observed; this was defined as normal or pattern 1. In pattern 2, platelet aggregation was induced by low concentrations of all agonists (0.1 μg/ml epinephrine, 0.2 μg/ml collagen, or 1.0 μM ADP). In pattern 3, the platelets responded to low concentrations of epinephrine or ADP, but collagen aggregation was normal. Pattern 4 was characterized by an increased sensitivity to epinephrine or collagen, but ADP aggregation was normal. In pattern 5, epinephrine aggregation increased, but collagen and ADP aggregation were normal.

Increased sensitivity in IBD patients

Aggregation patterns in the controls and IBD patients are shown in Table 2. In the healthy controls, 1 out of 22 showed increased sensitivity to epinephrine (pattern 5), but 21 were normal (pattern 1). Similarly, the aggregation pattern was almost normal in patients with infectious colitis (data not shown).

All of the active UC patients showed an increased sensitivity of platelet aggregation. In particular, 8 out of 12 active UC patients were sensitive to all agonists (pattern 2), whereas 4 patients showed an increased sensitivity to epinephrine/collagen or epinephrine/ADP (patterns 3 or 4). Increased sensitivity was observed even in the inactive UC patients. Only 2 out of the 12 inactive UC patients were normal, but 8 patients showed an increased sensitivity to epinephrine (pattern 5). Two inactive UC patients showed pattern 3 or 4.

Similarly, 10 out of 12 active CD patients were sensitive to all agonists (pattern 2), and 2 active CD patients showed pattern 3. One characteristic of the CD patients we observed was that many inactive CD patients (CDAI < 150) were still sensitive to two or all agonists.

Platelet aggregation patterns with disease activity

We evaluated the relationship between the platelet aggregation patterns and disease activity (Table 3). In

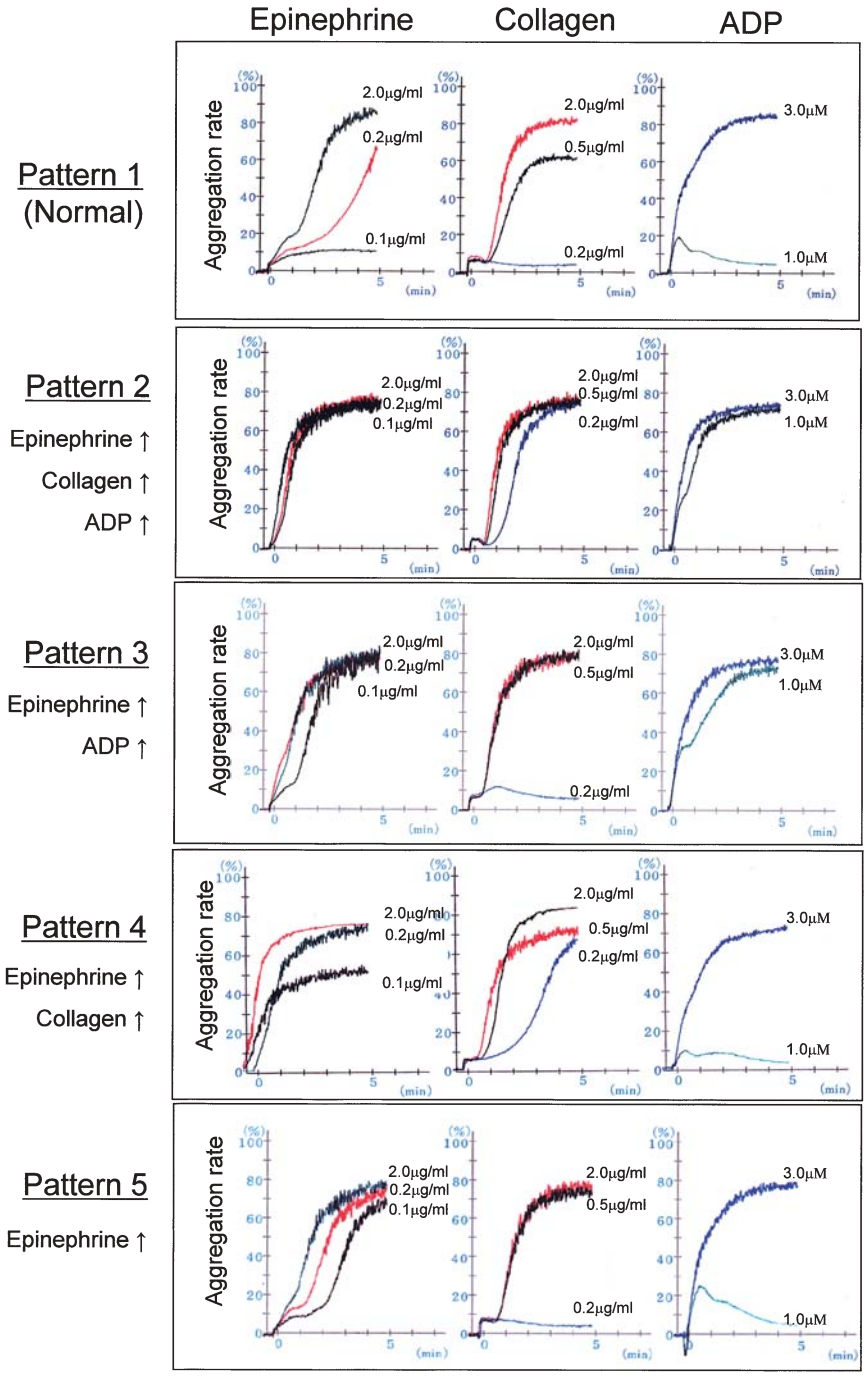

Fig. 1. Platelet aggregation patterns. The platelet number in the platelet-rich plasma (PRP) was adjusted to $30 \times 10^4/\mu l$ using platelet-poor plasma (PPP). A 200-μl aliquot of PRP was added to each channel of the platelet aggregometer, and agonist solution (epinephrine, 0.1, 0.2, or 2.0 μg/ml; collagen, 0.2, 0.5, or 2.0 μg/ml; or ADP, 1.0 or 3.0 μM) was added. Platelet aggregation was measured by a turbidometric method. The light transmission of the PPP was regarded as the 100% aggregation value, and light transmission before the addition of the agonist was regarded as 0% aggregation

patients with mild-active UC ($5 \leq CAI < 12$), the platelet aggregation distributed among patterns 2, 3, 4, and 5, but in the severe UC patients, the aggregation patterns concentrated into pattern 2 (Table 3).

We divided the inactive CD patients into two groups: a low CDAI group (CDAI < 100) and a high CDAI group ($100 \leq CDAI < 150$) (Table 3). In the inactive CD patients with a low CDAI ($CDAI \leq 100$), the aggregation patterns distributed into patterns 1, 3, 4, and 5. Like the active CD patients, many of the inactive CD patients with a high CDAI ($100 \leq CDAI < 150$) exhibited pattern 2.

Changes in aggregation pattern after remission

We analyzed the changes in the aggregation patterns after remission induction in 12 UC and 12 CD patients. Platelet aggregation was determined both during the

Table 2. Platelet aggregation patterns in inflammatory bowel disease (IBD) patients

	Pattern 1 (normal)	Pattern 2	Pattern 3	Pattern 4	Pattern 5
	Epinephrine (→) Collagen (→) ADP (→)	Epinephrine (↑) Collagen (↑) ADP (↑)	Epinephrine (↑) Collagen (→) ADP (↑)	Epinephrine (↑) Collagen (↑) ADP (→)	Epinephrine (↑) Collagen (→) ADP (→)
Healthy control (n = 22)	21 (95.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (4.5%)
Infectious colitis (n = 7)	6 (83.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (16.7%)
Active UC (n = 12)	0 (0.0%)	8 (66.7%)	2 (16.7%)	2 (16.7%)	0 (0.0%)
Inactive UC (n = 12)	2 (16.7%)	0 (0.0%)	1 (8.3%)	1 (8.3%)	8 (66.7%)
Active CD (n = 12)	0 (0.0%)	10 (83.3%)	2 (16.7%)	0 (0.0%)	0 (0.0%)
Inactive CD (n = 13)	2 (15.3%)	5 (46.2%)	2 (15.3%)	1 (7.6%)	3 (23.0%)

Table 3. Platelet aggregation patterns with disease activity

	Pattern 1 (Normal)	Pattern 2	Pattern 3	Pattern 4	Pattern 5
	Epinephrine (→) Collagen (→) ADP (→)	Epinephrine (↑) Collagen (↑) ADP (↑)	Epinephrine (↑) Collagen (→) ADP (↑)	Epinephrine (↑) Collagen (↑) ADP (→)	Epinephrine (↑) Collagen (→) ADP (→)
UC					
0 ≤ CAI ≤ 4 (n = 12)	3 (25.0%)	0 (0.0%)	1 (8.3%)	1 (8.3%)	7 (58.3%)
5 ≤ CAI < 10 (n = 7)	0 (0.0%)	2 (28.5%)	2 (28.5%)	2 (28.5%)	1 (14.2%)
10 ≤ CAI (n = 5)	0 (0.0%)	5 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
CD					
CAI ≤ 100 (n = 7)	2 (28.4%)	0 (0.0%)	1 (14.2%)	1 (14.2%)	3 (42.8%)
100 < CDAI ≤ 150 (n = 6)	0 (0.0%)	5 (83.3%)	1 (16.7%)	0 (0.0%)	0 (0.0%)
150 < CDAI ≤ 200 (n = 6)	0 (0.0%)	4 (66.7%)	2 (33.3%)	0 (0.0%)	0 (0.0%)
200 < CDAI (n = 6)	0 (0.0%)	6 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

active phase and 1 month after remission induction. As shown in Table 4, many of the active UC patients with pattern 2 switched to pattern 5 after remission. After remission, almost all UC patients still demonstrated an increased sensitivity to epinephrine. In contrast, many of the CD patients with pattern 2 still remained in pattern 2 at 1 month after remission (Table 4).

Relationship between platelet aggregation response and other markers

As shown in Fig. 2A, the number of platelets was significantly elevated in the pattern 2 group ($42.5 \pm 11.0 \times 10^4/\mu l$) as compared with the healthy controls ($20.1 \pm 4.0 \times 10^4/\mu l$; pattern 1 group). Similarly, in the pattern 3 and 4 groups ($31.6 \pm 3.9 \times 10^4/\mu l$), the platelet number was significantly higher than in the control group. On the other hand, there was no significant difference between the control group and the pattern 5 group ($24.9 \pm 4.1 \times 10^4/\mu l$).

Table 4. Changes in aggregation patterns of active IBD patients

Active phase → Remission	UC (n = 12)	CD (n = 12)
Pattern 2 → pattern 5	6 (50.0%)	2 (16.6%)
Pattern 2 → pattern 3	1 (8.3%)	1 (8.3%)
Pattern 2 → pattern 2	1 (8.3%)	7 (58.3%)
Pattern 3 → pattern 5	2 (16.7%)	2 (16.6%)
Pattern 4 → pattern 5	1 (8.3%)	0 (0.0%)
Pattern 4 → pattern 1	1 (8.3%)	0 (0.0%)

Changes in platelet aggregation were evaluated at both the active and inactive phase in 12 UC and 12 CD patients. In UC patients, assessment in the remission phase was determined 1 month after remission induction (CAI ≤ 4). In CD patients, assessment in the remission phase was performed 1 month after reaching remission (CDAI ≤ 150)

Recently, we reported that circulating PDMPs, a novel marker of platelet activation, were elevated in active IBD patients.[18] As shown in Fig. 2B, circulating PDMP levels were significantly higher in the pattern 2

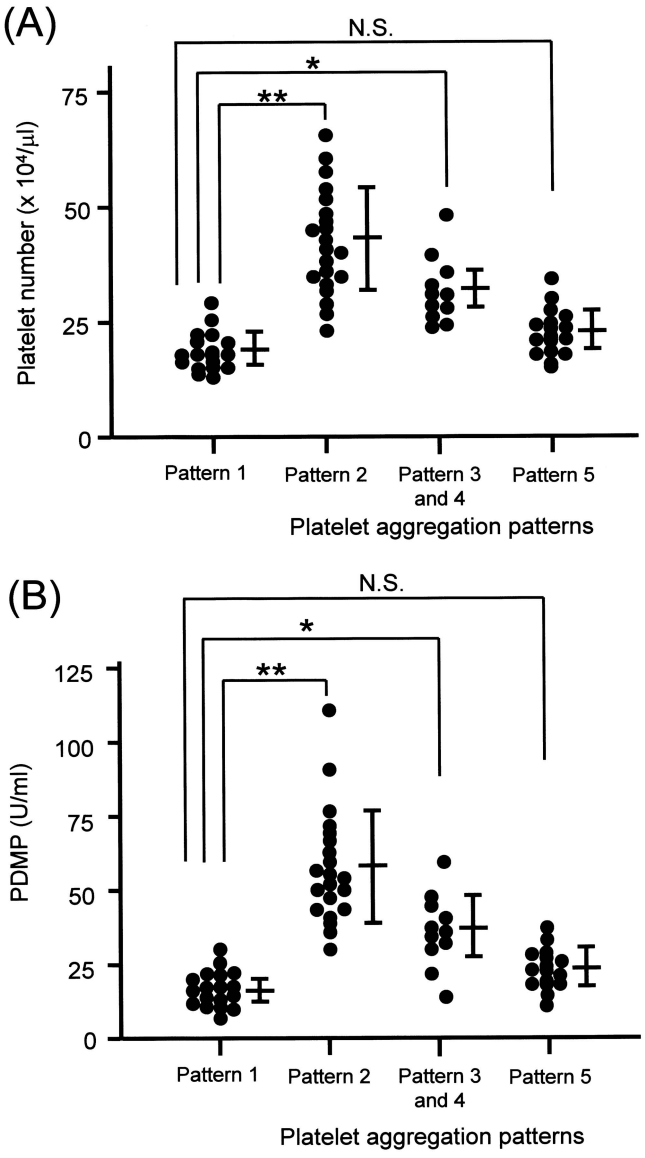

Fig. 2. Platelet number and plasma platelet-derived microparticle (*PDMP*) levels in different groups of aggregation patterns. Data points are means ± SD. $*P < 0.05$, $**P < 0.01$

group (58.3 ± 17.2 U/ml) than in the control group (17.2 ± 2.4 U/ml). PDMP levels were also increased in the pattern 3 and 4 groups (37.5 ± 2.8 U/ml). However, the PDMP levels were not significantly elevated in the pattern 5 group (24.8 ± 6.6 U/ml).

Patients with spontaneous aggregation

In five patients not included in above assessment, platelet aggregation was observed without any stimuli (spontaneous aggregation). As shown in Table 5, all of these patients had relatively higher activity. All of the UC patients with spontaneous aggregation were resistant to drugs and received a total colectomy. At 1 month after the colectomy, their aggregation patterns were still increased. Two CD patients with spontaneous aggregation exhibited pattern 2 at 1 month after remission induction.

Discussion

When platelets are exposed to aggregating agents such as epinephrine, collagen, or ADP, they change from disk shaped to a more rounded form.[13,19] In this altered form, they release their granule contents and adhere to each other. Epinephrine, collagen, and ADP are routinely used as aggregating stimuli, but the mechanisms by which they induce platelet activation have not been fully identified. Various studies have demonstrated that one common mechanism is to induce endogenous ADP from dense granules.

In the normal pattern, low concentrations of these agonists did not cause platelet aggregation. In IBD patients, increased aggregation was divided into four patterns. In these four patterns, epinephrine aggregation was characteristically involved, and combinations of the epinephrine response with a collagen response and/or an ADP response determined each pattern. Active UC and CD patients were sensitive to two or all agonists, and this indicated that their platelets were potentially highly activated during the active phase. Increased responses to these agonists tended to be asso-

Table 5. Clinical course of IBD patients with spontaneous aggregation

Case no.	Initial clinical activity	Clinical course	Final assessment of platelet aggregation
1. Case 1 (UC)	CAI 12	Total colectomy	Pattern 5 (after colectomy)
2. Case 2 (UC)	CAI 12	Total colectomy	Pattern 5 (after colectomy)
3. Case 3 (UC)	CAI 13	Total colectomy	Pattern 3 (after colectomy)
4. Case 5 (CD)	CDAI 230	Responded to infliximab	Pattern 2
5. Case 6 (CD)	CDAI 245	Responded to corticosteroid	Pattern 2

Spontaneous aggregation of platelets was observed in eight patients at initial assessment. In cases 1–3, final assessment was performed 1 month after total colectomy. In cases 4 and 5, final assessment was performed 1 month after the inactive phase was reached (CDAI ≤ 150)

ciated with disease activity, because many cases of mild disease activity were sensitive to two agonists (epinephrine/collagen or epinephrine/ADP), but all severe cases such as UC (10 ≤ CAI) and CD (200 ≤ CDAI) were sensitive to all agonists. It is possible that increased aggregation may be an effect of drugs such as corticosteroids. However, early studies have reported that corticosteroid inhibits platelet aggregation and release responses.[19]

We found that platelet aggregation responses increased even in inactive IBD patients. Many of the inactive UC patients (8 out of 12) were sensitive to epinephrine. Inactive CD patients (CDAI < 150) also showed increased sensitivity to the agonists. The subgroup of inactive CD patients with a higher CDAI (100 ≤ CDAI < 150) was sensitive to all agonists, and a subgroup with a lower inactive CDAI also showed increased sensitivity. In the inactive CD patients with a higher CDAI, gut inflammation might remain and thus stimulate platelet aggregation responses. These findings are compatible with the observations reported by Fagerstam et al.[20] They reported that platelet activation marker CD62P expression was enhanced even in inactive IBD patients. Thus, these findings indicate that platelets are potentially activated even during the inactive phase in IBD patients. Since recent studies have revealed that platelets play an active role in a variety of inflammatory and immune processes,[2] this exaggerated platelet activity even in the inactive phase could have a reinforcing and aggravating influence on the inflammatory response, and thus increase patient susceptibility to flare-ups of IBD.

One characteristic finding in this study was that the platelets of IBD patients are fundamentally sensitive to epinephrine. Even in those IBD patients with normal collagen and ADP aggregation, epinephrine aggregation was selectively enhanced. To our knowledge, such a specific elevation of epinephrine aggregation has not been reported in other diseases. The presence of α_2-adrenoceptors on human platelets has been previously reported,[21] and it is recognized that epinephrine aggregation is mediated by α_2-adrenoceptors since it is antagonized by α_2-antagonists but not by α_1-antagonists.[21] These findings suggest that the platelets of IBD patients are potentially sensitive to sympathetic stimuli, even during the inactive phase. In general, the sympathetic nervous system is stimulated by proinflammatory stimuli, which are secreted in the periphery and appear in the systemic circulation.[22–24] For example, the plasma levels of neuropeptide Y (NPY), a marker of the activity of the sympathetic nervous system,[25] are elevated in inactive and active IBD patients. Thus, some parts of platelet activation in IBD patients may be associated with excitation of the sympathetic nervous system.

Spontaneous aggregation is characterized by platelet aggregation without any stimuli, and this indicates hyperactivation of platelets. In this study, spontaneous aggregation was observed in five severe cases. In particular, three patients with UC were resistant to steroid and/or cyclosporine A, and received total colectomy. Although the number of cases is small, this finding suggests that spontaneous aggregation may be one factor predicting therapeutic intractability of UC patients. To confirm this speculation, further study is necessary.

Recently, we reported that platelet-derived microparticles are novel markers of platelet activation in IBD patients.[18] The generation of PDMPs is associated with platelet activation induced by stimuli such as high shear stress, thrombin, collagen, and calcium ions.[26–28] PDMPs play a role in hemostatic responses under normal and pathological conditions.[28] In addition, previous studies have reported a role for PDMPs during inflammatory responses.[28] As shown in Fig. 2B, the PDMP levels were significantly elevated in groups 2, 3, and 4. Since almost all of these patients were in the active phase, PDMP elevation in these groups is compatible with the results of our recent study showing that circulating PDMP levels are correlated with the disease activity of IBD patients. On the other hand, in group 5, in which epinephrine aggregation was elevated, the PDMP levels were within the normal range. Since PDMPs are generated as a consequence of platelet activation,[28] these results indicate that the selective increase of epinephrine aggregation in the inactive phase reflects a potentially activated state of the platelets, but not ongoing platelet aggregation.

In conclusion, we demonstrated that platelet aggregation responses are enhanced in IBD patients. These responses were potentially enhanced even in inactive IBD patients. Since recent studies have demonstrated that platelets play a role not only in hemostasis but also in inflammatory and immune responses, the increased platelet activity observed in this study may be an important factor involved in the pathophysiology of IBD.

References

1. Danese S, de la Motte C, Fiocchi C. Platelets in inflammatory bowel disease: clinical, pathogenic, and therapeutic implications. Am J Gastroenterol 2004;99:938–45.
2. Weyrich AS, Zimmerman GA. Platelets: signaling cells in the immune continuum. Trends Immunol 2004;25:489–95.
3. Talstad I, Rootwelt K, Gjone E. Thrombocytosis in ulcerative colitis and Crohn's disease. Scand J Gastroenterol 1973;8:135–8.
4. van Wersch JW, Houben P, Rijken J. Platelet count, platelet function, coagulation activity and fibrinolysis in the acute phase of inflammatory bowel disease. J Clin Chem Clin Biochem 1990;28: 513–7.

5. Kapsoritakis AN, Koukourakis MI, Sfiridaki A, Potamianos SP, Kosmadaki MG, Koutroubakis IE, et al. Mean platelet volume: a useful marker of inflammatory bowel disease activity. Am J Gastroenterol 2001;96:776–81.
6. Wakefield AJ, Sawyerr AM, Dhillon AP, Pittilo RM, Rowles PM, Lewis AA, et al. Pathogenesis of Crohn's disease: multifocal gastrointestinal infarction. Lancet 1989;2:1057–62.
7. Quera R, Shanahan F. Thromboembolism—an important manifestation of inflammatory bowel disease. Am J Gastroenterol 2004;99:1971–3.
8. Miehsler W, Reinisch W, Valic E, Osterode W, Tillinger W, Feichtenschlager T, et al. Is inflammatory bowel disease an independent and disease specific risk factor for thromboembolism? Gut 2004;53:542–8.
9. Collins CE, Cahill MR, Newland AC, Rampton DS. Platelets circulate in an activated state in inflammatory bowel disease. Gastroenterology 1994;106:840–5.
10. Vrij AA, Rijken J, Van Wersch JW, Stockbrugger RW. Platelet factor 4 and beta-thromboglobulin in inflammatory bowel disease and giant cell arteritis. Eur J Clin Invest 2000;30:188–94.
11. Danese S, de la Motte C, Sturm A, Vogal JD, West GA, Strong SA, et al. Platelets trigger a CD40-dependent inflammatory response in the microvasculature of inflammatory bowel disease patients. Gastroenterology 2003;124:1249–64.
12. Danese S, Katz JA, Saibeni S, Papa A, Gasbarrini A, Vecchi M, et al. Activated platelets are the source of elevated levels of soluble CD40 ligand in the circulation of inflammatory bowel disease patients. Gut 2003;52:1435–41.
13. Adamus GA. Platelet aggregation. In: Longenecker GL, editor. The platelets, physiology and pharmacology. New York: Academic; 1985. p. 1–11.
14. Webberley MJ, Hart MT, Melikian V. Thromboembolism in inflammatory bowel disease: role of platelets. Gut 1993;34:247–51.
15. Rachmilewitz D. Coated mesalazine (5-aminosalicylic acid) versus sulphasalazine in the treatment of active ulcerative colitis: a randomised trial. BMJ 1989;298:82–86.
16. Best WR, Becktel JM, Singleton JW, Kern F Jr. Development of a Crohn's disease activity index. National Cooperative Crohn's Disease Study. Gastroenterology 1976;70:439–44.
17. Osumi K, Ozeki Y, Saito S, Nagamura Y, Ito H, Kimura Y, et al. Development and assessment of enzyme immunoassay for platelet-derived microparticles. Thromb Haemost 2001;85:326–30.
18. Andoh A, Tsujikawa T, Hata K, Araki Y, Kitoh K, Sasaki M, et al. Elevated circulating platelet-derived microparticles in patients with active inflammatory bowel disease. Am J Gastroenterol 2005;100:2042–8.
19. Packham MA, Mustard JF. Clinical pharmacology of platelets. Blood 1977;50:555–573.
20. Fagerstam JP, Whiss PA, Strom M, Andersson RG. Expression of platelet P-selectin and detection of soluble P-selectin, NPY and RANTES in patients with inflammatory bowel disease. Inflamm Res 2000;49:466–72.
21. Yun-Choi HS, Park KM, Pyo MK. Epinephrine induced platelet aggregation in rat platelet-rich plasma. Thromb Res 2000;100: 511–8.
22. Straub RH, Herfarth H, Falk W, Andus T, Scholmerich J. Uncoupling of the sympathetic nervous system and the hypothalamic-pituitary-adrenal axis in inflammatory bowel disease? J Neuroimmunol 2002;126:116–25.
23. Nakajima A, Sendo W, Tsutsumino M, Koseki Y, Ichikawa N, Akama H, et al. Acute sympathetic hyperfunction in overlapping syndromes of systemic lupus erythematosus and polymyositis. J Rheumatol 1998;25:1638–41.
24. Perry F, Heller PH, Kamiya J, Levine JD. Altered autonomic function in patients with arthritis or with chronic myofascial pain. Pain 1989;39:77–84.
25. Morris MJ, Russell AE, Kapoor V, Cain MD, Elliott JM, West MJ, et al. Increases in plasma neuropeptide Y concentrations during sympathetic activation in man. J Auton Nerv Syst 1986; 17:143–9.
26. VanWijk MJ, VanBavel E, Sturk A, Nieuwland R. Microparticles in cardiovascular diseases. Cardiovasc Res 2003;59:277–87.
27. Gemmell CH, Sefton MV, Yeo EL. Platelet-derived microparticle formation involves glycoprotein IIb-IIIa. Inhibition by RGDS and a Glanzmann's thrombasthenia defect. J Biol Chem 1993;268:14586–9.
28. Nomura S. Function and clinical significance of platelet-derived microparticles. Int J Hematol 2001;74:397–404.