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IgG inhibits the increase of platelet-associated C_3 stimulated by anti-platelet antibodies

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SUMMARY

We investigated the increase of platelet-associated IgG and complement component 3 (C_3) caused by the *in vitro* action of anti-platelet MoAbs, and the effect of mouse and human IgG on these events. Anti-glycoprotein IIb/IIIa and anti-glycoprotein Ib MoAbs caused a slight increase of C_3, but not of platelet-associated IgG. In contrast, anti-CD9 and anti-Fcγ II receptor MoAbs caused an increase of both platelet-associated C_3 and IgG. In particular, three MoAbs which activated the complement system caused a marked increase of C_3. When platelet-rich plasma was treated with aspirin and prostaglandin E_1 before incubation with antibodies, the increase of platelet-associated IgG was inhibited in all cases. In contrast, the increase of platelet-associated C_3 was scarcely influenced. These results suggest that the binding to platelets of platelet-activating antibodies caused the increased expression of IgG molecules on the platelet surface and a possible increase of platelet-associated IgG. However, the increase of platelet-associated C_3 appeared to depend on specific characteristics of the antibodies tested, such as a complement-activating effect. In addition, intact mouse or human IgG inhibited the increase of platelet-associated C_3 caused by complement-activating antibodies, while $F(ab')_2$ mouse or human IgG had no such effect. This suggested that the Fc portion of IgG may block the increase of C_3 mediated by anti-platelet antibodies.

Keywords IgG Fc portion platelet-associated C_3 anti-platelet antibody idiopathic thrombocytopenic purpura

INTRODUCTION

Idiopathic thrombocytopenic purpura (ITP) is a syndrome which is caused by circulating antibodies that react with the platelet membrane [1,2]. It is thought that platelet-associated IgG (PAIgG) has an important role in the mechanism of ITP, because an increase in PAIgG is closely correlated with a decrease in the platelet count in this disease [1–5]. Furthermore, high levels of PAIgM and platelet-associated complement component 3 (PAC_3) are also considered to be of pathogenetic relevance to thrombocytopenia in ITP [2,6–9].

The short-term beneficial effect of high-dose intravenous IgG in children and adults with chronic ITP is widely accepted [10,11], but the reason for the success of this therapy remains unclear. We have recently found that some anti-platelet MoAbs can activate the complement system [12,13]. In this study, we investigated whether or not PAIgG and PAC_3 were increased by the action of anti-platelet MoAbs and also assessed the effect of IgG on these events.

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MATERIALS AND METHODS

Monoclonal antibodies

The following MoAbs were used: NNKY1-32 (anti-glycoprotein (GP) IIb/IIIa) [14,15], NNKY2-11 (anti-GPIIb/IIIa) [15], NNKY5-4 (anti-GPIb/IX) [15], NNKY5-5 (anti-GPIb) [15], NNKY1-19 (anti-CD9) [13], MALL13 (anti-CD9) [13], and NNKY3-2 and NNKY4-7 (anti-Fcγ II receptor) [12].

Platelet activation

Platelet-rich plasma (PRP) was obtained as described previously [14,15] and stimulation with MoAb was performed by incubating PRP for 15 min at 22°C without stirring in the presence of each MoAb at final concentrations of 5–10 μg/ml. Following incubation, the samples were immediately treated without centrifugation using 1% paraformaldehyde in EDTA/PBS (pH 7·2). Activated platelets were then incubated with saturating concentrations of fluorescein-conjugated anti-human IgG (5 μg/ml; Cappel Products, Westchester, PA) or anti-human C_3 (5 μg/ml; Cappel). After washing, the samples were subjected to flow cytometry.

Table 1. The increase of platelet-associated IgG (PAIgG) and platelet-associated complement component 3 (PAC_3) caused by various MoAbs

Antibody				
Epitope	Designation	Subclass	PAIgG(%)	PAC_3(%)
No antibody	(Control)		9·8±3·3	5·4±3·9
GPIIb/IIIa				
	NNKY1-32	IgG2a	10·4±4·4	15·6±5·5
	NNKY2-11	IgG2a	10·3±3·6	12·3±2·3
GPIb				
	NNKY5-4	IgG1	10·8±5·2	12·4±3·3
	NNKY5-5	IgG2b	13·4±4·8	20·5±5·2
CD9				
	NNKY1-19	IgG1	18·4±6·3	22·4±4·5
	MALL13	IgG2a	19·5±6·8	39·4±5·6
FcγII receptor				
	NNKY3-2	IgM	20·3±8·3	42·3±6·5
	NNKY4-7	IgM	20·9±4·8	41·9±7·2

Results are the mean±s.d. of three experiments.

Effects of inhibitors on PAC_3 and PAIgG

The effects of two inhibitors on PAC_3 and PAIgG were tested by adding aspirin (500 μM; Wako Pure Chemical Industries, Osaka, Japan) plus prostaglandin E_1 (5 μg/ml; Sigma Chemical Co., St Louis, MO) to PRP before incubation with the MoAbs.

Effects of IgG on PAC_3

PRP was incubated with IgG under various conditions before stimulation with MoAbs. The PRP was then processed as described above for platelet activation. Intact mouse IgG and intact human IgG were used (1 mg/ml; Zymed Laboratories, Inc., San Francisco, CA), as were $F(ab')_2$ fragments of mouse and human IgG (1 mg/ml; Cappel).

Flow cytometry [16–18]

A Becton Dickinson (Oxnard, CA) FACScan was used for flow cytometry, and forward light scatter was measured as an index of particle size and fluorescence. All data were collected using four-decade logarithmic amplication. Fluorescence was used to trigger the instrument, and the threshold was set so that only particles having a fluorescence at least equal to that of a single platelet were measured. The data were collected in list mode files and were analysed at a later time. Beads with a diameter of 2 μm were used for calibration of forward light scatter, and Calibrate beads (Becton Dickinson) were used to calibrate the fluorescence parameters. The negative control (platelets incubated with fluorescein-labelled goat anti-mouse IgG; Kirkegaard & Perry Labs, Inc., Gaithersburg, MD) was set so that the fluorescence-positive rate was infinitely close to 1%. The fluorescence-positive rate was then calculated from the total platelet count above the negative line. Ten thousand events were analysed in the one-colour analysis.

RESULTS

The anti-GPIIb/IIIa and anti-GPIb antibodies caused a slight increase of PAC_3, but no increase of PAIgG (Table 1). On the

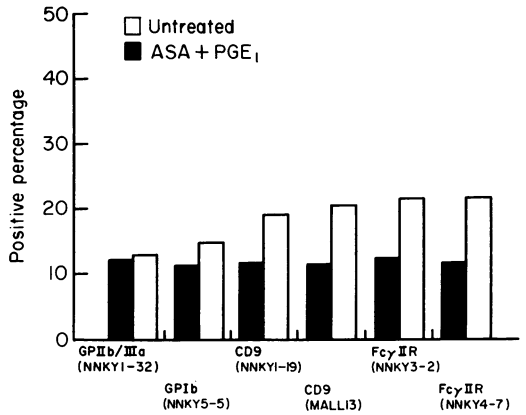

Fig. 1. Effects of aspirin (ASA) and prostaglandin E_1 (PGE_1) on the increase of platelet-associated IgG (PAIgG) (■). When platelet-rich plasma (PRP) was treated with aspirin and prostaglandin E_1 before incubation with the MoAbs, the increase of PAIgG was inhibited in all cases.

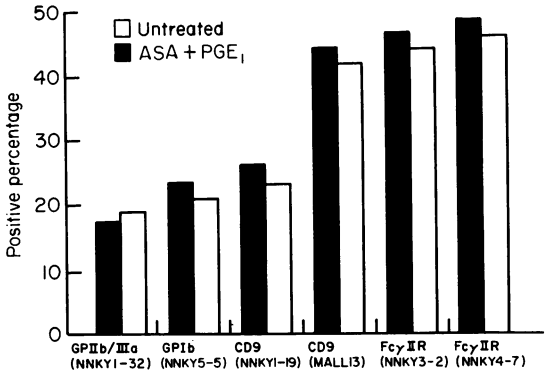

Fig. 2. Effects of aspirin (ASA) and prostaglandin E_1 (PGE_1) on the increase of platelet-associated complement component 3 (PAC_3) (■). When platelet-rich plasma (PRP) was treated with aspirin and prostaglandin E_1 before incubation with the MoAbs, there was virtually no influence on the increase of PAC_3 caused by each antibody.

Table 2. Effect of IgG on the increase of platelet-associated complement component 3 (PAC_3) caused by various complement-activating MoAbs

	IgG-treated PAC_3/untreated PAC_3		
Antibodies	MALL13	NNKY3-2	NNKY4-7
Mouse IgG			
Intact	0·56±0·09	0·28±0·05	0·31±0·08
$F(ab')_2$	1·01±0·18	1·04±0·15	1·03±0·16
Human IgG			
Intact	0·49±0·06	0·30±0·05	0·29±0·06
$F(ab')_2$	1·02±0·21	0·98±0·19	1·02±0·22

Results are the mean±s.d. of five experiments.

other hand, the anti-CD9 and anti-FCγII receptor antibodies caused an increase of both PAC_3 and PAIgG. In particular, the three MoAbs which activate the complement system (MALL13), NNKY3-2 and NNKY4-7) caused a marked increase of PAC_3 (Table 1).

Next, we studied the effects of aspirin and prostaglandin E_1 on the increase of PAIgG (Fig. 1). When PRP was treated with aspirin plus prostaglandin E_1 before incubation with the MoAbs, the increase of PAIgG was inhibited in all cases. On the other hand, the response of PAC_3 to the MoAbs was little influenced by the addition of aspirin and prostaglandin E_1 (Fig. 2).

The effect of IgG on the increase of PAC_3 caused by the MoAbs is shown in Table 2. Both intact mouse and human IgG inhibited the increase of PAC_3 caused by MALL13, NNKY3-2, or NNKY4-7. In contrast, $F(ab')_2$ mouse or human IgG had no such effect.

DISCUSSION

Our results indicate that when platelet-activating antibodies bind to platelets, there is an increase of IgG on the platelet surface, and PAIgG may also increase. This increase was inhibited by anti-platelet agents (aspirin and prostaglandin E_1). IgG appears to be present inside normal platelets, and George [19] has reported that intraplatelet IgG is stored in the α-granules. Thus, intraplatelet IgG may be released from platelets after their activation by the anti-platelet antibodies. PAC_3 also increased with the binding of the MoAbs to platelets, and this increase was scarcely influenced by the anti-platelet agents. Thus, this increase appeared to depend on effects of the MoAbs such as complement activation, rather than on platelet activation *per se* as in the case of PAIgG.

Participation of PAIgM and PAC_3 in the mechanism of thrombocytopenia in ITP has been reported in addition to the role of PAIgG [9], suggesting that prevention of an increase in PAC_3 could be a possible target of therapy in this disease. Intravenous IgG is one of the representative therapies for ITP, and its mechanism of action has been speculated on for many years. Previous studies have raised the possibility that intravenous IgG acts via an influence on the IgG Fc receptors of phagocytic cells and B lymphocytes [20]. In the present study, intact IgG inhibited the increase of PAC_3, while $F(ab')_2$ fragments of IgG could not do so. This finding suggests that the Fc portion of IgG may inhibit the increase of PAC_3.

It was previously reported that fluid-phase IgG can competitively block C_3 uptake by particles after the *in vitro* activation of C_3 by trypsin [21]. Since deposition of opsonic C_3 fragments onto antibody-coated targets has an important role in the *in vivo* clearance of foreign material, inhibition of this process may have a bearing on at least the acute response of ITP to intravenous IgG [22]. It has been discovered that intravenous IgG also inhibits Forssman shock, which is a type II allergic reaction [20]. Animal studies of Forssman shock have suggested the possibility that the Fc portion of IgG might inhibit the binding of C_{3b} or C_{4b} to immune complexes, and consequently prevent their uptake by macrophage, or reduce immune complex-mediated damage to cells and tissues [23–25].

We investigated the increase of PAIgG and PAC_3 in response to anti-platelet antibodies and the effect of IgG on this process. Platelet activation by the anti-platelet antibodies did not cause an increase of both PAIgG and PAC_3. In particular, the increase of PAC_3 seemed to depend on complement activation by the anti-platelet antibodies, and the Fc portion of IgG seemed to inhibit this increase of PAC_3.

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