PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 186, 378-383 (1987)

RAPID COMMUNICATIONS

INHIBITION OF HUMAN TUMOR CELL INDUCED PLATELET AGGREGATION BY ANTIBODIES TO PLATELET GLYCOPROTEINS Ib AND IIb/IIIa

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Abstract: Tumor cell induced platelet aggregation was shown to be inhibited in a dose dependent manner by preincubation of human platelets with antibodies to platelet glycoprtein Ib and the IIb/IIIa complex. Combination of antibody to Ib and antibody to the IIb/IIIa complex at concentrations which produced half maximal inhibition of platelet aggregation alone caused complete inhibition of tumor cell induced platelet aggregation. Antibodies to platelet glycoproteins Ib and the IIb/IIIa complex also inhibited platelet synthesis of thromboxane A2, but not synthesis of 12-hydroxyeicosatrienoic acid. Inhibition of tumor cell induced platelet aggregation with antibodies against platelet glycoproteins suggests a role for these glycoproteins in tumor cell-platelet interactions and possibly platelet facilitated tumor cell metastasis.

Introduction

Arrest and adhesion of tumor cells within the host microvasculature has been suggested to be one of the rate limiting steps in the metastatic cascade. Studies have demonstrated a correlation between the ability of tumor cells to induce platelet aggregation in vitro and their ability to metastasize in vivo (1). Numerous human and animal tumor cells induce platelet aggregation and this interaction of host platelets with circulating tumor cells is proposed to facilitate tumor cell attachment to endothelial cells and subendothelial matrix (2-4). Although the role of platelets in metastasis is presently unclear, various mechanisms have been proposed. For example, upon platelet stimulation the released contents of alpha granules may provide tumor cell attachment factors (i.e., fibronectin, thrombospondin, and von Willebrand factor). Platelet membrane components may also be involved as tumor cell induced platelet aggregation (TCIPA) results in extensive platelet-tumor cell membrane interactions (3). Although the mechanism(s) is still largely unknown, platelet surface glycoproteins have been shown to play a role in aggregation, adhesion, and surface contact interactions (5). Of the five known platelet surface glycoproteins, platelet glycoprotein Ib (GpIb) and the glycoprotein IIb/IIIa complex (GpIIb/IIIa) were selected for investigation because of their reported involvement in platelet aggregation and platelet adhesion to subendothelial matrix. GpIb has been established as the von Willebrand receptor (6) and as the thrombin receptor (7). Thus it seems reasonable to believe GpIb could play a key role in the interaction of platelets with subendothelial surfaces. The GpIIb/IIIa complex exists in the presence of calcium as a heterodimer composed of glycoprotein IIb and glycoprotein IIIa (8,9) and functions as the receptor for fibrinogen, fibronectin, and also von Willebrand factor on activated platelets (5). It is also required during aggregation as the fibrinogen receptor (10), since fibrinogen is proposed as a linking molecule in platelet-platelet interactions (11). In the present study we used antibodies against the platelet glycoproteins Ib and the IIb/IIIa complex to investigate their role in TCIPA.

Methods

Cell Cultures: Human cervical carcinoma (MS751) cells were obtained from American

0037-9727/87 $1.50

Type Cell Culture, (Rockville, MD) and grown in Eagles minimal essential medium (MEM) supplemented with essential and nonessential amino acids and 10% fetal bovine serum, (Gibco, Grand Island, NY). Human colon carcinoma (Clone A) cells were generously supplied by Dr. D. Dexter (E.I. Du Pont De Nemours, Wilmington, DE), and were grown in Dulbeccos modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (Gibco, Grand Island, NY) in a humidified atmosphere of 5% CO_2. Both cell lines were passaged every 5 days and only cells with greater than 90% viability (trypan blue exclusion) were used for the aggregometry studies.

Isolation of Human Platelets: Blood (150 ml) was drawn from the antecubital vein of normal healthy volunteers who had not ingested drugs (48 hours prior) into heparin (5 units/ml final conc.). The blood was centrifuged at 500 xg (25 min; 20°C) and the supernatant aspirated with a polypropylene pipet and set aside. The blood was then centrifuged at 700 xg (15 min; 20°C) and the supernatant was aspirated, combined with the first supernatant, and designated platelet rich plasma (PRP). The remaining blood was centrifuged at 3500 xg (20 min; 20°C) and the supernatant removed and designated platelet poor plasma (PPP).

Antibodies: Monoclonal antibody against human platelet GpIb (mAbIb) was obtained from Dakopatts Co. (Glostrup, Denmark). Polyclonal antibodies against platelet GpIIb/IIIa (pAbIIb/IIIa) were elicited in rabbits with purified platelet IIb/IIIa as previously described (12). Immunoglobin G fraction of anti-transferrin antibody was obtained from Cooper Biomedical (Malvern, PA). Thrombin and apyrase were obtained from Sigma (St.Louis, MO). D-Phenylalanyl-L-L-prolyl-L-arginine chloromethyl ketone (PPACK) was obtained from Calbiochem (La Jolla, CA).

Aggregation Studies: PRP was adjusted to $1.9x10^8$ platelets/ml with PPP. Tumor cells were adjusted to $2.0x10^7$ cells/ml. Platelets ($4.5x10^7$/cuvette) were preincubated in aggregometry cuvettes with varying concentrations of antibodies at 37°C. Platelets were challenged with $1.5x10^5$ tumor cells and the aggregation observed with a model DP-247E Aggregometer (Sienco, Morrison, CO).

Radioimmunoassay: Radioimmunoassay was used to measure the major in vitro eicosanoid metabolites of platelets [i.e., thromboxane A_2, measured as TXB_2 and 12-hydroxyeicosa-tetraenoic acid (12-HETE)]. Samples were terminated by transferring the contents of the aggregometry cuvette into 1 ml of cold (4°C) acetone, extracted and assayed as previously described (13).

Results

The addition of either MS751 or Clone A cells to homologous platelets induced aggregation after a lag time of approximately 2 min. Platelets pretreated with apyrase or PPACK were challenged with MS751 or Clone A cells. Apyrase degrades ADP while PPACK is a selective thrombin inhibitor. Complete inhibition of aggregation was observed in PPACK treated platelets, but not in apyrase treated platelets suggesting MS751 and Clone A cells induce platelet aggregation via a thrombin dependent mechanism (data not shown). PRP preincubated with varying concentrations of either mAbIb or pAbIIb/IIIa demonstrated dose dependent inhibition of TCIPA by both MS751 and Clone A cells (Fig. 1). Preincubation of PRP with concentrations of mAbIb and pAbIIb/IIIa which produced half maximal inhibition of TCIPA alone completely inhibited TCIPA by MS751 and Clone A cells (Fig. 2). ADP (9 μM) induced platelet aggregation was not inhibited by either mAbIb or pAbIIb/IIIa. However, thrombin (0.7 μM) induced platelet aggregation was partially inhibited by either mAbIb or pAbIIb/IIIa (data not shown). We have previously demonstrated that TXA_2 and 12-HETE are required for the induction of TCIPA (14). Therefore we measured the extent of platelet eicosanoid metabolism during TCIPA in the presence and absence of mAbIb and pAbIIb/IIIa. In the absence of antibodies platelets stimulated with MS751 or Clone A **Clone A cells synthesized 11.5±1.4 ng/ml (X±S.D.) TXB_2 and 272±112 ng/ml and 450±7 ng/ml 12-HETE, respectively. Pretreatment of platelets with pAbIIb/IIIa prior to tumor cell stimulation resulted in a decrease in TXB_2 biosynthesis. Under similar conditions results with 12-HETE were inconsistent. However, mAbIb inhibited thrombin stimulated TXB_2 and 12-HETE biosynthesis by platelets from two donors (Table 1), while pAbIIb/IIIa was ineffective in inhibiting thrombin stimulation of TXA2 and 12-HETE (data not shown). To demonstrate that the inhibitory effect of these antibodies was specific, platelets were pretreated with antibodies to an unrelated antigen (transferrin) prior to tumor cell stimulation. Antitransferrin antibodies had no significant effect on TCIPA or TXB_2 and 12-HETE biosynthesis when used at 2 fold higher concentration than mAbIb or pAbIIb/IIIa (data not shown).**

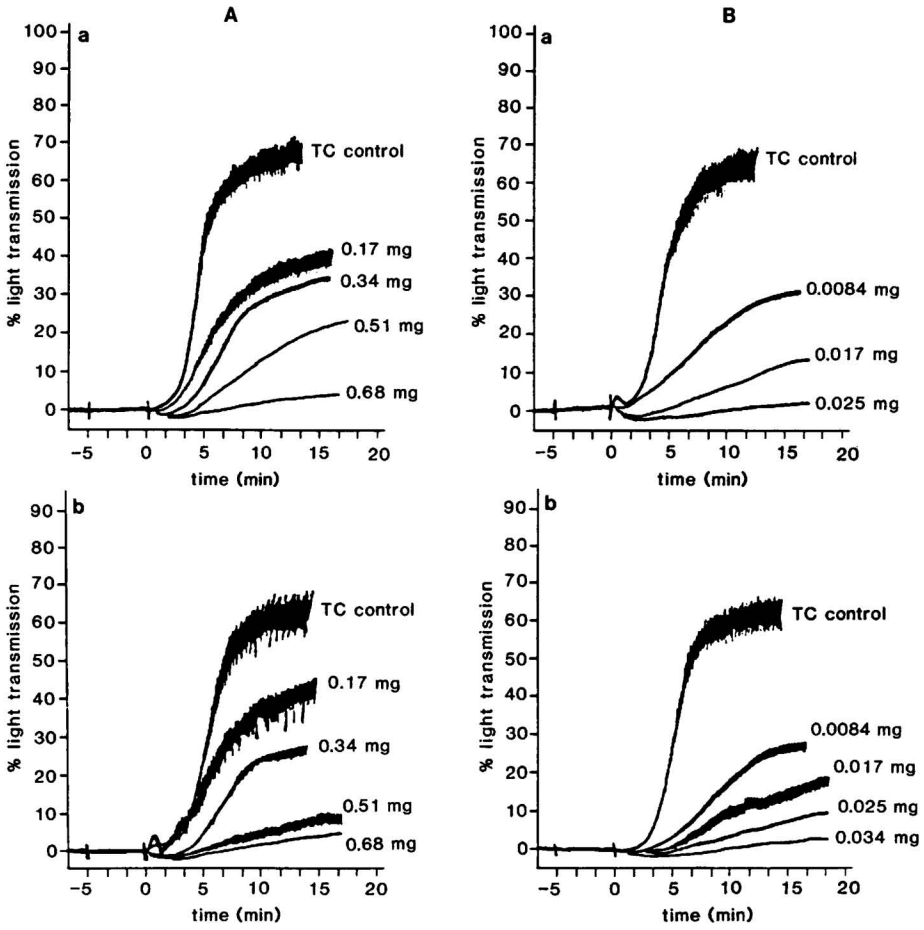

Fig. 1 Effects of antibodies to platelet glycoprotein Ib and the IIb/IIIa complex on TCIPA. Platelets preteated with various concentrations of mAbIb (A) and pAbIIb/IIIa (B) 5min prior to TC addition demonstrate a dose dependent inhibition of TCIPA by MS751 (a) and Clone A (b) cells (mg equals total antibody protein (mAbIb) or IgG fraction protein (pAbIIb/IIIa) per aggregometry cuvette).

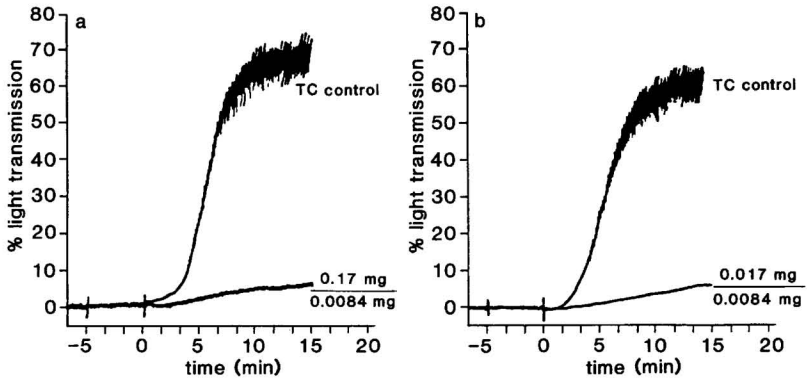

Table 1. Effects of Antibodies to Platelet Glycoproteins Ib and IIb/IIIa on Platelet Synthesis of TXA_2 and 12-HETE during TCIPA by MS751 and Clone A Cells

	MS751		Clone A	
Condition	TXB_2*	12-HETE*	TXB_2*	12-HETE*
Plt/PRP	1.8±0.6**	120± 0	1.5±0.1	64± 5
TC/Plt control	11.5±1.4	277±112	11.5±1.0	450± 74
TC/Plt:				
+0.17mg*** mAbIb	2.5±1.4	339 ± 20	0.5±0.3	146± 6
+0.34mg mAbIb	1.8±0.2	207± 81	0.4±0.1	211± 5
+0.51mg mAbIb	1.2±0.3	216± 79	0.3±0.2	74± 5
+0.0084mg pAbIIb/IIIa	6.2±0.6	331±207	2.7±1.5	284± 26
+0.017mg pAbIIb/IIIa	3.4±0.1	188± 90	2.3±0.2	324±135
+0.025mg pAbIIb/IIIa	1.7±0.2	264±130	1.4±0.4	376± 20
+0.17mg mAbIb and 0.0084mg pAbIIb/IIIa	2.3±1.0	228± 31	1.2±0.6	348±248
Thrombin/Plt	31.4±9.4	1200±121	33.7±0.8	443± 46
+0.34mg mAbIb	21.4±9.0	696±476	17.8±1.8	433± 63
±1.36mg mAbIb	5.6±3.5	393± 103	6.7±3.3	286± 78

[*ng/ml concentration; **n=3; ***Total mg of antibody protein (mAbIb) or IgG fraction protein (pAbIIb/IIIa) per cuvetteng/ml concentration; Plt, platelet; TC, tumor cell]

Discussion

We previously demonstrated both in vivo and in vitro that TCIPA involves extensive platelet-tumor cell membrane interactions (3). Ultrastructural studies have shown that platelets become associated with distinct areas on tumor cell surfaces rather than their entire circumference. We interpret this as a focal interaction between platelets and tumor cells (3,15). In the absence of overt platelet aggregation (washed platelets in the absence of PPP), platelets were adherent to the tumor cell surface without a platelet release reaction or aggregation (16). This provides further evidence for a selective interaction between the tumor cell plasma membrane and the platelet plasma membrane prior to the initiation of aggregation. Factors responsible for this interaction are as yet unknown. However, platelet-platelet interactions and platelet interaction with the subendothelial matrix are mediated by platelet surface glycoproteins (5). Therefore it is reasonable to predict that these platelet glycoproteins may mediate tumor cell-platelet interactions and possibly platelet facilitated tumor cell adhesion to subdendothelial matrix (4).

Platelet glycoprotein Ib is known to function as a thrombin receptor (7). We have demonstrated (this study) that MS751 and Clone A tumor cells induce platelet aggregation via the generation of thrombin. Therefore the inhibition of aggregation in the presence of mAbIb may be due to the inhibition of thrombin stimulated platelet aggregation. However this does not exclude a role for GpIb in mediating tumor cell platelet adhesion. Glycoprotein IIb/IIIa is a receptor for fibrinogen. Fibrinogen possesses divalent binding sites for GpIIb/IIIa and serves as a binding molecule during platelet-platelet aggregation (11). Glycoprotein IIb/IIIa is also a receptor for components of the extracellular matrix (i.e., fibronectin and von Willebrand factor), thus mediating platelet interaction with the vessel wall. The observed inhibition of TCIPA with pAbIIb/IIIa is probably due (in part) to an inhibition of fibrinogen binding to GpIIb/IIIa and subsequent platelet-platelet interactions. However, this receptor also mediates platelet adhesion to the tumor cell plasma membrane (17 and unpublished observations).

Hemostatic metastasis is the successful intravasation, dissemination, arrest and extravasation of tumor cells and involves a series of cell-cell interactions within the vasculature. Circulating tumor cell association with host platelets is one of the cell-cell interactions which is believed to facilitate

Fig. 2 Effects of the combination of antibodies to platelet glycoproteins on TCIPA. Platelets pretreated with mAbIb (0.17mg) and pAbIIb/IIIa (0.0084mg) at concentrations which produced half maximal inhibition alone were shown to completely inhibit TCIPA by MS751 (a) and Clone A (b) cells (mg equals total antibody protein (mAbIb) or IgG fraction protein (pAbIIb/IIIa) per aggregometry cuvette).

metastasis (1). We have postulated that platelets function during the early arrest stage of metastasis by stabilizing the bond between the tumor cell and the endothelial cell and/or the subendothelial matrix (2-4). In addition we demonstrated that platelet facilitated tumor cell adhesion to subendothelial matrix is not mediated by platelet release reaction products(18), but is dependent upon an intact platelet membrane, platelet membrane receptor mobility, and an intact platelet cytoskeleton (4,18). The receptor(s) responsible for tumor cell attachment to platelets, endothelial cells and subendothelial matrix is still largely unknown. We have demonstrated (this study) that GpIb and the GpIIb/IIIa complex mediate TCIPA. It is conceivable that they are also causal factors in subsequent tumor metastasis: i.e., platelet enhanced tumor cell adhesion to endothelium and subendothelial matrix.

Acknowledgment

This work was supported by Public Health Service Grants CA-29997 (K.V.H) B.F. Sloane is the recipient of Research Career Development Award CA-00921 from the National Cancer Institute, to whom reprint requests should be sent.

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Received July 17, 1987.
P.S.E.B.M. 1987, Vol. 186.
Accepted September 21, 1987.