Histol Histopathol (1999) 14: 579-585

http://www.ehu.es/histol-histopathol

Histology and Histopathology

Invited Review

Collagen-platelet interaction: platelet non-integrin receptors

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Summary. Platelet-collagen interaction is a complex event that involves ligand-receptor interaction. There are many adhesive non-integrin receptors for platelets to interact with various types of collagens. These non-integrin receptors also serve as signal transducers both from the outside of platelets to the inside and possibly vice versa. The present review covers basic aspects of non-integrin receptor function and various signal transduction pathways.

Key words: Receptor, Platelet, Collagen, Signal transduction, Protein phosphorylation

Introduction

Platelets play an important role in the process of hemastasis and thrombosis. Following injury to blood vessels and in certain pathologic conditions, platelets adhere to the exposed subendothelial connective tissue, collagen in particular, and aggregate, releasing several biological active substances. The adherence of platelets to the endothelial component-collagen has been demonstrated to be a receptor mediated event (Puett et al, 1973; Brass and Bensusan, 1974; Chiang et al., 1975).

The addition of collagen to human platelets leads to platelet protein phosphorylation either on tyrosine and serine/threonine residues, the activation of phospholipase A2 that generates thromboxane A2, or phospholipase C that generates tris-inositol phosphate and mobilize calcium. These biochemical reactions are closely associated with platelet shape change, release granular contents, and platelet aggregation. Collagen also stimulates protein phosphatases 1 and 2 A to dephosphorylate platelet proteins to modify platelet reactivity. Okadaic acid, a protein phosphatases 1 and 2 A inhibitor, and phenyl arsine oxide, a tyrosine phosphatase inhibitor, both inhibit collagen-induced platelet aggregation and the release reaction.

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Platelet collagen receptors

Two groups of platelet proteins have been proposed as collagen receptors which can initiate platelet aggregation through collagen receptor interaction. One group, represented by members of the integrin family in which the receptor contains heterodimeric proteins, includes VLA2 (very late activation antigen, α2ß1) (Kunicki et al., 1988; Staatz et al., 1989) and glycoproteins (GP) IIb-IIIa (αIIbß3), GPIa-IIa, GPIc-IIa, and GPIc-IIa (Body, 1996). Other investigators (Springer, 1990; Santoro and Zutter, 1995) have reviewed the platelet integrin receptor for collagen. The other group, composed of monomer proteins or two identical subunit proteins, includes collagen glycosyl transferase (Barber and Jameison, 1971), membrane-bound fibronectin (Bensusan et al., 1978), a 65 kDa protein described by our laboratory (Chiang and Kang, 1982), GPIIb (Shadle et al., 1984), a 61 kDa protein (Kotite and Cunningham, 1986), a 160 kDa protein (Santoro, 1986), a 80 kDa protein (Lahav, 1987), a 85/90 kDa protein (Deckmyn et al., 1992), GPIa (Takada and Helmer, 1989), factor XIII (Saito et al., 1986), GP IV (Tandon et al., 1989), GP VI (Moroi et al., 1989), factor VIII (Aihara et al., 1984), and 62 kDa protein (Ryo et al., 1992) (Table 1). It is currently accepted that GPIIb and GPIa are integrin subunits αIIb and α2, respectively, and that they exist only as heterodimers with integrin ß subunits. Both integrin and non-integrin receptors have been proposed as having a role in platelet-collagen interaction. The role of the integrin receptors has been well worked out, but considerable number of non-integrin receptors i.e. (glycoprotein IV, glycoprotein VI, 80/90 kDa protein, 62 kDa protein, and 65 kDa protein) have been studies extensively and proposed as having a role in this interaction.

Platelet receptor(s) for type III collagen

It has been reported that different peptides identified in types I and III collagens are involved with platelet interaction. A nona peptide [located at α1(III)-CB4] was reported to be specific for type III collagen interaction

Table 1. Platelet proteins responsible for binding of collagen

PLATELET PROTEIN	HOW ISOLATED	REFERENCE
Glucosyl transferase	enzyme substrate	Barber and Jameison, 1971
Fibronectin	binding and antibody	Bensusan et al., 1978
65 kDa protein	affinity column, adherence and poly- and monoclonal antibodies	Chiang and Kang, 1982
Glycoprotein Ib	antibody study	Shadle et al., 1984
von Willebrand factor	binding study	Aihara et al., 1984
61 kDa protein	affinity column	Kotite and Cunningham, 1986
Factor XIII	binding	Saito et al., 1986
80 kDa glycoprotein	affinity column	Lahav, 1987
Glycoprotein IV	patient study	Tandon et al., 1989
Glycoprotein Ia	patient study	Takada and Helmer, 1989
Glycoprotein VI	patient study	Moroi et al., 1989
85-90 kDa glycoprotein	patient study	Deckmyn et al., 1992
62 kDa glycoprotein	patient study	Ryo et al., 1992

All of these proteins bind to type I collagen.

with platelets (Legrand et al., 1980). Two CNBr peptides, α1(I)-CB3 and α1(I)-CB5 were also reported to be reactive sites for type I collagen interaction with platelets (Katzman et al., 1973; Fauvel et al., 1978). In addition, Balleisen et al. (1979) suggest that collagen-induced platelet aggregation is inhibited by antibodies to distinct types of collagen. Furthermore, the complexity of collagens has become increasingly apparent. More than 19 genetically distinct types of collagen have been described. The walls of vessels contain relatively large amounts of type I and type III collagens which can aggregate human platelets either in the soluble or the fibril form (Balleisen et al., 1976; Hughes et al., 1976). Types IV, V, and XI collagen also can cause platelets to aggregate but only in the fibril form (Fitzsimmons et al., 1986; Morton et al., 1987). Because types I and III collagen are the predominant collagen types in vascular walls, most investigators have investigated the mechanism of interaction of platelets with types I and III collagen. Reports suggest that there are multiple reactive sites on type III collagen to interact with platelet (Morton et al., 1987; Chiang et al., 1993a,b; Glattauer et al., 1997), but the reactive site(s) on platelets has not been determined. We have purified a protein from isolated platelet membranes (Mr 47 kDa) that binds to type III collagen-Sepharose 2B column. A polyclonal antibody (anti-47p) was raised against the purified protein. The anti-47p inhibits type III collagen but not type I collagen-induced platelet aggregation and the release of ATP (Chiang et al., 1993a,b). These results suggest that there is (are) platelet protein(s) to interact with other types of collagen.

Reactive site(s) of collagen

It has been established that a tetra peptide segment, Arg-Gly-Asp-Ser (RGDS), of fibronectin is the cell attachment site for cell-matrix interaction (Pytela et al., 1986; Ruoslahti and Pierschbacher, 1987; Yamada, 1991). This sequence has been found in other proteins including type I collagen, von Willebrand factor, and the α chain of fibrinogen. Gartner and Bennett (1985) have reported that this tetra peptide sequence inhibits platelet aggregation stimulated by ADP, collagen, and thrombin without inhibiting platelet shape change, or serotonin release and does not interfere with platelet adhesion, perhaps because the tetra peptide inhibits the GPIIb/IIIa-fibrinogen interaction. The tetra peptide and its cyclo derivative have also been reported to inhibit the binding of platelet to fibronectin, von Willebrand factor (Plow et al., 1985) and platelet aggregation (Coller et al., 1995; Norikazu et al., 1996). Another active site for integrin-matrix interaction is Asp-Gly-Glu-Ala (DGEA), defined from collagen sequence (Staatz et al., 1990, 1991). In addition, Morton et al. (1995a,b) have reported that peptides containing a repeat of Gly-Pro-HyPro sequence are extremely platelet reactive, more active than collagen fibers in inducing platelet aggregation. These peptides express α2β1-independent activity and it is believed that these peptides recognize a crucial signaling collagen receptor directly that is not the integrin α2β1. The relationship among these proteins is unknown, but different experimental approaches may be the cause of the large number of collagen receptors reported. For example, in the presence of Mg^{2+}, the adherence of platelets to collagen is mediated by platelet VLA2 (Staatz et al., 1989), but in the absence of metal ions, platelets adhere to many non-integrin proteins as described above. In an *in vitro* study, Mg^{2+} inhibits platelet reactivity to collagen- and ADP-induced platelet aggregation and increases bleeding time in healthy volunteers (Ravn et al., 1996). The same laboratory also reports that aspirin and Mg^{2+} have synergistic inhibitory effects on collagen-induced platelet aggregation. Thus far, we have isolated and purified a receptor for type I collagen (Mr 65 kDa) from isolated platelet membranes using collagen affinity column chromatography and preparative gel electrophoresis. Type I collagen-induced platelet aggregation is inhibited by preincubation of platelets with anti-65m and anti-65p reactive to our receptor (Chiang and Kang, 1982; Chiang et al., 1984, 1988). The purified receptor does not react with either

anti-fibronectin or anti-GPIIb/IIIa, suggesting that the receptor is not a part of fibronectin or GPIIb/IIIa (Chiang et al., 1984). Other unpublished data also suggest that the 65 kDa protein does not react with anti-von Willebrand factor or anti-GPIa. In addition, collagen-receptor interaction causes phosphoinositide hydrolysis and mobilization of calcium (Chiang et al., 1988). Flow cytometry studies show that anti-65m and polyclonal antibody raised against 47 kDa bind to the platelet surface (Chiang and Kang, 1982; Chiang et al., 1993a,b). The 65 kDa receptor has been cloned and sequenced by our laboratory (Chiang et al., 1997). Database (gene and protein) searches failed to find homology. The recombinant protein was expressed in prokaryotic and eukaryotic vectors. The recombinant protein blocks type I collagen but not type III collagen-, ADP-, and thrombin-induced platelet aggregation and the release of ATP (Chiang et al., 1997).

Inhibitors of collagen-induced platelet aggregation

Along with a large number of compounds such as aspirin, prostaglandin I2 (PGI2), heparin, etc., antibodies of collagen receptors (GPIIb/IIIa, fibronectin, 65 kDa, 85/90 kDa, 47 kDa, α2ß1, GPIV, and factor VIII) are also inhibitors of collagen-platelet interaction in addition to the peptides RGD and DGEA mentioned above. Other inhibitors are leech protein (Henrita van Zanten, 1995), hemorrhagin catrocollastatin (snake-venom) (Rahman et al., 1995, Zhou et al., 1996), RG13965, a novel platelet fibrinogen receptor antagonist (Bostwick et al., 1996), a mutant of echistatin (Yamada and Kidera, 1996), a non-peptide GPIIb/III inhibitor, L-734217 (Cook et al., 1996), and a mimetic of the RGDF-peptide (Pueyo et al., 1996). Each of these proteins and peptides is required at high concentrations to inhibit collagen-induced aggregation. For example, complete inhibition of collagen induced platelet aggregation requires 250 μM RGDS (Gartner and Bennett, 1985). Other inhibitors require high concentrations resulting in adverse effects, such as leech protein. The DGEA peptide requires 5 mM to completely inhibit platelet adhesion to collagen and its derivative, (GPHyPro)5-GADGEA(GPHyPro)5 which is 10 to 30 fold more effective than the parent peptide (Santoro et al., 1994). In contrast, our peptide-1 (18 amino acids) completely inhibits type I collagen-induced platelet aggregation at 20 to 40 μM (Chiang and Kang, 1997). Recently, I have defined the peptide-1 to a smaller fragment of 5 amino acids (unpublished data).

Two distinct pathways of collagen-induced signal transduction

Collagen-platelet interaction involves specific receptors as mentioned in the earlier section. The participation of such specific molecules in physiological collagen-platelet interaction remains largely unclassified and the molecular mechanism by which collagen induces platelet activation has yet to be elucidated. Evidence indicates that the integrin α2ß1 is the principal platelet adhesion receptor for collagen (Kunicki et al., 1988; Staatz et al., 1989). Collagen binding domain is located within the α2 subunit (Takada and Helmer, 1989). Clinical deficiency of platelet α2ß1 results in a mild to severe bleeding tendency, accomplished by defective platelet aggregation in response only to collagen (Nieuwenhuis et al., 1985; Kehrel et al., 1988). It is still not established whether the binding of collagen to the receptor is sufficient for stimulating a full picture of collagen-induced platelet activation. Using a patient's platelets that lack GP IV with normal integrin α2ß1 content, Ichinohe et al. (1997) have demonstrated and established that signaling pathways between the integrin and the non-integrin (GP IV) are different.

Protein tyrosine phosphorylation is now considered the key signaling event in the activation of platelets (Clark et al., 1994). Collagen is known to stimulate tyrosine protein kinases, c-Src (Liebenhoff et al., 1993), Syk (Clark et el., 1994), and focal adhesion kinase (Lipfert et al., 1992), and to promote rapid tyrosine phosphorylation of platelet proteins (Blake et al., 1994; Clark et al., 1994; Cichowski et al., 1996). Collagen-stimulated protein tyrosine phosphorylation is unique in that it is insensitive to inhibition by cAMP-increasing agents (Ichinohe et al., 1995) which are known to inhibit platelet activation by most agonists. Although integrin α2ß1-mediated cell adhesion is believed to be essential for the induction of tyrosine phosphorylation (Shattil et al., 1993), other described receptors may also represent candidates responsible for the collagen-stimulated protein tyrosine phosphorylation (Suigiyama et al., 1987; Moroi et al., 1989).

Presently, the precise role of specific receptors for the various collagen types in platelet adhesion has not yet been fully established. Some insight has been obtained regarding the platelet receptors (integrin and non-integrin), their reactive sites, the role of divalent ions, the signal generated, and the contribution of cofactors (von Willebrand factor and fibronectin) in collagen-platelet interaction. Much remains to be studied, particularly regarding the details of molecular interactions that occur. Investigations may lead to selective approaches to inhibit platelet function and adhesion to collagen in atherosclerotic plaques without interfering with hemostasis.

Receptor phosphorylation and phosphatidylinositol turnover

Protein phosphorylation in relation to its physiological function has been studied in many systems (for detailed review - reference Krebs and Beavo, 1979). Receptor phosphorylation and its role on the ligand binding are altered in several systems (Kasuga et al., 1982; Roth and Karlsson, 1982; Garcia et al., 1983; Huganir et al., 1986; Yarden and Schlessinger, 1987). We noted that the response of platelets to collagen in terms of thromboxane A2 production was enhanced by

pretreatment with protein kinase purified from human plasma. In addition, these kinases were shown to catalyze phosphorylation of platelet outer surface proteins including the collagen receptor (Chiang et al., 1988). Phosphorylation of platelet surface proteins enhances the response of platelets to subthreshold amount of collagen and also increases the content of thromboxane A2 (Chiang et al., 1991). Type I collagen can be phosphorylated by the purified human plasma protein kinases (Fig. 1).

Collagen stimulates phospholipase A2 activity

The precise mechanism by which collagen leads to the generation of free arachidonic acid, presumably by activation of phospholipase A2, is not clear. Although it has generally been assumed that increases in intracellular Ca^{2+} during platelet stimulation are responsible for phospholipase A2 activation, there is no definite evidence to support this hypothesis (Siess et al., 1983). It has been reported that phospholipase A2 requires high, non-physiological concentration of Ca^{2+} for activation (Rittenhouse and Sasson, 1985). Recently, observation of a novel phospholipase A2 from human and sheep platelet is a Ca^{2+} independent (Irvine, 1982) or exquisitely sensitive to physiological concentration of Ca^{2+} (Rittenhouse and Sasson, 1985); it may be responsible for the initial mobilization of arachidonic acid.

Various physiological stimuli have been suggested to activate phospholipase A2; angiotensin, bradykinin, prolactin, and thrombin, for example, release arachidonic acid when added to responsive cells (Aarsman et al., 1985; Ballou et al., 1986). In human platelets, collagen and thrombin have been known to activate phospholipase A2 to release arachidonic acid and subsequently to form thromboxane A2 (B2). The release of arachidonic acid is tacitly assumed to be an indication of phospholipase A2 action. This may or may not be the case since arachidonic acid can also be derived from non-phospholipase A2 mediated phospholipid hydrolysis, as previously reported. A better proof on phospholipase A2 activation occurs after physiological stimulation was obtained in chondrocytes and synovial fibroblasts (Loeb and Gross, 1986). Recently, we have observed that 0.09N H_2SO_4 extracts from collagen-treated platelets or platelet membrane enhances 2-fold arachidonic acid release from phosphatidylcholine (Chang et al., 1986).

Role of protein phosphatase in collagen-platelet interaction

There are three protein phosphatases in human platelets (Gratecos et al., 1977; Burchell et al., 1987; Edelman et al., 1987). The role of each protein phosphatase has not yet to be defined. It has been reported that okadiac acid inhibits phosphoprotein phosphatases (Biolojan and Takai, 1988). Lerea et al. (1989) has reported that vanadate and molybdate increase a specific platelet protein phosphorylation (Lerea, 1991). He further demonstrated that thrombin can be selectively inhibited by okadiac acid in electropermeabilized platelets (Chiang, 1992). I have reported that okadiac acid and vanadate also inhibit collagen-induced platelet aggregation and the release of ATP. These reports suggest that phosphoprotein

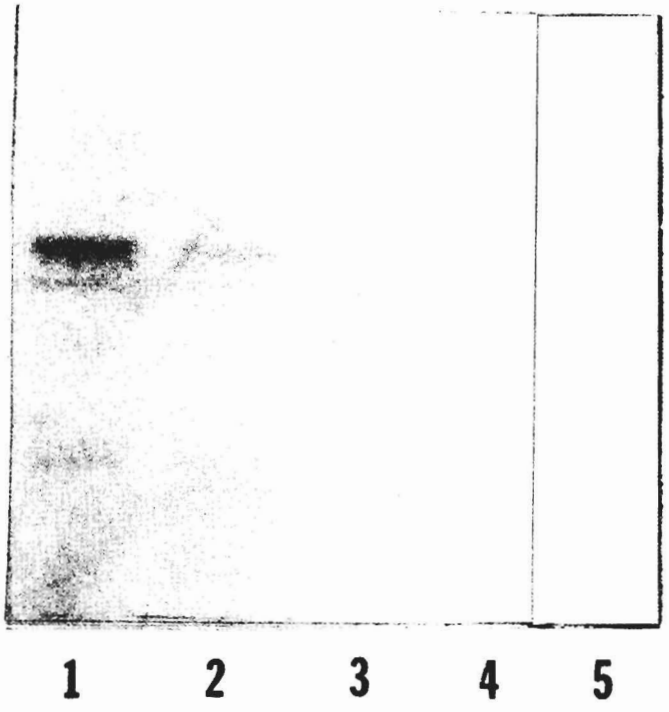

Fig. 1. Radioautogram of the phosphorylated soluble collagen type I collagen. Soluble type I collagen (10 μg). was incubated with the purified human plasma protein kinases (10 μg of fraction I, lane 1 and fraction II, lane 2). in a reaction mixture contained: 2 mM $MgCl_2$, 2 mM NaF, and 10 μM (r-$^{32}PO_4$).ATP in a final volume of 0.025 ml. Control experiments were: collagen (10 μg) plus reaction mixture (lane 3), human plasma protein kinase fraction I (10 μg) plus reaction mixture (lane 5), and human plasma protein kinase fraction II (10 μg). plus reaction mixture (lane 5). These samples were incubated at 30 °C for 5 minutes and were stopped with adding equal volume of SDS-PAGE sample buffer and boiled for 3 minutes. These samples were analyzed with 7.5% SDS-PAGE and radioautography.

phosphatases are involved in platelet function. In subsequent study, we have observed that collagen stimulates phosphoprotein phosphatase 1 and 2A (Chiang et al., 1993a,b). Recently, we have observed that protein phosphatase 1 is coprecipitated with anti-type I collagen receptor (65 kDa) (Chiang, 1993). The inhibitory effect of okadaic acid and phenyl arsine oxide may be mediated by inhibiting phosphorylation/dephosphorylation of platelet protein phosphatase 1 (Chiang, 1998). I am not attempting to cover all of the related literatures. There is a more detailed review on protein kinases and phosphatases in platelet activation (Watson et al., 1993).

Conclusion remarks

Collagen-platelet interaction is a complex process. It involves various signal transduction components existed in platelets. Most studies are focused on receptors, platelet proteins phosphorylation, phospholipase A2, and phospholipase C. The role of protein phosphatase in this interaction has not yet established. There are several platelet proteins; glycoprotein IIIa, annexin, moesin, talin, etc are substrates for protein kinases. Collagen is also a substrate for cAMP-dependent protein kinase. The function of the phosphorylation and/or dephosphorylation of these proteins needs to be elucidated.

Acknowledgements. Results described in this article are from the author's laboratory and have been supported by Department of Veterans Affairs Medical Research and American Heart Association Southern Research Consortium.

References

Aarsman A.J., Rosenboom C.F.P., Van Geffen G.E.W. and Van Den Bosch H. (1985). Some aspects of rat platelet and serum phospholipase A2 activities. Biochem. Biophys. Acta 937, 288-295.

Aihara M., Cooper H.A. and Wagner R.H. (1984). Platelet-collagen interactions: increase in rate of adhesion of fixed washed platelets by factor VIII-related antigen. Blood 63, 495-501.

Balleisen L., Gay S., Max R. and Kuhn K. (1976). Platelet-collagen interaction. The influence of native and modified collagen on the aggregation of human platelets. Haemostasis 5, 155-164.

Balleisen L., Nowack H., Gay S. and Timple R. (1979). Inhibition of collagen-induced platelet aggregation by antibodies to distinct types of collagen. Biochem. J. 184, 683-687.

Barber A.J. and Jameison G.A. (1971). Platelet collagen adhesion characterization of collagen glucosyl transferase of plasma membranes of human blood platelets. Biochem. Biophys. Acta 252, 533-545.

Ballou L.R., Dewitt L.M. and Cheung W.Y. (1986). Substrate-specific forms of human platelet phospholipase A2. J. Biol. Chem. 261, 3107-3111.

Bensusan H.B., Koh T.L., Henry K.G., Murray B.A. and Culp L.A. (1978). Evidence that fibronectin is the collagen receptor on platelet membranes. Proc. Natl. Acad. Sci. USA 75,5864-5868.

Bialojan C. and Takai A. (1988). Inhibitory effect of a marine-sponge toxin, okadiac acid, on protein phosphatase. Biochem. J. 256, 283-290.

Blake R.A., Schieven G.L. and Watson S.P. (1994). Collagen stimulates tyrosine phosphorylation of phospholipase C gamma 2 but not phospholipase C gamma 1 in human platelets. FEBS Lett. 353, 212-216.

Body S.C. (1996). Platelet activation and interactions with the microvasculature. J. Cardiovasc. Pharmacol. 27, S13-S25.

Bostwick J., Kasiewski J.S., Chu V., Klein S.I., Sabatino R.D., Perrone M.H., Dunwiddie C.T., Cook J.J. and Leadley Jr. (1996). Antithrombotic activity of RG13965, a novel platelet fibrinogen receptor antagonist. Thromb. Res. 82, 495-507.

Brass L.F. and Bensusan H.B. (1974). The role of collagen quaternary structure in the platelet-collagen interaction. J. Clin. Invest. 53, 875-883.

Burchell A., Foulkes J.G., Cohen P.T.W., Gordon G.D. and Cohen P. (1987). Evidence for the involvement of protein phosphatase-1 in the regulation of metabolic processes other than glycogen metabolism. FEBS Lett. 92, 68-72.

Chang J., Gilman S.C. and Lewis A.J. (1986). Interleukin 1 amplifies receptor-mediated phospholipase A2 in rabbit chondrocytes: a possible signal for IL-1 action. J. Immunol.136, 1283-1287.

Chiang T.M. (1992). Okadaic acid and vanadate inhibit collagen-induced platelet aggregation; the functional relation of phosphatases on platelet aggregation. Thromb. Res. 67, 345-354.

Chiang T.M. (1993). The role of protein phosphatase 1 and 2A in collagen-platelet interaction. Arch. Biochem. Biophys. 302, 56-63.

Chiang T.M. (1998). Collagen-platelet interaction: Active form of phosphoprotein phosphatase 1. Thromb. Res. 92, 233-238.

Chiang T.M. and Kang A.H. (1982). Isolation and purification of collagen α1(I) receptor from human platelet membranes. J. Biol. Chem. 257, 7581-7586.

Chiang T.M. and Kang A.H. (1997). A peptide inhibitor of type I collagen-mediated platelet aggregation. J. Clin. Invest. 100, 2079-2084.

Chiang T.M., Beachey E.H. and Kang A.H. (1975). Interaction of a chick skin collagen fragment (α1-CB5) with human platelets. Biochemical studies during the aggregation and release reaction. J. Biol. Chem. 250, 6916-6924.

Chiang T.M., Kang A.H., Dale J.B. and Beachey E.H. (1984). Immunological studies of the purified human platelet receptor for the α1(I)-chain of chick skin collagen. J. Immunol. 133, 872-876.

Chiang T.M., Wojcikiewicz R.J.H., Kang A.H. and Fain J.N. (1988). Phosphorylation of outer surface of platelet proteins enhances the effects of collagen on aggregation, ATP release, calcium translocation and phosphoinositide hydrolysis. Thromb. Res. 50, 719-731.

Chiang T.M., Kang A.H. and Fain J.N. (1991). Stimulation of phospholipase A2 activity in human platelets by trypain and collagen. Arch. Biochem. Biophys. 284, 47-52.

Chiang T.M., Seyer J.M. and Kang A.H. (1993a). Collagen-platelet interaction: Separate receptor sites for types I and III collagen. Thromb. Res. 71, 443-456.

Chiang T.M., Kang E.S. and Kang A.H. (1993b). Involvement of phosphoprotein phosphatase 1 in collagen-platelet interaction. Thromb. Res. 84, 399-409.

Chiang T.M., Rinaldy A. and Kang A.H. (1997). Cloning, characterization, and functional studies of a platelet receptor for type I collagen. J. Clin. Invest. 100, 514-527.

Cichowski K., Brugge J.S. and Brass L.F. (1996). Thrombin receptor activation and integrin engagement stimulate tyrosine phosphorylation of the proto-oncogene product, p95vav, in platelets. J. Biol. Chem. 271, 7544-7550.

Clark E.A., Shattil S.J. and Brugge J.S. (1994). Regulation of protein tyosine kinases in platelets. Trends Biochem. Sci. 19, 464-469.

Clark E.A., Shattil S.J., Ginsberg M.H., Bolen J. and Brugge, J.S. (1994). Regulation of the protein tyrosine kinase pp72syk by platelet agonists and the integrin αIIbß3. J. Biol. Chem. 269, 28859-28864.

Coller B.S., Anderson K. and Weisman H.F. (1995). New antiplatelet agents: platelet GPIIb/IIIa antagonists. Thromb. Haemost. 74, 302-308.

Cook J.J., Holahan M.A., Lyle E.A., Ramjit D.R., Sitko G.R., Stranieri M.T., Stupienski III, R.F., Wallace A.A., Hand E.L., Gehret J.R., Kothstein T., Drag M.D., McCormick G.Y., Perkins J.J., Ihle N.C., Duggan M.E., Hartman G.D., Gould R.J. and Lynch Jr. J.J. (1996). Nonpeptide glycoprotein IIb/IIIa inhibitors. 8. Antiplatelet activity and oral antithrombotic efficacy of L-734,217. J. Pharmaco. Expt. Therap. 278, 62-73.

Deckmyn E., von Houtte E. and Vermylen J. (1992). Disturbed platelet aggregation to collagen associated with an antibody against an 85/90 kDa platelet glycoprotein in a patient with prolonged bleeding time. Blood 79, 1466-1471.

Edelman A.M., Blumenthal D.K. and Krebs E. G. (1987). Protein serine/threonine kinase. Annu. Rev. Biochem. 56, 567-613.

Fauvel F., Legrand Y.J., Bentz H., Fietzek P.P., Kuhn K., and Caen J.P. (1978). Platelet-collagen interaction: adhesion of human blood platelets to purified (CB4) peptide for type III collagen. Thromb. Res. 12, 841-850.

Fitzsimmons C.M., Cawston T.E. and Barnes M.J. (1986). The reactivity of collagen type I: Evidence for multiple platelet-reactive sites in type I collagen molecule. Thromb. Haemost. 56, 95-99.

Garcia T., Tuohima P., Mester J., Buchou T., Renoid J.M. and Boulieu E.E. (1983). Protein kinase activity components of the chicken oviduct progesterone receptor. Biochem. Biophys. Res. Commun. 113, 960-966.

Gartner T.K. and Bennett J.S. (1985). The tetrapeptide analogue of the cell attachment site of fibronectin inhibits platelet aggregation and fibrinogen binding to activated platelets. J. Biol. Chem. 260, 11891-11894.

Glattauer V., Werkmeister J.A., Kirkpatrick A. and Ramshaw J.A.M. (1997). Identification of the epitope for a monoclonal antibody that blocks platelet aggregation induced by type III collagen. Biochem. J. 323, 45-49.

Gratecos D., Detwiler T.C., Hurd S. and Fisher E.H. (1977). Rabbit muscle phoshorylase. I. Purification and chemical properties. Biochemistry 16, 4812-4817.

Henrita van Zanten G., Connolly T.M., Schiphorst M.E., de Graaf S., Slootweg P.J. and Sixma J.J. (1995). Recombinant leech antiplatelet protein specifically blocks platelet deposition on collagen surfaces under flow conditions. Arterioscler. Thromb. Vasc. Biol. 15, 1424-1431.

Hughes J., Herion F., Nusgens B. and Lapiere C.M. (1976). Type II collagen and probably not type I collagen aggregates platelets. Thromb. Res. 9, 223-231.

Huganir R.L., Dekour A.H., Greengard P. and Hess G.P. (1986). Phosphorylation of nicotinic acetylcholine receptor regulates its rate of desensitization. Nature 321, 774-776.

Ichinohe T., Takayama H., Ezumi Y., Yanagi S., Yamamura H. and Okuma M. (1995a). Cyclic AMP-insensitive activation of c-Src and Syk protein-tyrosine kinases through platelet membrane glycoprotein VI. J. Biol. Chem. 270, 28029-28036.

Ichinohe T., Takayama H., Ezumi Y., Arai M., Yamamoto N., Takahashi H. and Okuma M. (1995b). Collagen-stimulated activation of Syk but not c-Src is severely compromised in human platelet lacking membrane glycoprotein VI. J. Biol. Chem. 272, 63-68.

Irvine R.F. (1982). How is the level of free arachidonic acid controlled in mammalian cells? Biochem. J. 204, 3-16.

Kasuga M, Karlsson F.A. and Kahn C.R. (1982). Insulin stimulation of phosphorylation of the B subunit of the insulin receptor: Formation of both phosphoserine and phosphotyrosine. J. Biol. Chem. 257, 9891-9894.

Katzman R.L., Beachey E.H. and Kang A.H. (1973). Collagen-induced platelet aggregation: Involvement of an active peptide fragment (α1-CB5). J. Biol. Chem. 181, 670-672.

Kehrel B., Balleisen L., Kokott R., Mesters R., Stenzinger W., Clemetson K.J. and van de Loo J. (1988). Deficiency of thrombo spondin and membrane glycoprotein Ia in platelet with defective collagen-induced platelet aggregation and spontaneous loss of disorder. Blood 71, 1074-1078.

Kotite N.J. and Cunningham L.W. (1986). Specific adsorption of a platelet membrane glycoprotein by human insoluble collagen. J. Biol. Chem. 261, 8342-8347.

Krebs E.G. and Beavo J.A. (1979). Phosphorylation-dephosphorylation of enzymes. Annu. Rev. Biochem. 48, 923-959.

Kunicki T.J., Nugent D.J., Staatz S.J., Okchekowski R.P., Wayner E.A. and Carter W.G. (1988). The human fibroblast class II extracellular matrix receptor mediates platelet adhesion to collagen and it's identical to the platelet GPIa-IIa complex. J. Biol. Chem. 263, 4516-4519.

Lahav J. (1987). Identification of a surface protein of the rabbit blood platelets with high affinity for collagen. Exp. Cell. Res. 168, 447-456.

Legrand Y.J., Karniguian A., Le Francier P., Fauvel F. and Caen J.P. (1980). Evidence that a collagen-peptide is a specific inhibitor of platelet-collagen interaction. Biochem. Biophys. Res.Commun. 96, 1579-1585.

Lerea K.M., Tonks N.K., Kerbs E.G., Fisher E.H. and Glomset J.A. (1989). Vanadate and molybdate increase tyrosine phosphorylation in 50 kilodalton protein and stimulate secretion in electro-permeabilized platelets. Biochemistry 28, 9286-9292.

Lerea K.M. (1991). Thrombin increased effects are selectively inhibited following treatment of intact platelet with okadaic acid. Biochemistry 30, 6819-6824.

Liebenhoff U., Brockmeier D. and Presek P. (1993). Substrate affinity of the protein tyrosine kinasec-src is increased on thrombin stimulation of human platelets. Biochem. J. 295, 41-48.

Lipfert L., Haimovich B., Schaller M.D., Cobb B.S., Parson J.T. and Brugge J.S. (1992). Integrins dependent phosphorylation and activation of the protein tyrosine kinase pp125 fak in platelets. J. Cell Biol. 119, 905-912.

Loeb L.A. and Gross R.W. (1986). Identification and purification of sheep platelet phospholipase A2 isoforms. J. Biol. Chem. 261, 10467-10470.

Moroi M., Jung S.M., Okuma M. and Shinmyozu K. (1989). A patient with platelets deficient in glycoprotein VI that lack both collagen-induced aggregation and adhesion. J. Clin. Invest. 84, 1440-1445.

Morton L.F., Fitzsimmons C.M., Rauterberg J. and Barnesd M.J. (1987). Platelet-reactive sites in collagen: Collagens I and III possess

different aggregatory sites. Biochem. J. 248, 483-487.

Morton L.F., Hargreaves P.G., Farndale R.W., Young R.D. and Branes M.J. (1995). Integrin α2β1-independent activation of platelets by simple collagen-like peptides: collagen tertiary (triple-helical) and quaternary (polymeric) structures are sufficient alone for α2β1-independent platelet activity. Biochem. J. 306, 337-344.

Morton L.F., Peachey A.R., Knight C.G., Farndale R.W. and Barnes M.J. (1997). The platelet reactivity of synthetic peptides based on the collagen III fragment α1(III)CB4. J. Biol. Chem. 272, 11044-11048.

Nieuwenhui H.K., Akkerman J.W.N., Houdijk W.P.M. and Sixma J.J. (1985). Human blood platelets showing no response to collagen fail to express surface glycoprotein Ib. Nature 318, 470-472.

Norikazu N., Hayashida J., Arai T., Mihara H., Ueno Y. and Kumagai H. (1996). Cyclo(-arginyl-sarcosyl-asparatyl-phenylartyl-phenylglycyl-). 2. Simple synthesis of an RGD-related peptide with inhibitory activity for platelet aggregation. J. Chem. Soc. Perkin Trans. 1, 939-946.

Plow E.F., Pierschbacher M.D, Ruoslahti E., Marguire G.A. and Ginsberg M. (1985). The effect of Arg-Gly-Asp-containing peptides on fibronectin and von Willebrand factor binding to platelets. Proc. Natl. Acad. Sci. USA 82, 8057-8061.

Puett D., Wasserman B.K., Ford J.D. and Cunningham L.W. (1973). Collagen mediated platelet aggregation: effects of collagen modification involving the protein and carbohydrate moieties. J. Clin. Invest. 52, 2495-2506.

Pueyo C., Badimon J.J., Royo T., Feigen L.P. and Badimon L. (1996). A mimetic of the RGDF-peptide [Arginine-Glycine-Aspartic acid-Phenylalanine] blocks aggregation and flow-induced platelet deposition on severely injured stenotic arterial wall. Effects on different animal models and in humans. Thromb. Res. 81, 101-112.

Pytela R.P., Pierschbacher M.D., Ginsberg M.H., Plow E.F. and Ruoslahti E. (1986). Platelet membrane glycoprotein IIb-IIIa: Member of a family of Arg-Gly-Asp-specific adhesion receptors. Science 231, 1559-1562.

Rahman S., Lu X., Kakkar V.V. and Authi K. (1995). The integrin αIIbβ3 contains distinct and interacting binding sites for snake-venom RGD proteins: Evidence that the receptor-binding characteristics of snake-venom RGD proteins are related to the amino acid environment flanking the sequence of RGD. Biochem. J. 312, 223-232.

Ravn H.B., Vissinger H., Kristensen S.D. and Husted S.E. (1996). Magnesium inhibits platelet reactivity - an in vitro study. Thromb. Haemost. 76, 88-93.

Rittenhouse S.E. and Sasson J.P. (1985). Mass changes in myoinositol trisphosphate in human platelets stimulated by thrombin: inhibitory effects of phorbol ester. J. Biol. Chem. 260, 8657-8660.

Roth R.A. and Cassell D.J. (1982). Insulin receptor evidence that it is a protein kinase. Science 219, 299-301.

Ruoslahti E. and Pierschbacher M.D. (1987). New prespectives in cell adhesion: RGD and integrins. Science 238, 491-497.

Ryo R., Yeshiva A., Sugano W., Yasunga M., Nakayama K., Saigo M., Adachi N., Yamaguchi N. and Okuma M. (1992). Deficiency of P62, a putative collagen receptor, in platelets from a patient with defective collagen-induced platelet aggregation. Am. J Hematol 39, 25-31.

Saito Y., Imada T., Takagi J., Kikuchi T. and Inada Y. (1986). Platelet factor XIII. The collagen receptor? J. Biol. Chem. 261, 1355-1358.

Santoro S.A. (1986). Identification of a 160,000 dalton platelet membrane protein that mediates the initial divalent cation-dependent adhesion to platelets to collagen. Cell 46, 913-920.

Santoro S.A. and Zutter M.M. (1995). The α2β1 integrin: A collagen receptor on platelets and other cells. Thromb. Haemost. 74, 813-821.

Santoro S.A., Zutter M.M., Wu J.E., Staatz W.D., Saelman E.U.M. and Kelley P.J. (1994). Analysis of collagen receptors. Meth. Enzymol. 245, 147-183.

Shadle P.J., Ginsberg M.H., Plow E.F. and Barondes H. (1984). Platelet-collagen adhesion: inhibition by a monoclonal antibody that binds glycoprotein IIb. J. Cell. Biol. 99, 2056-2060.

Shattil S.J., Ginsburg M.H. and Brugge J.S. (1993). Adhesive signaling in platelets. Curr. Opin. Cell Biol. 6, 695-704.

Siess W., Cuatrecasas P., and Lapetina E.G. (1983). A role for cyclooxygenase products in the formation of phosphatidic acid in stimulated human platelets: differential mechanisms of action of thrombin and collagen. J. Biol. Chem. 254, 4683-4686.

Springer T.A. (1990). Adhesion receptors of the immune system. Nature 346, 425-434

Staatz W.D., Rajpara S.M., Wagner E.A., Carter W.G. and Santoro S.A. (1989). The membrane glycoprotein Ia-IIa (VLA-2). complex mediates the Mg^{2+}-dependent adhesion of platelets to collagen. J. Cell. Biol. 108, 1017-1024.

Staatz W.D., Walsh J.J., Pexton T. and Santoro S.A. (1990). The α2β1 integrin cell surface collagen receptor binds to the α1(I)-CB3 peptide of collagen. J. Biol. Chem. 265, 4778-4781.

Staatz W.D., Fok K.F., Zutter M.M., Adams S.P., Rodriguez B.A. and Santoro S.A. (1991). Identification of a tetrapeptide recognition sequence for the α2β1 integrin in collagen. J. Biol.Chem. 266, 7363-7367.

Suigiyama T., Okuma M., Ushikubi F., Sensaki S., Kanaji K. and Uchino H. (1987). A novel platelet aggregating factor found in a patient with defective collagen-induced platelet aggregation. Blood 69, 1712-1720.

Takada Y. and Helmer M.E. (1989). The primary structure of the VLA2/collagen receptor α2 subunit (platelet GPIa): homology to other integrins and the presence of a possible collagen-binding domain. J. Cell Biol. 109, 397-407.

Tandon N.N., Kralisz U. and Jameison G.A. (1989). Identification of glycoprotein IV (CD 36) as a primary receptor for platelet-collagen adhesion. J. Biol. Chem. 264, 7576-7583.

Watson S.P., Blake R.A., Lane T. and Walker T.R. (1993). The use of inhibitors of protein kinases and protein phosphatases to investigate the role of protein phosphorylation in platelet activation. Adv. Exp. Med. Biol. 344, 105-118.

Yamada K.M. (1991). Adhesion recognition sequence. J. Biol Chem. 266, 12809-12812.

Yamada T. and Kidera A. (1996). Tailoring echistatin higher affinity for integrin αIIbβ3. FEBS Lett. 387, 11-15.

Yarden Y. and Schlessinger J. (1987). Self-phosphorylation of epidermal growth factor receptor: evidence for a model of intermolecular allosteric activation. Biochemistry 26,1434-1442.

Zhou Q., Dangelmaier C. and Smith B.J. (1996). The Hemorrhagin Catrocollastatin inhibits collagen-induced platelet aggregation by binding to collagen via its distintegrin-like domain. Biochem. Biophys. Res. Commun. 219, 720-726.

Accepted September 18, 1998