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REGULAR ARTICLE

Increase in platelet non-integrin type I collagen receptor in patients with systemic sclerosis

Thomas M. Chiang[a,*], Hiroshi Takayama[b], Arnold E. Postlethwaite[a]

[a]*Department of Veterans Affairs, Memphis VA Medical Center, and Departments of Medicine and Molecular Sciences, University of Tennessee-Health Science Center, Memphis, TN, United States*
[b]*Department of Hematology and Oncology, Clinical Sciences for Pathological Organs, Graduate School of Medicine, Kyoto University of Hematology and Oncology, Kyoto University, Kyoto, Japan*

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Abstract Microvascular injury is one of the major pathogenetic processes involved in systemic sclerosis (SSc). Interaction of the platelet types I and III collagen receptors with their respective ligand in the exposed subendothelial stroma as a result of ongoing microvascular injury in SSc patients results in platelet activation and aggregation with the release of mediators, which contribute to vascular damage and inflammation. We have found that there is a twofold increase in radiolabeled type I collagen binding to washed platelets from patients with SSc compared to platelets obtained from normal volunteers. Western blot analyses showed that the non-integrin platelet type I collagen receptor protein (65 kDa) is increased dramatically in lysates of platelet from patients with SSc. However, the integrin ($\alpha_2\beta_1$) and other non-integrin receptors such as glycoprotein VI, glycoprotein IV, and the platelet receptor for type III collagen remain unchanged. In addition, platelet lysates from rheumatic disease controls (rheumatoid arthritis, osteoarthritis, gout, and systemic lupus erythematosus) do not show any significant increases. There is no nitrotyrosylation on 65 kDa in patients with SSc compared to controls, suggesting this might also contribute to binding of CI to the 65-kDa CIR. These results suggest that there is a specific increase in the number of platelet type I collagen receptors in SSc patient's platelets. In addition, the activity of nitric oxide synthase is decreased in

Abbreviations: SSc, systemic sclerosis; ADP, adenosine diphosphate; ATP, adenosine triphosphate; Pf4, platelet factor 4; TXA, thromboxane A2; PDGF; platelet-derived growth factor; TGF-β, tumor growth factor-βCI, type I collagen; CIII, type III collagen; PRP, platelet-rich plasma; Tris–EDTA; 20 mM Tris/130 mM NaCl (pH 7.2); TBS, 20 mM Tris–0.5 M NaCl–0.05% Tween 20; ECL, enhanced chemiluminescence; GP, glycoprotein; ELISA, enzyme-linked immunosorbent assay.

* Corresponding author. Research Service (151), Veterans Administration Medical Center, 1030 Jefferson Avenue, Memphis, TN 38104, United States. Tel.: +1 901 523 8990x7608; fax: +1 901 577 7273.
E-mail address: tchiang@utmem.edu (T.M. Chiang).

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patients' platelet lysates compared to controls. The increase in platelet expression of the 65-kDa non-integrin platelet type I collagen receptor may explain the enhanced aggregation of platelets from patients with SSc to CI in vitro and microvascular thrombosis in the disease in vivo.

Introduction

Platelets play an important role in hemostasis and thrombosis. Following injury to blood vessels and in certain pathologic conditions, platelets adhere to the exposed sub-endothelial connective tissue, particularly to collagen, before aggregating and releasing several biologically active substances. Many proteins such as integrin ($\alpha_2\beta_1$) and non-integrins (65 kDa, 47 kDa, 90 kDa, GP IV, and GP VI) appear to modulate both the adherence of platelets to the exposed collagen and the ensuing aggregation process [1—7]. The interaction of platelets with vessel wall matrix components has been the topic of numerous studies [8—12]. Other investigators as well as we have demonstrated that platelets possess different receptors for different types of collagen [2,3,8—10]. Platelets possess a variety of receptors that interact with type I and type III collagens (CI and CIII, respectively) [1—3,8—10].

In systemic sclerosis (SSc), vascular lesions are characterized by an arteriolar—capillary perivasculitis with mononuclear cell infiltration that leads to arterial intimal proliferation and obliteration of arterioles and capillaries with attrition of endothelial cells and basal lamina [13]. A recurring pattern of injury to the endothelial cells or basal lamina, or both, is characteristic of SSc [14—16]. It is also unclear whether the changes in platelet function, which are observed in patients with SSc, are a primary characteristic of this disease or whether they are secondary to vascular changes. Platelets from patients with SSc undergo accelerated aggregation when exposed to CI [17]. It has been reported that SSc is associated with a significant enhancement of the sensitivity of platelets to CI [18]. Antibodies to such platelet proteins as glycoprotein (GP) IIIa and GPIIb/IIIa have been found in some patients with SSc [19].

Platelets first adhere to the matrix and then aggregate, releasing their granular contents, including adenosine diphosphate (ADP), adenosine triphosphate (ATP), platelet factor 4, thromboxane A2, platelet-derived growth factor (PDGF), and transforming growth factor-β (TGF-β). PDGF and TGF-β are potent chemotactic and fibrogenic factors thought to be important in the activation of fibroblasts and also smooth muscle cells, which migrate into the intima [20,21]. These smooth muscle cells proliferate and synthesize new matrix components, including CI and CIII, that are deposited in close proximity to the endothelium, resulting in thickened intima and narrowed lumina of vessels [22]. Vascular damage in the lungs and other organs of patients with SSc is almost always associated with extensive fibrosis [23]. Sondergaard et al. [23] have reported that there are increased CI and CIII levels in the skin of patients with SSc compared to normal skin. Others have also reported that fibroblasts grown from involved skin from patients with SSc have increased constitutive production of collagen when cultured in vitro and that they overexpress the collagen α2(I) gene [24,25]. We have reported that a platelet non-integrin protein with a molecular weight of 65 kDa can serve as a receptor for CI. The protein binds specifically to CI and mediates platelet aggregation. Soluble CI receptor protein inhibits CI-induced platelet aggregation and the release of ATP [2,26]. In this investigation, we present evidence suggesting that there is a dramatic increase in the expression of a non-integrin CI receptor in platelets from patients with SSc compared to platelets from normal donors or from those with other rheumatic diseases.

Materials and methods

Materials

Antibodies used in this investigation included anti-65-kDa (anti-platelet receptor for CI [26]), anti-47-kDa (anti-platelet receptor for CIII [3]), and anti-90-kDa antibodies (platelet receptor for CI and CIII [4]) from our laboratory; anti-GP VI (anti-platelet glycoprotein VI for CI) and anti-GP IV (anti-platelet glycoprotein IV for CI) from Dr. Takayama (Tokyo, Japan); and anti-$\alpha_2\beta_1$ (anti-platelet integrin receptor for CI; purchased from