The role of platelets in the pathogenesis of systemic sclerosis

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Systemic sclerosis (SSc) is an inflammatory disease of unknown etiology characterized by widespread organ dysfunction due to fibrosis and ischemia. Its nebulous pathogenic background and the consequent absence of an etiologic therapy prevent the adoption of satisfying treatment strategies, able to improve patients’ quality of life and survival and stimulate researchers to identify a unifying pathogenic target. Platelets show a unique biological behavior, lying at the crossroads between vascular function, innate and adaptive immunity, and regulation of cell proliferation. Consequently they are also emerging players in the pathogenesis of many inflammatory diseases, including SSc. In the setting of SSc platelets are detectable in a persistent activated state, which is intimately linked to the concomitant presence of an injured endothelium and to the widespread activation of the innate and adaptive immune system. As a consistent circulating source of bioactive compounds platelets contribute to the development of many characteristic phenomena of SSc, such as fibrosis and impaired vascular tone.

Keywords: platelets, systemic sclerosis, inflammation, autoimmunity, vascular injury, HMGB1, serotonin, collagen

PLATELETS, IMMUNITY, AND VESSEL INTEGRITY

Platelets are anucleate cellular fragments emitted by a larger nucleate precursor, the megakaryocyte. Differently from other myeloid or lymphoid precursors this latter progenitor usually resides in the bone marrow, but can also be detected in the lung (Kosaki, 2005). During the evolution of the hemopoietic system, platelets progressively acquired a functional specialization in the control of vessel integrity and repair (Semple et al., 2011). However, as probably happened for other circulating innate mediators such as pentraxins (Manfredi et al., 2008; Martinez de la Torre et al., 2011), an ancestral link between vessel repair and patrolling (i.e., between hemostasis and immunity) still seems to characterize platelet biology in humans and “upper” species (Semple et al., 2011). In the setting of injured vessels, a tripartite network between the endothelium, platelets, and rolling neutrophils develops (Maugeri et al., 2012). Subsequently extensive physical and biochemical cross-talks between these cellular species take place: selectins provide a dual anchorage system to rolling neutrophils, since they are both expressed by the endothelium and by platelets (Maugeri et al., 2012). Coagulation factors recruited at sites of vessel injury also constitute a common binding platform for leukocytes and platelets (Maugeri et al., 2012); finally a large array of membrane-bound and soluble alarmins and immune mediators described in the T cell subset (Austrup et al., 1997), suggesting that T-helper 1 lymphocytes (Th1) to interact with P-selectin has been described in the T cell subset (Austrup et al., 1997), suggesting that platelets could actively regulate the characteristics of the immune infiltrate during chronic inflammation. Circulating platelets are also armed with a large array of other immune signaling quanta (e.g., the alarmin High Mobility Group 1 protein (HMGB1), IL18, and many chemokines), as well as defensive mediators (the so-called thrombocidins), which exert direct killing actions against invading bacteria (Yraman, 2010; Semple et al., 2011). Furthermore platelets store large amounts of growth factors like PDGF, VEGF, and many chemokines, as well as defensive mediators (the so-called thrombocidins), which exert direct killing actions against invading bacteria (Yraman, 2010; Semple et al., 2011). Further more platelets store large amounts of growth factors like PDGF, VEGF, and TGFβ, and, when recruited “at sites of inflammation and vessel injury through hemostatic mechanisms” (Nurden, 2011), platelets are able to release them and affect local tissue tropism.
CHARACTERISTIC FEATURES OF SYSTEMIC SCLEROSIS

Systemic sclerosis (SSc) is an autoimmune disease, in which excessive connective tissue deposition, inflammation and autoimmunity and vascular dysfunction (the three main pathophysiological hallmarks of the disease), rise from a largely unknown pathogenic background. The clinical fallout of such complex pathophysiological phenomena consists in a large spectrum of pathophysiological hallmarks of the disease), which has the “aim” of preventing hemorrhage or the impairment of motility or substance exchange (including lung, kidney, heart, gastrointestinal tract, and skin), due to ischemia or to the impairment of motility or substance exchange with the environment after substitution of functional tissues with amorphous fibrotic tissue. Due to the scarcity of effective therapies and to the frequent involvement of major organs, SSc significantly impacts on the patients quality of life and is associated with higher overall mortality (Pattanaik et al., 2011).

Signs of systemic inflammation and autoimmunity are detectable in almost all patients with SSc and comprise the activation of cellular and humoral as well as innate and adaptive immune responses (Kahaleh and Lefky, 1999; Postlewaite and Chiang, 2007; Yoshizaki et al., 2004; Pattanaik et al., 2011). A characteristic antiviral-like interferon-mediated response has been recognized in patients with SSc (Lafyatis and York, 2009) and constitutes a shared pathogenic hallmark of many connective tissue diseases (Banerjee and Pascual, 2006). Marked signs of humoral innate immunity activation are also detectable in SSc both in circulating blood (Yoshizaki et al., 2009) and affected tissues (Luchetti et al., 2004; see also below), and have been shown to correlate with disease activity (Yoshizaki et al., 2009). On the other hand, extensive antibody and cellular autoimmunity against vessel and connective tissue constituents is thought to directly contribute to tissue damage, stimulate the development of aberrant repair responses (Pattanaik et al., 2011) and enhance the activation of innate immune players (including platelets, Postlewaite and Chiang, 2007).

Vascular impairment is one of the main and early appearing hallmarks of SSc and develops as a consequence of the concurrent action of autoimmune reactions against vessel walls (Holt et al., 1989; Kahaleh and Fan, 1997), endothelial dysfunction (with prominent imbalance between vasconstrictors and vasodilators; Pattanaik et al., 2011), abnormalities in neural control of the vascular tone (Freedman et al., 1999) and possibly alterations in the hemostatic function. The development of ischemia, which ultimately follows vascular impairment in SSc, leads to the release of large amounts of active mitogens. These latter mediators of cell proliferation, instead of initiating a physiological formation of neo-vessels, give rise to aberrant and dysfunctional neangiogenic responses (see below) and further stimulate fibrogenesis (Tinjovovska, 2008; Pattanaik et al., 2011).

Platelet activation and pathogenesis of SSc

Platelet activation and increased tendency to aggregate have long been observed in SSc patients (Kahaleh et al., 1982; Goodfield et al., 1993) and are generally attributed to the concomitant dysfunction of the endothelium (Pattanaik et al., 2011). Moreover the elevation of markers of platelet activation like the HMGB1 or P-selectin has been shown to correlate with disease activity in SSc (Agache et al., 2007; Yoshizaki et al., 2009), as well as in other diseases characterized by prominent vascular inflammation (Harris et al., 2012). Several studies have revealed an increased responsiveness of SSc platelets to 5-hydroxytryptamine (5-HT), adrenaline, ADP, and collagen (Fridhoff et al., 1984; Goodfield et al., 1993; Postlewaite and Chiang, 2007). More recently SSc platelets were shown to upregulate a specific non-integrin receptor for type I collagen (Chiang et al., 2006). Type I collagen is in turn abundantly expressed in (injured) vessel walls and is one of the main constituent of fibrotic tissues in SSc. Overexpression of the non-integrin receptor for collagen I together with enhanced down-stream signaling ultimately leads to further pro-coagulant activation of platelets, which undergo extensive cytoskeletal remodeling and mobilization of intracellular calcium (Postlewaite and Chiang, 2007; Figure 1).

Lungs, Platelets, and SSc: Pathogenesis

The involvement of the lung is the leading cause of mortality in SSc (Steen and Medsger, 2007). Pulmonary disease usually encompasses interstitial lung disease and pulmonary arterial hypertension and it is clinically evident as progressive respiratory insufficiency. Moreover histologic evidence of lung injury can be detected in up to 80% of patients with SSc (D’Angelo et al., 1969). In fact as the lung vasculature is the main portal system in the body, it is highly exposed to the activity of circulating inflammatory humoral and cellular mediators (including platelets) and thus it constitutes the ideal setting for the complete expression of some of the most characteristic pathogenic features of SSc, i.e., vascular dysfunction and subsequent aberrant vessel and tissue remodeling. Moreover, as suggested by the pathogenesis of transfusion-related
FIGURE 1 | Involvement of platelets in the pathogenesis of SSc. Platelets are stimulated by the injured endothelium and contribute to vascular dysfunction and ischemia by participating in thrombotic events and by releasing vaso-active molecules (such as TXA2, increased expression of type I collagen as a consequence of tissue fibrosis further enhances platelet activation. On the other hand platelets actively stimulate tissue fibrosis by releasing fibrogenic mediators such as PDGF, TGF-β, lysophospholipids, and serotonin. The interactions of platelets with immune cells in SSc are less clearly understood. Platelets are known sources of active pro-inflammatory mediators like HMGB1, and interestingly increased circulating levels of this prototypic alarmin have been shown to correlate with disease activity in SSc. Moreover evidence is growing about the prominent role of circulating heterotypic aggregates (between platelets and innate immune cells) in the pathogenesis of inflammatory and vascular diseases and recent studies are currently evaluating their impact in the pathogenesis of SSc. Furthermore other studies suggested the existence of a specific network between collagen-reactive T lymphocyte and platelets in SSc. In this latter setting platelet activation would be enhanced by the release of IFNγ and a wider array of cytokines from autoreactive T lymphocyte, which in turn would affect megakaryocyte maturation and platelet basal activation state.

Acute lung injury (TRALI; Nurden, 2011), by clinical reports of non-SSc interstitial lung disease after the onset of severe idiopathic thrombocytopenic purpura (ITP; Fontana et al., 2007) and finally by detection of platelet activation markers in bronchoalveolar lavages of SSc patients with active pulmonary involvement (Kosak-Blieckka et al., 2005), lungs constitute a preferential target for platelet-induced inflammatory injury. Lungs have long been recognized as secondary sites of thrombocytopoiesis (Kosaki, 2005). Differently from the bone marrow (which is mainly constituted by immature immune cells and is protected by the blood-marrow barrier), in the setting of the lung megakaryocytes are potentially exposed to powerful inflammatory stimuli, provided by resident or circulating mature immune cells, such as lymphocytes (Kosaki, 2005; Postlethwaite and Chiang, 2007; Nurden, 2011). A recent series of studies suggested that autoreactive T lymphocytes, directed against collagen I, could stimulate platelets through specific patterns of cytokines and induce them to increase their own response to collagen I and enhance their aggregation activity (Postlethwaite and Chiang, 2007). Although the precise way by which innate immune players (as platelets are) could elicit a selective activation against a single autoantigen in response to signals from adaptive immunity is still incompletely clear, this kind of evidence provides an interesting clue toward a better comprehension of the links between platelets abnormalities and autoimmunity.

Activated or destructed platelets are known to release large amounts of bioactive compounds in the bloodstream, most of which are actively metabolized by the lungs, which are thus chronically exposed to platelet-derived endogenous toxins. In particular the lungs are the main physiologic filters for circulating 5HT, a known marker of platelet activation and a key player in platelet aggregation (Nurden, 2011), whose aberrant activity in tissue and vessel remodeling is well-demonstrated by carcinoid heart disease (Palaniyappan et al., 2012). Excessive circulating 5HT and defective platelet content (i.e., increased release of 5HT by platelets) has been observed in patients with SSc (Klimiuk et al., 1989) and other connective tissue diseases (Biondi et al., 1988; maybe accounting for the presence of mild to severe pulmonary involvement in systemic lupus erythematosus or inflammatory myopathies). Moreover platelet-derived serotonin has recently been linked to the development of fibrosis (Dees et al., 2011; see below). Along with this line inhibition of platelet loading with 5HT by the administration of selective serotonin reuptake inhibitors (SSRI) has been proposed as a possible therapeutic option to face the
presence of extensive fibrogenic activity and vasoconstriction as well as increased incidence of mood disorders in SSc (Coleiro et al., 2001; Garcia-Porrua et al., 2004).

An imbalance between endothelial prostacyclin (PGI2) and platelet-derived thromboxane (TXA2) has long been recognized as a key factor in determining the risk of ischemia by means of platelet aggregation and vessel vasoconstriction (and it constitutes the main rationale for the use of low-dose aspirin for anti-thrombotic prevention). Diffuse microvascular dysfunction with sustained vasoconstriction, due to defective synthesis of PGI, is a characteristic feature of SSc lungs (Tuder et al., 1999) and accounts for the frequent development of pulmonary hypertension and the efficacy of prostaglandin analogs like iloprost (Herrick, 2011). Moreover this deficit in prostacyclin activity likely promotes platelets aggregation by acting synergistically with the known resistance of SSc platelets to PGI2 signaling (Belch et al., 1985) and the enhanced production of TXA2, due to collagen stimulation (Postlethwaite and Chiang, 2007; Figure 1). Accordingly the use of prostacyclin analogs as well as other vasodilators has been suggested to affect and improve also platelet function (Gandella et al., 2001; Herrick, 2011).

**KIDNEYS, PLATELETS, AND THROMBOSIS**

Kidney disease in SSc manifests itself as the so-called “scleroderma renal crisis,” the leading cause of death by SSc before the introduction of angiotensin converting enzyme inhibitors (ACEi; Stern and Medger, 2007). Although the hallmark of renal injury in SSc is the aberrant activation of the renin-angiotensin-aldosterone system, the initial sequence of vasoconstriction and ischemia (which gives rise to a vicious circle, broken by the administration of ACEi) appears to be primed by microangiopathic processes (Trotter et al., 1988) similar to those seen in other SSc-vessels (and characterized by deposition of platelet antigens within the vessel walls (Miller et al., 1980). These processes, which lead to the development of spongiosis, thrombocytopenia, renal insufficiency, proteinuria and an active urinary sediment, also resemble the pathophysiological features of thrombotic thrombocytopenic purpura (TTP), from which scleroderma renal crisis is often hardly distinguishable. Platelet consumption due to defective von Willebrand factor (vWF) turnover is the hallmark of TTP, but notably the presence of “supranormal” multimers of vWF has also been recognized in sera from SSc patients (Manucci et al., 1989).

**PLATELETS AND ABNORMALITIES OF CELL PROLIFERATION AND SECRETION IN SSc**

Aberration in cell proliferation and secretion is a hallmark of SSc and expresses on the one hand as “frustrated angiogenesis,” a process ultimately leading to chronic ischemia, and on the other hand as excessive myeloblast proliferation and deposition of connective tissue. Such anomalies in the control of the cell life-cycle are globally due to excessive release of mitogens like TGF-β, PDGF, VEGF, EGF, FGF, IGF-1, endothelin 1, IL-8, IL-6, and IL13 in response to inflammation and/or ischemic injury (Mausriel, 2005; Trojanowska, 2008). The importance of these molecules in the pathogenesis of SSc has been confirmed recently by studies utilizing DNA microarray technologies (Whitfield et al., 2003; Gardner et al., 2006). However such kind of studies can only suggest the nature of the complex intercellular interactions underlying the development of such humoral alterations and may even fail to adequately evaluate subtle functional variations in the biological behavior of cytoplasts containing small amounts of RNA, as are platelets (Postlethwaite and Chiang, 2007).

Several other studies have indeed recognized the fundamental role of platelets in determining tissue remodeling in SSc (Silieri et al., 2011) as in many other diseases related to vascular injury (May et al., 2002; Donners et al., 2008; Nurden, 2011; Maugeri et al., 2012):

1. as the main constituents of blood clots, platelets are directly responsible for the development of ischemia by means of (micro)-vascular thrombosis;
2. by their productive interactions with leukocytes (Maugeri et al., 2012), they actively drive the subsequent inflammatory and (mi)-repair response (Figure 1);
3. in the setting of injured vessels, they also directly release active mitogens like TGF-β, PDGF, or lysophospholipids (Pattanaik et al., 2011) and stimulate fibroblasts sensitivity by releasing serotonin (Dees et al., 2011; Figure 1).

Defective vasculogenesis characterizes SSc (Kuwana et al., 2004) and develops as a consequence of the overwhelming activity of anti-angiogenic factors such as the long pentraxin PTX3 (which is released by the endothelium and by activated myeloblasts in SSc; Lucetti et al., 2004; Margheri et al., 2010) upon a yet enhanced VEGF/FGF-mediated angiogenic response (Giusti et al., 2006; Margheri et al., 2010), which shows the important contribution of circulating platelets (Solanilla et al., 2009). PTX3, an emerging marker and a central player in vascular/inflammatory injury (Bottazzi et al., 2010; Maugeri et al., 2012), besides its widespread regulatory functions in innate immunity (Manfredi et al., 2008), acts in the circulating blood as a key modulator of neutrophil-platelet cross-talk: after release by neutrophil secondary granules PTX3 inhibits platelet-leukocyte aggregation and prevents the binding of fibrinogen to activated platelets (Maugeri et al., 2012). Less is known about the role of tissue-derived PTX3 on platelets, especially in the setting of SSc.

**CONCLUSION**

Besides their ancient role in hemostasis, platelets exert a complex set of interactions with circulating and tissue residing cells, which connotes them as circulating regulators of vascular homeostasis, immune function, and tissue remodeling. Along with this line they are actively involved in the development of the main pathological phenomena of SSc, although variations in their biological behavior are probably underestimated markers of disease activity and treatment response to date. Further researches about the involvement of platelets in the initiation and perpetuation of the pathogenic phenomena of SSc would possibly disclose new therapeutic perspectives for such a nebulous and still health-impacting disease.

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REFERENCES

Agache, I., Radiv, M., and Duca, L. (2007). Platelet activation in patients with systemic sclerosis – pattern and significance. J. Intern. Med. 193, 183–191.

Austri, P., Forstner, D., Bogos, E., Lehnig, M., Bruner, R., Hett, U., Rens, H., Hallmann, R., Scheffold, A., Radbruch, A., and Hanusa, H. (1997). P- and selectin-mediated recruitment of T-helper but not T-helper 2-cells into inflamed tissues. Nature 385, 81–83.

Banchereau, J., and Pascual, V. (2005). Type 1 interferon in systemic lupus erythematosus and other autoimmune diseases. Arthritis. Rheum. 53, 383–391.

Belch, J. J., O'Donnell, A., Darba, D. B., and Sterneck, R. D. (1985). Platelet sensitivity to a prostacyclin analogue in systemic sclerosis. Br. J. Rheumatol. 24, 346–350.

Biondi, M. L., Mariani, B., Biondi, E., and Agostoni, A. (1988). Plasma free adrenenephrine and platelet count in patients with Raynaud's phenomenon. J. Intern. Med. 19, 335–339.

Böttcher, B., Dond, A., Garlands, C., and Mantovani, A. (2010). An integrated view of humoral innate immune-constituencies as a paradigm. Arznei. Res. J. 55, 177–183.

Bottazzi, B., Doni, A., Garlanda, C., and Banchereau, J., and Pascual, V. (2006). Endothelial and mesenchymal cells in platelet non-integrin type I collagen interactions. Thromb. Res. 120, 176–182.

Clark, S. R., Ma, A. C., Tavener, S. A., Coleiro, B., Marshall, S. E., Denton, C. et al. (2007). Platelet-derived serum links vascular disease and tissue fibrosis. J. Exp. Med. 206, 961–972.

Dacor, G., Togari, C. M., Soguld, M. H., and von Andrian, U. H. (1998). Circulating activated-platelets nonsyntenic hemophila homozygous and immunity in L-selectin-deficient mice. J. Exp. Med. 187, 197–204.

Dacor, T. G., Puri, K. D., Warnock, R. A., Springer, T. A., and von Andrian, U. H. (1996). Platelet-mediated homophila delivery to high endothelial venules. Science 275, 252–255.

Demers, M., Becker, L. R., Liowens, D., Marzini, I., Herne, J., Janson, B. J., Wijnands, E., Clauw, J., Zamecki, A., Niber, G., Ahonen, C. L., Babooh, U., Newby, A. C., Noble, R. J., Diemer, M. J., and Langers, E. (2010). The CD46-TRAP39K array is the key regulator of the CD40/CD40L system in autoimunity formation and arterial remodeling. Blood 111, 4596–4604.

Denex, V., Homest, L. D., Donna, E., Dallakzhan, P., Alin, E. R., and Ahn, Y. S. (2007). Interstitial lung disease (ILD) and severe ITP. Thromb. Res. 120, 75–80.

Dřízeň, P. R., Gerg, P., and Myers, M. D. (1999). Endothelial and adenosine-based dephosphatase in Raynaud's phenomenon and sclerosis. J. Rheumatol. 26, 2286–2288.

Ehara, H., Akino, Y., and Sowilo, T. (2008). Immuno- and Stem. Mark. K. (2002). Matura vascularization endothelial cells can give rise to smooth muscle cells via endothelium–mesenchymal transdifferentiation in vitro analysis. Circ. Res. 90, 1189–1196.

Feldhoff, L. T., Sebold, J. B., Kim, H. C., and Simonett, K. S. (1984). Serum induced platelet aggregation in systemic sclerosis. Clin. Exp. Rheumatol. 1992, 219–223.

Garra Porto, F., Margarit, C., and Gento, I. S. (2004). Raynaud's phenomenon and autoinmune markers. J. Rheumato. 31, 2004, author reply 2006–2007.

Garra Porto, F., Gennari, S., Raiti, B., Broccini, T., Lynes, M., Tzakis, E., Smith, D., Jafarzadeh, S., Blazik, M., Tan, F. K., and Myers, J. R. (1997). Gene profiling of scleroderma skin reveals robust signatures of diseases that are imperfectly reflected in the transcript profiles of exfoliated fibroblasts. Arthritis. Rheum. 54, 1901–1915.

Ganti, B., Fifis, G., Margheri, F., Settar, S., Rossi, L., Poggi, F., Lapini, L., Mapi, A., Del Rossa, A., Cefalì, M., Guiducci, S., Kahahd, B., Bancheri, L., Bombardieri, S., Marucci-Carrici, M., Genuini, G. F., Del Rosso, M., and Abbracchio, P. (2010). A model of anti-angiogenic differential tran-scription profiling of peripheral vascular cells under diffuse systemic skin patients. Arthritis. Rheum. 62, R1115.

Goldsworthy, M. J., Orchard, M. A., and Rowell, N. R. (1987). Increased platelet sensitivity to collagen-induced aggregation in whole blood patients with systemic sclerosis. Clin. Exp. Rheumatol. 5, 285–290.

Goldsworthy, M. J., Orchard, M. A., and Rowell, N. R. (1991). Whole blood platelet aggregation and coagulation factors in patients with systemic sclerosis. Br. J. Haemtol. 4, 677–680.

Harris, E. H., Anderson, U., and Pouls, D. S. (2012). HMB-45, a multifunctional alarmin driving autoimmunity and inflammatory disease. Nat. Rev. Rheumatol. 8, 195–202.

Herrick, A. L. (2011). Contemporary management of Raynaud’s phenomenon and digital ischemia. Curr. Opin. Rheumatol. 23, 555–561.

Holt, C. M., Lindsey, N., Moull, J., Rapp, G., Groves, M., Hame, A., Rowell, N. E., and Hughes, P. (1998). Antibody-dependent cellular cytotoxicity of vascular endothelium: characterization and pathogenic associations in systemic sclerosis. Clin. Exp. Immunol. 78, 359–365.

Homann, M., Stein, I., Joosuv, M., Marchesi, P., Castro, M. G., Lowenstein, J., and Ruggieri, Z. M., and Guidotti, L. G. (2001). Platelet-mediated cytokine- and hypothermia-induced liver damage. J. Exp. Med. 198, 197–204.

Kawakami, Y., and Ikeda, Y. (1998). Platelet sensitivity to collagen-induced aggregation in whole blood patients with systemic sclerosis. Clin. Exp. Rheumatol. 16, 170–174.

Kawana, M., Okada, Y., Nakanishi, K., Kawa, K., and Ikeda, Y. (2004). Defective vasculogenesis in systemic sclerosis. Lancet 364, 650–652.

Lazaridis, R., and Yock, M. (2003). Immune unimmunity and inflammation in systemic sclerosis. Curr. Opin. Rheumato. 21, 617–622.

Luchetti, M. M., Sambo, P., Malinigi, P., Svegliati Baroni, S., Neri, G., Panacci, P., Intorno, M., Soppacceus, A., Mantovani, A., and Garff, A. (2006). Scleroderma fibroblasts constituently express the long pentraxin PTX3. Clin. Exp. Rheumatol. 24 Suppl. 35, 566–572.

Lindberg, B. J., Bergman, P., Garfahari, L., von Stucke, E., Radeke, H., Gil, D., Iwah, S., Hend, K., Henschel, B., Kaufmann, B., Pfeiffer, J. M., and Bochow, W. H. (2010). Platelet, not endothelial, P-selectin expression contributes to recruitment of immunity in cutaneous contact hypersensitivity. Am. J. Pathol. 176, 1339–1345.

Martodjojo, A. A., Revers, Querini, P., Battani, G., and Jayson, M. I. (1989). Platelet production from mature megakaryocytes in patients with systemic sclerosis. J. Rheumato. 16, 503–507.

Maurice, P. M., Lambe, R., Latuda, A., Pietracusto, E., Wach, C., and Ramins, G. (1989). Supersensitive-Cell Wall factor multimers in scleroderma. Rheum. Dis. J. 40, 1038–1043.

O'Donnell, O. A., Fiacco, F. J., Mai, A. T., and Shulman, I. E. (1969). Pathologic observations in systemic sclerosis (scleroderma). A study of fifty-eight autopsy cases and fifty-eight matched controls. Am. J. Med. 46, 428–440.

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