SLE Thrombocytopenia: From Peripheral Platelet Destruction to Central Hemopoietic Defect

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Abstract: Thrombocytopenia in Systemic Lupus Erythematosus is a common clinical manifestation affecting up to one third of patients in published cohorts. Antiplatelet antibodies, antithrombopoietin antibodies and faulty hemopoiesis have been implicated among other immunologic and non-immunologic causes. This mini review is an uptodate summary of these immunologic phenomena.

INTRODUCTION

Thrombocytopenia, a generally benign complication of Systemic Lupus Erythematosus (SLE), largely reflects the disease activity in other systems and has recently been associated with long-term, irreversible organ damage [1-4]. The field of pathophysiology is rendered even more intriguing with autoantibodies against platelets, autoantibodies against thrombopoietin and bone marrow abnormalities constituting a puzzle of interactions related to this clinical phenomenon [5].

ANTIPLATELET ANTIBODIES: NOT ALWAYS TO BLAME

Immune platelet destruction in SLE is mediated through autoantibodies against their membrane glycoproteins. Humoral platelet destruction was first described by Harrington in 1951 [6] and the specific binding of autoantibodies to major platelet glycoproteins as previously described by Kurata, is considered as the basic immunologic disturbance related to thrombocytopenia [7].

Antigenic Targets

The main antigenic target is GpIIb/IIIa complex, which mediates platelet aggregation by binding adhesion proteins, including fibrinogen and von Willebrand factor (wWF), whilst autoantibodies against GpIb/IX and GpIa/IIa are detected less often [8,9]. The GpIIb/IIIa (CD41/CD61) complex is unique for the megakaryocyte (MK) lineage with 50,000 molecules being expressed on the platelet surface [10], constituting 3% of its total mass and 17% of its membrane mass. Both GpIIb and GpIIIa are glycosylated [11,12] and form a heterodimeric complex non-covalently linked to the rough endoplasmic reticulum [13]. In Glanzmann's thrombasthenia they are both absent, leading to a lack of aggregation in response to common platelet agonists [14,15]. The GpIb/IX/V (CD42), otherwise vWF receptor, is also unique for the MK lineage, even though similar proteins may be expressed by activated endothelial cells in response to cytokines [16]. The Bernard-Soulier syndrome is characterized by bleeding diathesis, thrombocytopenia with giant platelets, no response to ristocetin-induced aggregation and lack of the receptor [17]. Finally the GpIa/IIa complex (CD49b/CD29) (collagen receptor) facilitates collagen adhesion and is being expressed in various cell subtypes, with 1000 molecules present on the platelet surface [18].

Antibody-Mediated Clearance of Platelets

Sensitized platelets are removed from the circulation through interaction of autoantibodies Fc portion with macrophages. Their Fc Receptors (FcR) recognize the Fc part of IgG immunoglobulins, bridging humoral with cell-mediated immunity. Among the three distinct subtypes of FcR, i.e. FcRI (CD64), FcRII(CD32) and FcRIII(CD16) [19,20], only FcRII and FcRIII are implicated in platelet clearance from the circulation [21-23]. *Fehr* was the first to show that human immunoglobulin increases survival of anti-D sensitized erythrocytes in patients with immunologic purpura, a finding conceived as direct proof of phagocytic inhibition through FcR in so far as sensitized erythrocytes are cleared through this pathway only [24]. The net impact of the FcR system is determined by the balance between the stimulating effect of FcRIIA and FCRIIIA, and the inhibitory effect of FcRIIB of macrophage. Although the regulatory mechanism is unclear, each receptor effect seems to correlate with a discrete response to treatment. Specifically anti-D globulin response depends on FcRIIA [25], and rituximab response on FcRIIIA (at least in lymphomas). Experimental models have shown that response to intravenous immunoglobulin (IVIG) depends on FcRIIB [26] and mice not expressing FcRIIB have no therapeutic benefit. In humans, the treatment effect is regulated through upregulation of expression of the inhibitory FcRIIB [27].

Detection of Antiplatelet Antibodies

The last four decades have seen the advent of several methods for detecting antiplatelet antibodies. Chronologically they can be classified in three groups. The first group, that measured functional deviations of normal platelets incubated with patients' sera, was abandoned due to low sensitivity and specificity [28-30]. The second group measured the IgG bound to platelets (Platelet associated IgG, PAIgG) [31]. High levels of PAIgG characterized patients with immu-

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nologic purpura and were considered to represent antiplatelet antibodies located on the platelet surface [32,33]. However, accumulated evidence suggested that normal platelets have two IgG pools, one located on platelet surface (100 IgG molecules) and one intracellular pool (20,000 IgG molecules) [34,35]. Older methods also measured the IgG of α-granules, leading to falsely elevated results for surface IgG [36-38]. Surface immunoglobulin was later measured with I131-labeled monoclonal antibody for all IgG subclasses or labeled staphylococcal protein A [39-41]. In AITP surface immunoglobulin may be elevated, but this finding is also observed in non-immunologic thrombocytopenias and marrow failure. Therefore, these methods whilst sensitive (80-90%) were not specific, as they were unable to discriminate between normal and abnormal IgG.

The third group involved current methods (ELISA, MAIPA) which detect antiplatelet antibodies against specific surface glycoproteins and are considered specific, although lacking in sensitivity (47-60%) [42].

Direct MAIPA (Monoclonal Assay Immobilization of Platelet Antigens) has an estimated sensitivity of 49-66% and a specificity of 78-92%, with a positive predictive value of 80-83% [43-45]. A negative test can not exclude the diagnosis [44,46], and detection of circulating serum autoantigens is even less sensitive. Concordance between various laboratories for binded autoantibody is 55-67% and less for plasma [47]. Their prognostic value is narrowed when SLE emerges among the differential diagnosis of thrombocytopenia, as well as chronic hepatitis, myelodysplasia and lymphomas [48,49] and AITP cannot be differentiated from secondary immunologically-mediated thrombocytopenias [50]. New generation, antigen-specific ELISAs can also detect circulating autoantibodies, using specific monoclonal antibodies, bearing the limitations of MAIPA including loss of unusual antigenic targets (should the specific monoclonal antibody is not available), or loss of usual antigenic targets if the binding epitope of the monoclonal antibody is the same as that of the with autoantibody.

Despite the high prevalence of these antibodies among thrombocytopenic subjects, a number of patients display thrombocytopenia without anti-PLT positivity whilst a significant proportion of anti-PLT positive patients have never developed thrombocytopenia. Moreover, a correlation between anti-PLT antibodies and lower platelet counts is not always evident. [7,51,52]. However the fact that anti-PLTs are absent in the sera of SLE patients who recovered from thrombocytopenia after receiving immunosuppressive treatment, confirms a potential pathogenetic role, though not an exclusive one. Circulating and platelet-bound anti-PLTs are noted in significant proportion of patients (SLE, AITP) who have active thrombocytopenia. In contrast, when platelets normalize in responders, they become undetectable or decrease significantly and reappear in relapse [44,47,53].

In a recent series of SLE patients, autoantibodies against platelet membrane antigens were detected in the sera of 29% of patients, 88% of which targeted the Gp IIb/IIIa complex and less frequently the Gp Ia/IIa, HLA I and Gp Ib/Ix complex. Almost 16% of patients had multiple antigenic targets. The prevalence of antiplatelet antibodies in the thrombocytopenic and non-thrombocytopenic groups did not differ, accounting for about 40% in each group. Notably, none of the post-thrombocytopenic individuals exhibited autoantibody activity against platelets. This latter group had received immunosuppressive therapy and achieved normal platelet counts [53].

SLE Thrombocytopenia vs AITP

Even though antiplatelet antibodies recognize the same membrane glycoproteins both in SLE and AITP, the immunopathogenesis appears different. AITP is considered as an organ-specific autoimmune disorder, related to molecular mimicry, cryptic epitope spreading, Th1/Th1 imbalance and an abnormal profile of cytokines that initiate the immunologic disturbance [54,55]. The latter is characterized by shift to Th1 response with depression of Th2 cells [56,57]. Conversely, when AITP is in remission, a Th2 shift is observed [58]. Moreover, AITP patients display increased HLA-DR expression, the recognition molecule for CD4+ TCR, which is considered to be the basic initiator of T-cell dependent immunologic response. Indeed, increased T-cell activity is observed in AITP whereas patients with SLE thrombocytopenia do not express HLA-DR on their platelets nor expand specific T-cell clones in the presence of platelets [59-61]. Finally, AITP patients show evidence that autoantibody production is mediated through the oligoclonal expansion of B-cells after exposure to platelet antigens, using genetically predetermined and specific rearrangements of heavy and light chain genes [62]. These characteristics are not evident in SLE, suggesting that SLE thrombocytopenia is not a direct immunologic response against platelets and therefore not a true AITP [63]. Even though antiplatelet antibodies remain an acceptable mechanism of platelet destruction [64], other mechanisms have attracted attention.

THROMBOPOIETIN & ANTI-THROMBOPOIETIN ANTIBODIES: THE NEW SUSPECTS

Human TPO is the crucial regulator of PLT production with five independent research groups having identified and established its significant role in normal hemopoiesis [65-69]. It acts as a growth factor in the commited progenitor cells [colony-forming units of megakaryocytes (CFU-MK)], differentiates immature megakaryoblasts, inhibits apoptosis and leads finally to the release of normal platelets. Morphologically, it increases the number, size and ploidy of MK**.** It has no effect on normal platelets [70-72]. *In vitro*, the appearance of functional platelets occurs after 10-12 days after TPO stimulation of human CD34+ cells [73]. Its ability to promote the expansion and maturation of megakaryocytic lineage and its synergistic effect with other cytokines has been confirmed in subpopulations of human progenitor cells from the bone marrow [74-77], fetal marrow [78], peripheral blood [79-81] and umbilical cord [82].

The C-mpl receptor of TPO belongs to Type 1 membrane receptors, being expressed in CD34+ progenitor cells, in megakaryocytes and in normal platelets [74,83]. In experimental knock-out models, the homozygous deletion of the c-mpl or TPO gene leads to peripheral thrombocytopenia with an 85-95% concurrent reduction of megakaryocytes in bone marrow, the remainder being characterized by nuclear immaturity, lack of cytoplasmic granules and organelles. A 70% reduction is also observed in both erythroid and granulocytic lineages, confirming TPO synergistic role in early hemopoi-

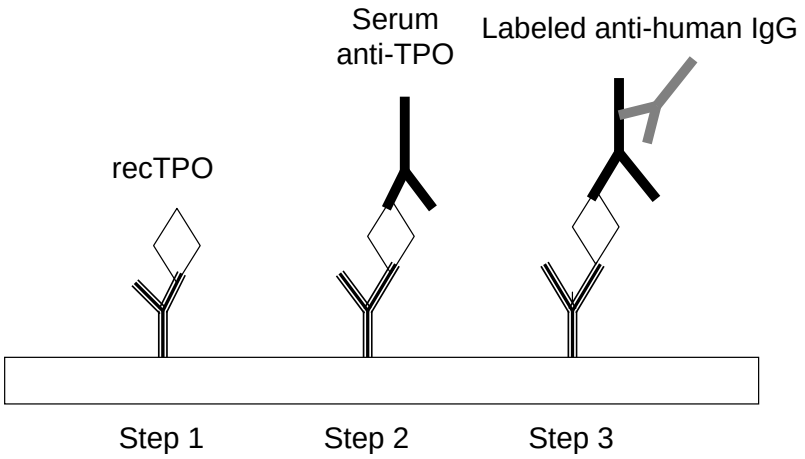

Fig. (1). Principles of sandwich ELISA for anti-TPO detection.

Anti-TPO precoated ELISA plates are saturated with recombinant human TPO (recTPO) (step 1). After washing, patient sera is added and anti-TPO (if present) binds to recTPO (step 2). Anti-human IgG conjugated with alkanine phosphatase is added (step 3) and color is measured at 405 nm after adding the substrate solution. Positive cut-off is set as the mean OD of 30 sera from healthy donors plus three standard deviations [53].

esis [84,85]. Single point c-mpl mutations characterize the rare congenital amegakaryocytic thrombocytopenia [86].

Experimental data have documented that TPO mRNA expression is stable in liver and kidneys and therefore serum levels are regulated by megakaryocyte mass [87,88]. Efforts to correlate peripheral platelet number with TPO levels in various disorders have initially faltered probably due to the wide range of values of both parameters. In the advent of newer quantitive ELISA, TPO levels were measured for both patients and healthy individuals, with levels for the latter varying usually below 200pg/ml. In contrast, TPO levels in thrombocytopenic patients are higher compared to normal sera. An impressive exception is AITP, with TPO levels being disproportionally lower than expected based on platelet count [89-93]. TPO levels are expected to be high in decreased platelet production (aplastic anemias, myelotoxic therapies) and inversely correlate with platelet count. On the contrary, in AITP where decreased platelet survival is expected but megakaryocyte mass is normal or increased, TPO levels are low [94-96]. In the only prospective study of TPO levels in AITP, its concentrations were comparable to normal volunteers and no negative correlation was documented with platelet numbers [97]. In chronic hepatic disease TPO levels are also low probably due to decreased hepatic synthesis [98,99].

Fureder [100] was the first to detect naturally occurring autoantibodies against thrombopoietin (anti-TPO) in 23% of SLE patients and correlate their presence with lower platelet counts, but he failed to display a difference in TPO levels. TPO levels, though high compared to normal controls and consistent with disease activity and complement level, did not correlate with platelet count. In contrast, anti-TPO autoantibodies are not a characteristic of AITP patients [97]. In the only reference of amegakaryocytic thrombocytopenia related to anti-TPO autoantibodies, TPO levels and platelet count increased as antibody titer decreased during immunosuppressive treatment with cyclosporine [101]. A recent analysis confirmed the presence of anti-TPO antibodies in SLE sera (Fig. **1**), and excluded their presence in rheumatoid arthritis and primary Sjogren patients who served as controls. Although anti-TPO positive patients showed no statistically significant differences in platelet count at specimen collection, longitudinal analysis revealed sustained lower platelet levels and lower circulating levels of TPO [53]. High concentrations of antibodies may bind avidly with natural TPO enabling systemic TPO to be stripped and eliminated by capturing the protein in immune complexes. Hence, a number of SLE patients produce antibodies that are likely to directly neutralize their own TPO, indicating their bimodal nature: *First*, by engendering immune-complexes, a non-specific mechanism that enhances peripheral platelet consumption [52, 102-104]. *Second*, by decreasing the effective TPO concentrations for stimulating megakaryopoiesis [53].

To support this hypothesis, SLE sera were incubated in vitro to assess the effect on CFU-MK. Anti-TPO positive sera significantly inhibited mature CFU-MK compared to anti-TPO negative SLE sera and were associated with lower circulating TPO concentrations. TPO-induced CFU-MK are small and mature, with low proliferative capacity, suggesting that TPO works mostly late in megakaryocyte progenitor development [71,105]. These findings suggest an antibody-associated restriction of the TPO-mediated effect on megakaryopoiesis. In conclusion, the capture and elimination/neutralization of circulating TPO by the antibodies may result in lower effective cytokine concentration to support megakaryopoiesis [106].

BONE MARROW IN SLE THROMBOCYTOPENIA: A TARGET OR A CAUSE OF AUTOIMMUNITY?

There are several indications for faulty hemopoiesis in SLE, most of which are attributed to the presence of autoreactive lymphocytes and the effect of proinflammatory cytokines [107,108]. The pathophysiologic disturbance includes the inhibition of colony formation and apoptosis through immunologic processes, as autoreactive lymphocytes are detected in bone marrow cultures [107,109] and their removal from bone marrow suspension increases the clonogenic potential of hemopoetic progenitor cells [110]. A recent study showed an increased expression of the FAS antigen in CD34+ cells of SLE patients [111] and a significant

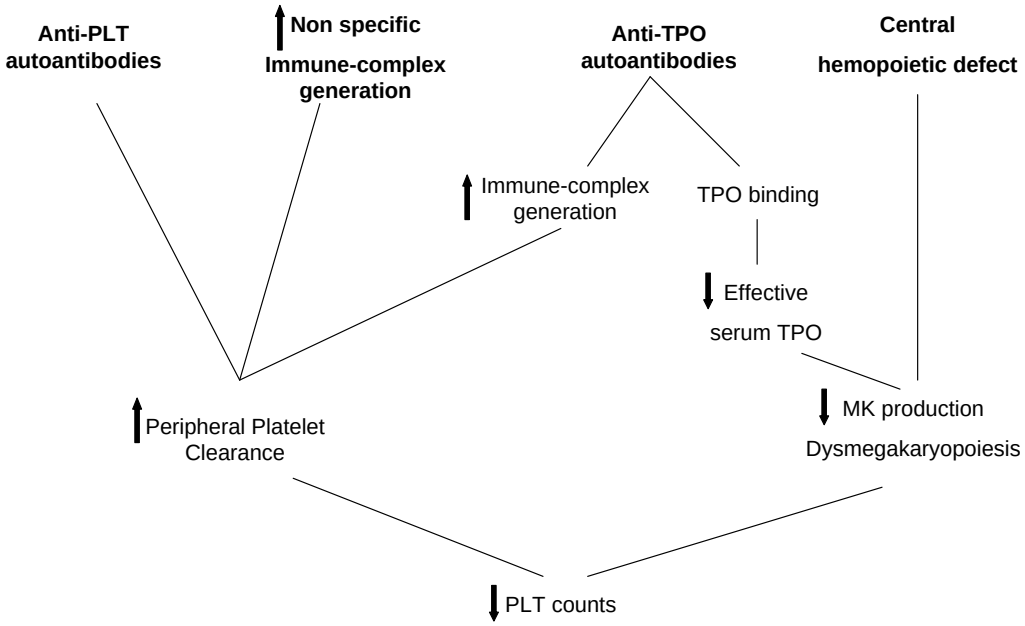

Fig. (2). Summary of mechanisms implicated in SLE thrombocytopenia.

correlation of apoptotic cells with FAS+ cells in the CD34+ compartment is observed, suggesting that the Fas signaling pathway is implicated in the induction of apoptosis of hemopoietic progenitor cells. Albeit increased expression of Fas does not invariably lead to apoptotic death [112], its implication in stem cell apoptosis is sufficiently documented in various diseases [113,114]. The same study documented a defect in the bone marrow microenvironment, leading to a decreased support of hemopoiesis in culture systems and a decrease in both primitive CD34+/CD38- and committed CD34+/CD38+ progenitor cells.

Current knowledge of Mesenchymal stem cells (MSCs) is primarily derived from studies performed on *ex vivo* expanded cells. There is a general consensus that MSCs are residents of the microenvironment and play a role in supporting hematopoiesis. An emerging body of data indicate that MSCs possess immunomodulatory properties and may play specific roles as immunomodulators in maintenance of peripheral tolerance, transplantation tolerance, tumor evasion, fetal-maternal tolerance, as well as autoimmunity [115,116]. In vitro studies suggest T-cell suppression and inhibition of differentiation of dendritic cells.

Recent observations suggested that MSCs from patients with autoimmune diseases are affected. MSCs derived from the BM of patients with severe aplastic anemia are deficient in their ability to suppress T-cell proliferation and cytokine release [117]. Whether these defects are relevant for the pathogenesis of aplastic anemia remains to be shown. Both stromal and endothelial progenitors in patients with systemic sclerosis also have been reported to be functionally impaired, showing a reduced proliferation and differentiation capacity. It has been suggested that the functional impairment of the BM microenvironment may be impicated in the impaired vasculogenesis in scleroderma [118]. It may therefore be hypothesized that the immunosuppressive capacities of MSCs might play a role in the BM microenvironment to create an immunoprivileged site that protects primitive stem cells from the bystander effects of local immune responses. Bone marrow MSCs of SLE patients display functional abnormalities and decreased hemopoietic recovery after MSCs infusion is observed in experimental models exposed to chemotherapy [119]. Theoretically, the immunosuppressive properties of MSCs create an immunoprivileged bone marrow microenvironment that protects hemopoietic progenitor cell from immune-mediated destruction, and these cells might be implicated in the pathogenesis of cytopenias in SLE [120].

Morphologically, in SLE patients with cytopenias, bone marrow changes include decreased cellularity, polyclonal B and T cell aggregations, and stromal changes (including oedema, fibrosis and vascular changes) [121]. Dysplastic changes were a uniform finding in all hemopoietic lineages (dysmyelopoiesis with maturation arrest, dyserythropoiesis and dysmegakaryopoiesis) with disorganized microarchitecture. Megakaryocytic atypias included hypolobulated nucleus, pyknotic appearance with denuded cytoplasms (naked MKs) and other morphologic abnormalities of differentiation. Finally, abnormal localization of immature precursors without a typical paratrabecular location (ALIP clusters) have been observed and correlated with the degree of anemia [53,121]. This finding has so far been linked to myelodyplastic syndromes. Therefore, bone marrow is considered as a target organ of autoimmunity in SLE and associated cytopenias.

Epilogue

The extent of interactions, the sequence of events and the magnitude of effect for each mechanism remain partially inconclusive. Antiplatelet antibodies are neither specific nor sensitive in SLE, despite macrophage mediated clearance being the best documented mechanism of platelet destruction. Anti-TPO antibodies have a persistent but weak effect on platelet counts through a presumed thrombopoietin-dependent, antibody-mediated inhibition of megakaryopoiesis. Bone marrow damage may largely determine the extent and severity of thrombocytopenia as it is a uniform finding in SLE thrombocytopenia and other cytopenias (Fig. **2**).

Bone marrow damage itself may be a secondary event in the context of SLE immunologic disturbance or alternatively, a primary defect through MSCs abnormalities that generate and perpetuate a central hemopoietic defect and participate in autoantibody generation and immunologic phenomena in SLE. The latter effect may be mediated through a disruption of normal immunomodulatory mechanisms in the bone marrow.

Immunosuppressive therapy has been regarded as the mainstay of treatment for SLE-associated thrombocytopenia [5]. In a recent review series, B-cell depletion using anti-CD20 (rituximab) has been regarded an effective alternative option in AITP [122] with an overall response rate of 62.5% in adults. An emerging body of data in SLE suggests that rituximab may also be highly effective in SLE cytopenias [123] through a mechanism that also involves the reduction of the pathogenic antibodies [123,124]. Second-generation thrombopoietic agents have been used in clinical trials to stimulate platelet production in AITP patients who are not responsive to standard treatments. These new molecules bear no structural resemblance to TPO but still bind and activate the TPO receptor. Romiplostin [125] and eltrombopag [126] have been successfully used in refractory AITP. Ongoing phase III clinical trials will reveal the potential of these agents in the management of AITP prior to splenectomy and for long-term maintenance therapy, as well as their relative benefit compared with standard care treatment [127]. These agents may also prove effective in SLE thrombocytopenia, where an impaired platelet production is one of the underlying mechanisms.

ABBREVIATION LIST

AITP	=	Autoimmune Thrombocytopenic Purpura
CFU	=	Colony Forming Unit
ELISA	=	Enzyme Linked Immunosorbent Assay
MAIPA	=	Monoclonal Assay Immobilazation of Platelet Antigens
MK	=	Megakaryocyte
MSCs	=	Mesenchymal Stem Cells
SLE	=	Systemic Lupus Erythematosus
TPO	=	Thrombopoietin
PLT	=	Platelets

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