A Sticky Story for STAT3 in Platelets

Running title: Chen et al.; A sticky story for STAT3 in platelets

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The Signal Transducer and Activator of Transcription 3 (STAT3) is a cytoplasmic protein that, upon appropriate signaling, translocates to the nucleus and binds DNA response elements of target genes\(^1\). As a result, STAT3 mediates the transcription of key mediators involved in mitogenesis, cell survival, apoptosis, cell cycle regulation, angiogenesis, and metastasis development\(^1,2\). STAT3 also regulates the transcription of several genes in megakaryocytes that are required for the formation of platelets (Figure 1 and Drachman et al\(^3\)).

As megakaryocytes form platelets, they transfer STAT3 to proplatelet tips. Consequently, STAT3 is found in platelets that circulate in the bloodstream (Figure 1). The presence of STAT3 in platelets raises the question of whether it regulates functional responses in platelets or is simply a vestigial remnant of megakaryocytes. An argument for the “leftover without function” hypothesis is the anucleate status of platelets: simply stated, “with no nucleus and no nuclear DNA there is no place for STAT3 to stick in platelets”. The problem with this argument is that simple is no longer a common word used to describe platelets. Moreover, why would platelets expend energy to carry a protein they do not need, especially since previous studies have shown that STAT3 undergoes signal-dependent phosphorylation in these anucleate cytoplasts\(^4\)? Well, any doubt regarding why STAT3 is present in platelets has been cleared up. Using a combination of pharmacologic and genetic based tools, Zhou and colleagues\(^5\) demonstrate that STAT3 affects how platelets stick to one another and extracellular matrices. In addition, the authors put forth a new role for interleukin-6 (IL-6) and its soluble receptor in enhancing platelet aggregation.

A major strength of the group’s findings is the plethora of evidence presented to make the story stick from men to mice and then back to men. First, they used two different types of STAT3 inhibitors to block collagen- and collagen-related peptide (CRP) dependent-dependent
aggregation as well as the formation of thrombi to a collagen substrate under flow conditions in human platelets. Neutralization of STAT3 also reduced collagen-dependent induction of P-selectin surface expression. STAT3 inhibitors, however, did not block ATP release nor did they dampen aggregation induced by adenosine diphosphate (ADP) or a thrombin receptor activating peptide (TRAP). Second, platelets from mice deficient in STAT3 aggregated poorly, had a low level of P-selectin surface expression and calcium influx in response to collagen, and formed smaller thrombi when exposed to a collagen matrix under arterial flow. The same platelets reacted normally to ADP and TRAP. Additional studies led to studies implying that GPVI is the primary collagen receptor on platelets linked to the STAT3 signaling pathway. Finally, Zhou and coworkers provided the first evidence that the IL-6 signaling complex can influence platelet function. They found that platelets constitutively express glycoprotein 130 (gp130), which is capable of interacting with exogenous IL-6 and the soluble IL-6 receptor (IL-6R). Together, but not individually, these IL-6 family members induce STAT3 phosphorylation and enhance collagen-dependent platelet aggregation.

A transcription-independent role for STAT3 builds on the growing appreciation that previously characterized transcription factors have diverse, non-canonical functions in platelets. In activated platelets, the Nuclear Factor kappa B (NF-κB) family member B cell lymphoma-3 (Bcl-3) interacts with Fyn-related tyrosine kinases to contract fibrin-rich clots. NF-κB itself also has roles in limiting platelet activation and NF-κB inhibitors attenuate the formation of lipodia in adherent platelets. Much like STAT3, peroxisome proliferator-activated receptor γ (PPARγ) regulates collagen-dependent platelet aggregation that is driven by GPVI. Ligand-dependent binding of retinoid X receptor (RXR) also controls guanosine triphosphate (GTP)-binding protein Gq function and thereby aggregation responses in platelets. Cumulatively,
these studies point to the sundry function of proteins that were originally thought to have a “sole-role” in transcription.

One of the most intriguing findings of Zhou’s work is the identification of an IL-6 signaling pathway that links inflammation to thrombosis. In response to inflammatory cues, IL-6 is synthesized and released by various types of nucleated cells. IL-6 exerts its activities through two molecules: the IL-6R (also known as IL-6Rα) and gp130 (also referred to as IL-6Rβ)\(^{12}\). The IL-6R is either membrane-bound (mIL-6R) or soluble (sIL-6R). As its name implies, sIL-6R is released into the extracellular milieu where it binds IL-6 and then forms a complex with membranous gp130. This unique receptor signaling system, termed IL-6 trans-signalling\(^{13}\), induces cellular activation including STAT3-dependent transcriptional responses. Until now there has been no evidence that IL-6 trans-signalling occurs in platelets. Zhou et al. demonstrate that in combination with the sIL-6R, IL-6 binds membrane expressed gp130 and primes platelets for collagen-induced cellular activation. This suggests that heightened IL-6 trans-signalling in response to inflammation may enhance thrombus formation in a variety of human diseases such as rheumatoid arthritis, lupus, and sepsis. Conversely, deficiencies in IL-6 production, which has been reported to occur in Common Variable Immune Deficiency (CVID)\(^{14}\), may lead to dampened thrombus formation and increased bruising and bleeding that is commonly observed in patients with this syndrome.

Selective inhibition of IL-6 trans-signalling has recently received considerable attention for the treatment of cancer and an IL-6R blocking antibody (tocilizumab) was recently approved for Castelman’s disease and rheumatoid arthritis (reviewed in \(^{15,16}\)). A STAT3 decoy inhibitor is currently being tested in patients with head and neck cancer and there is emerging evidence that STAT3 inhibitors may prove useful in treating disorders of cardiac-related inflammation\(^{15,17}\).
Thus, it will be important to consider “off-target” inhibition of platelet activity, which may be good or bad, when patients are treated with IL-6 trans-signalling and STAT3 inhibitors. Indeed, the authors speculate that inhibition of STAT3 may improve inflammation-induced platelet hyperreactivity and improve the efficacy of aspirin in patients with coronary artery disease. The interplay of IL-6 trans-signalling and STAT3 with GPVI will also have to be pondered as the efficacy of GPVI receptor antagonists are screened in the clinic.

Thrombosis is often linked to inflammation, but the reverse has received little attention until Zhou’s group unmasked the STAT3 signaling pathway in anucleate platelets. Their results challenge existing paradigms and, in doing so, reveal that we should never underestimate the resolve of platelets to use previously-described nuclear-based systems in alternative ways. Identification of a three-way bridge between IL-6 trans-signalling, STAT3, and GPVI that courses to aggregation adds to the fascinating biology of platelets (Figure 1). It also creates a “sticky story for STAT3” in platelets, and potentially the cytoplasm of nucleated cells, with clinical implications for human disease.

Conflict of Interest Disclosures: None.

References:


Figure Legend

**Figure 1.** Schematic representation of “traditional” and “non-traditional” roles of STAT3 in megakaryocytes and platelets, respectively, as described by Zhou et al. and reviewed here.
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