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Activated Platelets Induce Endothelial Cell Inflammatory Response in Psoriasis via COX-1

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Abstract

Objective: Patients with psoriasis have impaired vascular health and increased cardiovascular disease (CVD). Platelets are key players in the pathogenesis of vascular dysfunction in CVD and represent therapeutic targets in CV prevention. The object of this study was to define the platelet phenotype and effector cell properties on vascular health in psoriasis and evaluate whether aspirin modulates the platelet-induced phenotype.

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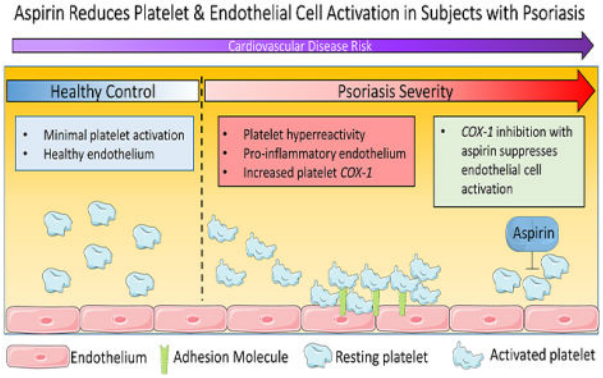
Approach and Results: Platelets from psoriasis patients (n=45) exhibited increased platelet activation (relative to age and gender-matched controls, n=18), which correlated with psoriasis skin severity. Isolated platelets from psoriasis patients demonstrated a 2 – 3-fold (p<0.01) increased adhesion to human aortic endothelial cells and induced pro-inflammatory transcriptional changes, including upregulation of interleukin (*IL*) *8*, *IL1β*, and cyclooxygenase *(COX)-2*. Platelet RNA sequencing revealed an interferon signature and elevated expression of *COX-1*, which correlated with psoriasis disease severity (r=0.83, p=0.01). In a randomized trial of psoriasis patients, two weeks of 81mg low-dose aspirin, a COX-1 inhibitor, reduced serum thromboxane (Tx) B_2, and reduced brachial vein endothelial pro-inflammatory transcript expression >70% compared to the no-treatment group (p<0.01). Improvement in brachial vein endothelial cell inflammation significantly correlated with change in serum TxB2 (r=0.48, p=0.02).

Conclusions: In patients with psoriasis, platelets are activated and induce endothelial cell inflammation. Low-dose aspirin improved endothelial cell health in psoriasis via platelet COX-1 inhibition. These data demonstrate a previously unappreciated role of platelets in psoriasis and endothelial cell inflammation and suggest aspirin may be effective in improving vascular health in patients with psoriasis.

Clinical Trial Registration: URL: https://clinicaltrials.gov Unique Identifier: NCT03228017

Graphical Abstract



Keywords

Psoriasis; Platelets; Endothelium; Inflammation; Aspirin

Subject terms:

Inflammation; Platelets; Translational Studies; Primary Prevention

Introduction

Psoriasis is a common chronic inflammatory skin disease found to increase cardiovascular disease (CVD) upwards of 50%.[1] Primary CVD prevention guidelines from the American Heart Association and American Academy of Dermatology each suggest incorporating a diagnosis of psoriasis when determining cardiovascular (CV) risk.[2, 3] The pathophysiology

of psoriasis includes abnormal keratinocyte proliferation along with dysregulation and interaction of both the innate and adaptive immune response.[1, 4] Key pathogenic cytokines upregulated in psoriasis including interferon (IFN) gamma and interleukin (IL)-17A, are also implicated in the pathogenesis of vascular inflammation and atherosclerosis development.[1, 5–8]

Platelets are key regulators of inflammation, immune function, and atherothrombosis with heightened platelet activity linked to CV events.[9–11] In addition to thrombosis, platelets perpetuate atherosclerosis by stimulating and secreting pro-inflammatory cytokines and facilitating leukocyte recruitment, tethering, and rolling along an activated endothelium. Aspirin inhibits cyclooxygenase (COX)-1 signaling and decreases platelet aggregation, thereby reducing CV events.[12–14] However, aspirin for the primary prevention of CVD is only recommended in certain patient populations where the benefits outweigh the risks.[14, 15] In psoriasis, a population at accelerated risk of CVD, the role of platelets in vascular inflammation and CVD, and whether aspirin may modulate CV risk is uncertain.

Previously, direct brachial venous endothelial sampling demonstrated that psoriasis patients exhibit impaired endothelial cell vascular health.[16] Prior data from our group reported that platelets induce endothelial activation in systemic pro-inflammatory conditions such as systemic lupus erythematosus (SLE) and human immunodeficiency virus (HIV).[17, 18] Therefore, to investigate the pathophysiology of impaired vascular health in psoriasis, we performed a comprehensive evaluation of the platelet: (1) phenotype, (2) transcriptome, and (3) effector cell properties on endothelial cell vascular health. Finally, to understand the clinical implications of our *in vitro* studies, psoriasis patients were randomly assigned to 81mg of aspirin/day vs. no-treatment to test the hypothesis that COX-1 inhibition with low-dose aspirin reduces endothelial pro-inflammatory activation, as measured *ex vivo* through brachial vein endothelial transcriptomic analysis.

Materials and Methods:

Additional data that support the findings of this study are available from the corresponding author upon reasonable request. A description of the full study methods are available within the article and online only supplemental methods and data files.

Study population

Patients with a diagnosis of psoriasis, free of clinical CVD, were recruited from New York University (NYU) Langone Health dermatology, phototherapy, and psoriatic arthritis specialty clinics between September 2017 and April 2019 as part of an ongoing study (NCT03228017) investigating endothelial vascular health in psoriasis. Inclusion criteria included ≥1% body surface area of psoriasis or psoriatic arthritis ≥1 swollen/tender joint confirmed and quantified by a board-certified Dermatologist and/or Rheumatologist. Healthy age- and sex-matched controls were consecutively recruited during the study accrual period. Study exclusion criteria included those with a recent (<1 month before study enrollment) change or planned change in psoriasis therapy, use of aspirin or lipid-lowering (statin) therapy, other autoimmune conditions aside from psoriatic disease, poorly controlled hypertension, diabetes and chronic kidney disease (details in supplemental methods). The

study protocol was approved by the NYU School of Medicine Institutional Review Board (i17–00692). All subjects provided written informed consent before participation in-line with the Declaration of Helsinki.

General study protocol

Fasting (>4 hours) psoriasis and control participants underwent a medical history, blood pressure, heart rate and anthropometrics all through established protocols.[19, 20] As previously described and published,[21] a 20-gauge angiocatheter was then inserted into the brachial forearm and three J – shaped endovascular guidewires (Teleflex Inc., Reading Pa) advanced into the brachial vein, removed, washed in endothelial dissociation buffer solution and kept at 4°C until further processing. Blood collection (serum-separator tube, 3.2% sodium citrate, EDTA tubes) occurred immediately post endothelial harvesting as per previously documented protocols.[16]

After baseline assessment psoriasis participants were next assigned using a random number generator to 81 mg of aspirin/day or no-treatment (supplemental Figure I and supplemental methods). A repeat visit and full assessment occurred at week two. For the randomized clinical trial component, the primary study endpoint was change in the composite brachial vein endothelial cell inflammatory transcriptome - defined as the mean of the combined expression values from all assessed endothelial cell pro-inflammatory transcripts. Serum thromboxane B_2 (TxB2, the inactive metabolite of thromboxane A_2 and the downstream product of COX-1 signaling[22]) and *ex vivo* platelet aggregation to 1600 μM of arachidonic acid confirmed aspirin compliance. All sample processing and post sample analyses were performed blinded to study assignment.

Sample processing

Blood samples were processed within 30 minutes of blood draw, and if not used centrifuged and stored at −80°C. Lipid profiles and high-sensitivity C-reactive protein (hs-CRP) were performed at the NYU clinical laboratory (Abbott Architect System). Complete blood count was assessed in a research laboratory setting using a Sysmex XN-1000 PR™ Automated Hematology Analyzer. Serum proteins were measured through the commercially available Olink Proteomics platform and values expressed as a Normalized Protein eXpression (NPX, on a log2 scale).[23]

Endothelial harvesting and isolation

Brachial vein endothelial cells were isolated using magnetic beads coated with CD146 antibody. Endothelial cells underwent mRNA extraction, cDNA conversion, and pre-amplification.[24, 25] Transcripts of interest using TaqMan (Life Technologies) probes and primers were assessed by real-time quantitative polymerase chain reaction on an Applied Biosystems 7500 Fast Real-Time PCR System. Any transcript with a cycle count >34 was considered to be 0 expression. To ensure reproducibility across analyses, results are normalized to human acidic ribosomal protein (hARP) for each sample and gene.[26] Samples whose reference gene (either hARP or beta-actin) expression was greater than 2 standard deviations from the mean were deemed inadequate and excluded.

Platelet analyses

Platelet activation was assessed using an Accuri C6 flow cytometer (BD Biosciences). Antibodies to CD61 (DAKO,) and CD45 (BD Biosciences) were incubated on fixed whole blood and leukocytes were collected based on side-scatter properties and positive staining for CD45. Neutrophil and lymphocyte platelet aggregates were identified based on their scatter properties and co-expression of CD45 and CD61 and expressed as a percentage positive for adherent platelets. The remaining whole blood was incubated with CD42b (BD Biosciences), CD62L (BD Biosciences) or appropriate controls to measure P-selectin in basal or thrombin (0.025 U/mL) conditions as previously published.[17, 27, 28] Light transmission aggregometry to 1600 μM of arachidonic acid was conducted on a Helena AggRAM light transmission aggregometer within 2 hours of collection. Serum Thromboxane B_2 was detected in serum by enzyme-linked immunosorbent assay in duplicate (Cayman Chemical, #10004023) at a dilution of 1:20 using manufacturer protocols.

Washed platelet, endothelial co-incubation, and MEG-01 studies

Washed platelets were obtained from citrated whole blood and resuspended in Tyrodes buffer (supplemental methods). Isolated washed platelets were standardized to 500,000 platelets/mL and incubated at a concentration of 100:1 ratio on cultured human aortic endothelial cells (HAECs) for 4 hours. When indicated, aspirin at 3mM was introduced to the platelet solution immediately after whole blood collection and inhibition confirmed by *ex vivo* inhibition of platelet aggregation to arachidonic acid. *In vitro* platelet activation occurred with 0.5 U/mL of thrombin just prior to co-incubation. For adhesion assays, washed platelets were incubated with 3 μM of CellTracker Green CMFDA Dye (ThermoFisher). Quantification of adherent plates were performed using ImageJ (National Institutes of Health, Bethesda, MD). These protocols have been previously published.[17] HAEC activation occurred with recombinant TNFα (10 ng/ml, R&D Systems) and/or IL-17A (200ng/ml, R&D Systems).[16] Basal HAECS or stimulated with thrombin, TNFα, arachidonic acid or aspirin were used as controls as needed (data not shown).

Meg-01 cells were obtained from American Type Culture Collection (ATCC). For gene expression assays, 150,000 Meg-01 cells were seeded in 12 well plates. The cells were then stimulated × 24 hours with the indicated concentrations of IFNγ (100 ng/ml, Miltenyi Biotec) and/or IL-17A (200ng/ml, R&D Systems). In all *in vitro* experiments (both MEG-01 and HAECs); RNA was extracted and standardized to 250 ng/ml, converted to cDNA and assessed using the SYBR Green system on an Applied Biosystems 7500 Fast Real-Time PCR System (Foster City, CA).[17, 29] Transcripts were normalized to the housekeeping gene GAPDH and are reported as fold change over control.

Correlative light and scanning electron microscopy (SEM)

Isolated and washed platelets incubated with CellTracker Green CMFDA Dye were co-incubated on HAECs as noted above. The cells were washed, fixed and imaged by Zeiss Observer Plan-Apochromat. Green Fluorescent Protein signal and DAPI staining were used as the reference to correlate light and SEM. The cells were then fixed, post fixed, dehydrated and critical point dried using Tousimis autosamdri931. The coverslips were put on SEM

stabs, and sputter coated with gold/palladium by DESK V TSC HP Denton Vacuum. Images were taken by Zeiss Gemini300 FESEM using an SE2 Detector at 5kv with 6mm working distance (all as noted in the supplemental methods).

Platelet RNA sequencing

Platelets were washed and then isolated by negative $CD45^+$ and Glycophorin A^+ selection. A relative purity of platelet/leukocyte ratio of 1×10^7 was confirmed by flow cytometry with protocols previously published.[27, 30] RNA extraction, library preparation and differential gene expression was performed as previously described and as noted in the supplemental methods.[27, 30] All downstream statistical analyses and generating plots were performed in R environment (v3.1.1).To evaluate pathways and differential gene expression a nominal p value <0.05 with base mean >20 was used to determine statistical significance. Ingenuity pathway analysis (Qiagen Bioinformatics) was used to discover differentially expressed canonical, and biological disease pathways. Gene count values are expressed as normalized count values (NCV).

Immunohistochemistry and immunofluorescence of human skin and platelets

Frozen skin sections were blocked and then incubated with 10E5 antibody (ITGA2B, Laboratory of Blood and Vascular Biology, The Rockefeller University). For immunohistochemistry, biotin-labeled horse anti-mouse antibody (Vector Laboratories) was used to detect the 10E5 antibody. For immunofluorescence, 10E5 antibodies were amplified with anti-mouse IgG2a Alexa Fluor 647 (Invitrogen) and with laminin alpha 5 antibody (Santa Cruz Biotechnology) and amplified with goat anti-mouse IgG1 Alexa Fluor 568 (Invitrogen). Negative controls were generated with their appropriate antibodies (full details in supplemental methods).

Data are reported as mean ± SEM or SD where appropriate. Non-normally distributed data are reported as median and IQR (Q1, Q3). Statistical significance between psoriasis and control was performed using parametric, non-parametric, or Spearman's test for correlations. Log transformation with paired sample t- or Wilcoxon tests for changes between baseline and follow-up data points were performed as appropriate. The randomized clinical trial component was designed to detect a >80% reduction in serum thromboxane, and 50% difference in the mean composite pro-inflammatory endothelial transcriptome with 80% statistical power at a two-sided p-value of 0.05 (G*Power 3.1.9.2). Sample size estimates were determined from our work demonstrating a (1) 2 – 3 fold increase in pro-inflammatory activation in endothelial cells isolated from participants with psoriasis vs. matched-controls,[16] (2) platelet-induced upregulation of endothelial cell activation from other pro-inflammatory conditions, including SLE and HIV,[17, 30] and (3) *in vitro* work demonstrating a reduction of platelet induced endothelial cell inflammation with aspirin. Statistical significance was determined using a two-tailed alpha <0.05 with all analyses performed in Stata v. 14 (College Station, TX: StataCorp LP).

Results:

Clinical characteristics of the recruited subjects

Clinical and anthropometric measurements are presented in Table 1. Demographic and traditional CV risk factors were similar between psoriasis and controls. Participants with psoriasis had, on average, 16 years of disease duration with a median psoriasis area and severity index (PASI) score of 4 (2.6–6.9) indicating mild to moderate active psoriasis at the time of enrollment. Nine participants (20%) had concomitant psoriatic arthritis, 15 (33%) were on biologics, and 18 (40%) were receiving phototherapy (Table 1). Total white blood cell count was higher in psoriasis than matched controls with a trend towards higher absolute neutrophil count (Table 1). Serum IL-6 and high sensitivity C-reactive protein (hs-CRP) did not differ between groups (data not shown), yet each correlated with PASI (IL-6; r=0.67, p<0.001, hs-CRP; r=80, p<0.001). As expected, serum IL-17A was significantly higher in psoriasis compared to matched controls (2.7 [1.8–5.1] vs. 1.8 [0.5 – 1.5] NPX, p<0.01).

Platelets are activated in psoriasis

Investigation of the skin has identified the key role keratinocytes, immune cells, and pro-inflammatory cytokines play in the pathophysiology of psoriasis.[1, 31] Activated platelets are central mediators of inflammation.[32] To investigate whether platelets are implicated in the pathophysiology of psoriatic inflammation, we performed platelet staining in human skin biopsies. Platelets were absent in the dermal layer of controls (Figure 1A). However, in psoriatic lesional skin, platelets were identified in the dermal layer (Figure 1B), with immunofluorescence staining suggesting their localization as extravascular (Supplemental Figure IIA–C).

We next evaluated the phenotype of circulating platelets in psoriasis. Clinical hematologic parameters, including platelet count, mean platelet volume, and immature platelet fraction, were similar between groups (Table 1). While basal P-selectin expression did not differ (Figure 1B), platelet surface expression of P-selectin after thrombin stimulation, and neutrophil- and lymphocyte platelet aggregates (Figure 1C, 1D, Supplement Figure IIIC–E) were all higher in psoriasis compared to matched controls. In support of an activated platelet phenotype in psoriasis, platelet activity correlated with PASI (Figure 1E, 1F) and circulating levels of IL-17A and IL-6 (Supplemental Figure IIIA, IIIB). Altogether, these data provide evidence for heightened platelet activity in psoriasis.

Psoriasis platelets activate endothelial cells

Activated platelets promote atherosclerosis and induce vascular dysfunction across disease states.[17, 30, 32] Previously, we found that patients with psoriasis have impaired vascular health, as assessed by elevated brachial vein endothelial cell expression of inflammatory transcripts including *IL8, IL1β*, and *COX-2*.[16] To investigate the clinical relevance of increased platelet activity in psoriasis, we next performed platelet-endothelial co-incubation studies by incubating HAECS with washed platelets from patients with active psoriasis or controls. Both resting and activated platelets in patients with psoriasis preferentially adhered to HAECs (Figure 2A) and induced upregulation of HAEC *IL8*, *IL1β*, and *COX-2* mRNA (Figure 2B) compared to control platelets. Scanning electron microscope images confirmed

immunofluorescence findings (Figure 2C), demonstrating that platelets establish tubular connections with endothelial cells providing direct evidence of platelet-endothelial interactions.

Psoriasis platelet transcriptome profiling reveals pro-inflammatory biological pathways

Exploration of the platelet transcriptional landscape allows mechanistic insight into the platelet phenotype. Because we noted an activated platelet phenotype in psoriasis, platelet RNA sequencing was performed in a subset of 6 psoriasis patients with active disease (PASI - 9.5 [5 – 15]) and six healthy age-, sex-matched controls (Supplemental Figure I). We found 163 genes upregulated and 159 genes downregulated (nominal $p<0.05$, Figure 3A, 3B, Supplemental Table I - top 10 upregulated and downregulated genes). Biological disease pathway analysis highlighted gene overlap in atherosclerotic related pathways such as CVD development, cell-to-cell signaling, and lipid metabolism (Figure 3C).

Canonical pathway analysis revealed IFN (IFNγ + IFNα/β) signaling to be the top upregulated pathway in psoriatic platelets (z-score = 1.342, $p=3.6\times10^{-4}$, Figure 3D, Supplemental Figure IV), a known pathogenic signature in psoriasis.[5, 33] Consistent with heightened psoriasis IFN-mediated inflammation, platelet IFN associated transcripts correlated with PASI (Table 2A). Since IL-17A and IFNɣ are central drivers of disease in psoriasis,[31] our prior publication supported a dominant IFN signature in psoriasis[16], and our current data showed differential expression of circulating IL-17A (Table 1), we investigated the correlation between platelet IFN induced transcripts with circulating IFNγ and IL-17A. A significant correlation was observed between platelet IFN induced transcripts, IFNγ (Supplemental Table IIA), IL-17A (Table 2B), and the product of IL-17A × IFNγ (Supplemental Table IIB). Less robust associations were seen between IFN induced transcripts with IL-6 and hs-CRP (Supplemental Table IIC, IID).

To determine if psoriasis-mediated inflammation is a significant contributor to the platelet transcriptome, we employed a megakaryocyte cell model (MEG-01) to investigate the effect of causal proteins in psoriasis on megakaryocyte mRNA.[34] MEG-01 cells were stimulated with IFNγ, IL-17A, and the combination of IFNγ and IL-17A (Figure 3E). Stimulation with IFNγ alone had a modest effect on IFN induced transcripts; however, the effect on megakaryocyte gene expression was significantly increased with the combination of IFNγ and IL-17A (Figure 3F). Collectively, our results suggest that the platelet transcriptome is influenced by psoriatic inflammation (e.g. IFNγ IL-17A).

Aspirin reduces endothelial cell inflammation *in vitro* via COX-1

COX-1 signaling is an important positive feedback mechanism for platelet activity.[22] In patients with psoriasis, platelet *COX-1* mRNA was upregulated compared to controls (Supplemental Figure VA). Furthermore, platelet-derived *COX-1* correlated with PASI, circulating IL-17A ($r=0.83$, $p=0.01$; $r=0.69$, $p=0.02$; respectively, Supplemental Figure VB, VC) and was upregulated *in vitro* following IL-17A stimulation of MEG-01s (Supplemental Figure VD). In addition, serum TxB2, the circulating downstream end product of COX-1 activity, correlated with PASI ($r=0.49$, $p<0.01$), further highlighting the association between platelet reactivity and psoriasis.

Our data thus far suggested that platelet COX-1 provided a critical mechanistic link between endothelial and platelet activation in psoriasis. To further explore this concept, we examined whether aspirin, a COX-1 inhibitor, could reverse platelet adhesion with HAECs and the inflammatory effect of platelets on vascular health. Prior data has shown that HAECs stimulated with TNFα+IL-17A, produce a psoriatic like endothelial pro-inflammatory profile.[16] We found a 2 −3 fold increase in platelet adhesion to TNFα+IL-17A stimulated HAECs (Figure 4A) while pre-treatment of platelets with aspirin reduced platelet adhesion (Figure 4A) and suppressed HAEC mRNA expression of pro-inflammatory transcripts (Figure 4B). These data provide evidence that *in vitro* inhibition of platelet-derived COX-1 reduces platelet-mediated endothelial cell inflammation.

Aspirin reduces endothelial cell inflammation *in vivo*

To evaluate the clinical implications of these findings, we next investigated if platelet COX-1 inhibition via *in vivo* administration of low-dose aspirin improves endothelial vascular health in patients with psoriasis. A subset of patients with psoriasis were randomized to aspirin 81mg daily (n=15) or no treatment (n=15) for two-weeks (Figure 5A, Supplemental Figure I). No significant differences were noted in the baseline comparisons between the aspirin and no-treatment groups (Supplemental Table IIIA, IIIB) and in psoriasis patients who were included or excluded in the clinical trial component (Supplement Table IV). As expected, two-weeks of low-dose aspirin reduced serum TxB2 (7.8 mg/dl to 1.0 ng/ml, $p<0.001$) and maximal platelet aggregation in response to arachidonic acid at 1600 μM (87% to 28%, $p<0.001$; Supplemental Figure VIA, VIB). Aspirin had no significant effect on hs-CRP, or leukocyte subsets (Supplemental Table V), suggesting that any observed impact of aspirin therapy is related to platelet inhibition rather than systemic inflammatory changes.

For the primary clinical trial endpoint, aspirin reduced the composite brachial vein endothelial inflammatory transcriptome in psoriasis patients >70% from baseline with no significant change in the no-treatment group (Figure 5B). Aspirin therapy decreased endothelial *IL1β* and *COX-2*, with a trend towards a reduction in *IL8* (Figure 5C, 5D, Supplemental Figure VIC). We noted a positive association between baseline serum TxB2 and baseline composite brachial vein endothelial transcript expression (Figure 5E) as well as with the individual transcripts *IL1β* and *IL8* (Figure 5F, 5G). Following the two-weeks of aspirin, there was no longer a significant correlation between serum TxB2 and brachial vein endothelial inflammation (Supplemental Figure VII). The change in brachial vein endothelial transcriptome significantly correlated with the change in TxB2 (Figure 5H–J). Altogether our results demonstrate that in the setting of psoriasis, inhibition of platelet COX-1 with low-dose aspirin reduces endothelial cell inflammation and improves vascular health.

Discussion:

Despite the accepted association between psoriasis, vascular dysfunction, and CVD, [2, 4, 8, 31, 35] mechanisms driving atherosclerosis in psoriasis require further elucidation. Moreover, therapeutic targets to improve vascular health and reduce CV risk remain uncertain.[31, 36–38] Platelets promote atherothrombosis across inflammatory and immune-

mediated diseases[39, 40] with cells of myeloid origin increasingly recognized as contributors to atherosclerosis in psoriasis.[41, 42] The present study demonstrates that platelets represent a contributor to psoriasis associated inflammation. We show that platelets are activated in psoriasis and correlate with psoriatic activity, display a pro-inflammatory platelet transcriptome, and act as effector cells to induce endothelial cell pro-inflammatory activation. We establish the association between platelet-derived COX-1 expression and psoriasis disease severity and using both *in vitro* and *ex vivo* methods with a prospective randomized trial, demonstrate that inhibition of platelet COX-1 with low-dose aspirin reduces endothelial inflammation.

To first understand the contribution of platelets to CV risk in psoriasis, we evaluated platelet specific markers of activation associated with a higher risk of CVD.[43] Platelet P-selectin expression and platelet-leukocyte aggregates are associated with a hyper-active platelet phenotype and higher prevalence of CVD.[44] We previously reported that neutrophil-platelet aggregates are elevated in patients with psoriasis and that endothelial injury and coronary noncalcified plaque were associated with these aggregates.[45] Our study extends this work by demonstrating a significant correlation between platelet activity, psoriasis skin along with circulating measures of disease severity.

We also noted that platelets isolated from psoriasis participants directly adhere to and induce an endothelial pro-inflammatory phenotype. Isolated platelets were also more likely to adhere to an activated rather than non-activated endothelium. These analyses are indicative that the interaction between platelets and the inflamed endothelium in psoriasis may be at least partially responsible for epidemiologic investigations of endothelial dysfunction and accelerated atherosclerosis in patients with psoriasis.[35]

The findings that psoriasis platelets are activated and induce endothelial inflammation led us to perform platelet RNA sequencing. Platelets contain mRNA and functional translational components derived from their megakaryocyte precursors.[46–48] Transcriptional profiling of isolated platelets from psoriasis vs. control revealed overlapping pathways involved in CVD and atherosclerosis and identified a gene signature centered on IFN signaling. MEG-01 validation studies recapitulated this psoriasis platelet RNA sequencing gene signature and identified a synergistic stimulatory effect of IFNɣ with IL-17A, a novel finding in a megakaryocyte cell model, yet complimentary to what others have described in keratinocytes.[49] These data suggest that pro-inflammatory cytokines upregulated in psoriasis may be educating and reprogramming megakaryocytes and their daughter platelets.

Psoriatic inflammatory signatures contain both type II (IFNɣ) and type I (IFNα/β) IFNs produced by multiple cellular sources in psoriatic lesions.[5, 50] IFNɣ is felt to be the most pathogenic IFN in psoriasis and most associated with chronic psoriatic activity.[5, 33, 50, 51] Consistently, platelet transcriptome profiling in SLE also identified a platelet specific type I IFN gene signature which associated with platelet activation and increased prevalence of vascular disease.[52] Our data highlight the importance of platelet IFN signaling in psoriasis but suggest that it is the combination of IFNs plus other pro-inflammatory cytokines such as IL-17A, which leads to altered platelet transcriptional profiles and heightened platelet

activity. However, more work and future clinical-translational studies are needed to address this hypothesis.

Platelet transcriptomic profiling also identified platelet COX-1 signaling as a critical link between psoriasis disease severity, platelet activity, and endothelial cell inflammation. Platelet activation leads to the downstream release of membrane-bound arachidonic acid.[53] Metabolism via COX-1 signaling drives the generation of thromboxane A2, which is a potent vasoconstrictor and platelet agonist.[53] Platelet-derived thromboxane A2 is an established mediator of CVD.[54] In our study, COX-1, in addition to TxB2, the inactive and measurable metabolite of thromboxane A2 was directly associated with psoriasis disease severity and impaired vascular health.

Aspirin for primary CVD prevention is controversial but recommended in certain patients where the benefits outweigh the risks.[13, 15] Because psoriasis is considered a CV risk enhancing condition and our platelet RNA sequencing and *in vitro* data suggested the contribution of COX-1 signaling to endothelial cell inflammatory activation, we investigated aspirin's efficacy to reduce endothelial inflammation.[16, 55] We noted that aspirin's ability to inhibit platelet-derived COX-1, as measured *in vivo* by TxB2, was directly related to the degree of improvement in the composite endothelial pro-inflammatory transcriptome. While aspirin may have platelet independent multi-cellular anti-inflammatory effects, low-dose aspirin preferentially inhibits COX-1 over COX-2.[12] Furthermore, the systemic anti-inflammatory effects of aspirin are observed at higher doses.[12] Thus we would not have expected aspirin to have a direct impact on the endothelium *in vivo*, and at 81mg/day of aspirin, aspirin predominantly impacts platelets.[56–58] In summary, these findings highlight that in patients with psoriasis there is a beneficial role to platelet COX-1 inhibition.

Limitations

To evaluate the role of platelets and aspirin's efficacy to reduce endothelial inflammation, we used platelet and endothelial clinical-translational investigations and not longitudinal outcomes-based assessments. Our study assessed potential mechanisms driving early atherosclerosis and did not assess measurements of CV risk itself. Because of this and our small sample size, we are not able to conclude that aspirin reduces CV risk in psoriasis. Additionally, psoriasis patients had relatively mild to moderate psoriasis disease severity, many of whom were on psoriasis biologic treatment which may influence the endothelial and platelet phenotype and transcriptional profiles. Thus, this study may actually underestimate the degree to which platelet activity relates to psoriasis and may impact the degree to which aspirin improves vascular health and endothelial cell inflammation in psoriasis.

In conclusion, in patients with psoriasis, we show evidence of heightened platelet activity with the degree of activity directly related to psoriasis severity. Psoriasis platelets preferentially induce endothelial cell pro-inflammatory activation *in vitro* with platelet transcriptomic analysis revealing a robust platelet-derived IFN signature associated with IFNɣ and IL17A along with upregulated COX-1 expression. Finally, a proof of concept randomized trial demonstrated that in psoriasis patients, aspirin reduces endothelial

inflammation most likely through its inhibitory effect on platelet COX-1 signaling. In summary, our findings demonstrate platelets in psoriasis to impair vascular health and suggest that platelet COX-1 inhibition with aspirin may be effective in improving vascular health in patients with psoriasis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments:

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Abbreviations

CVD	Cardiovascular Disease
COX	Cyclooxygenase
HAEC	Human Aortic Endothelial Cells
HIV	Human Immunodeficiency Virus
Hs-CRP	High sensitivity C-reactive Protein
IFN	Interferon
IL	Interleukin
NCV	Normalized Count Values
NPX	Normalized Protein eXpression
TNF	Tumor Necrosis Factor
NYU	New York University
PASI	Psoriasis Area and Severity Index
SLE	Systemic Lupus Erythematosus
TxB2	Thromboxane B_2

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Highlights

- In psoraisis, platelets are activated and induce endothelial cell pro-inflammatory actovation.
- Platalet RNA sequencing highlights a strong interferon signature and COX-1 upregulation.
- In a randomized controlled trial, low-dose aspirin improves endothelial cell health in psoriasis via platelet COX-1 inhibition.

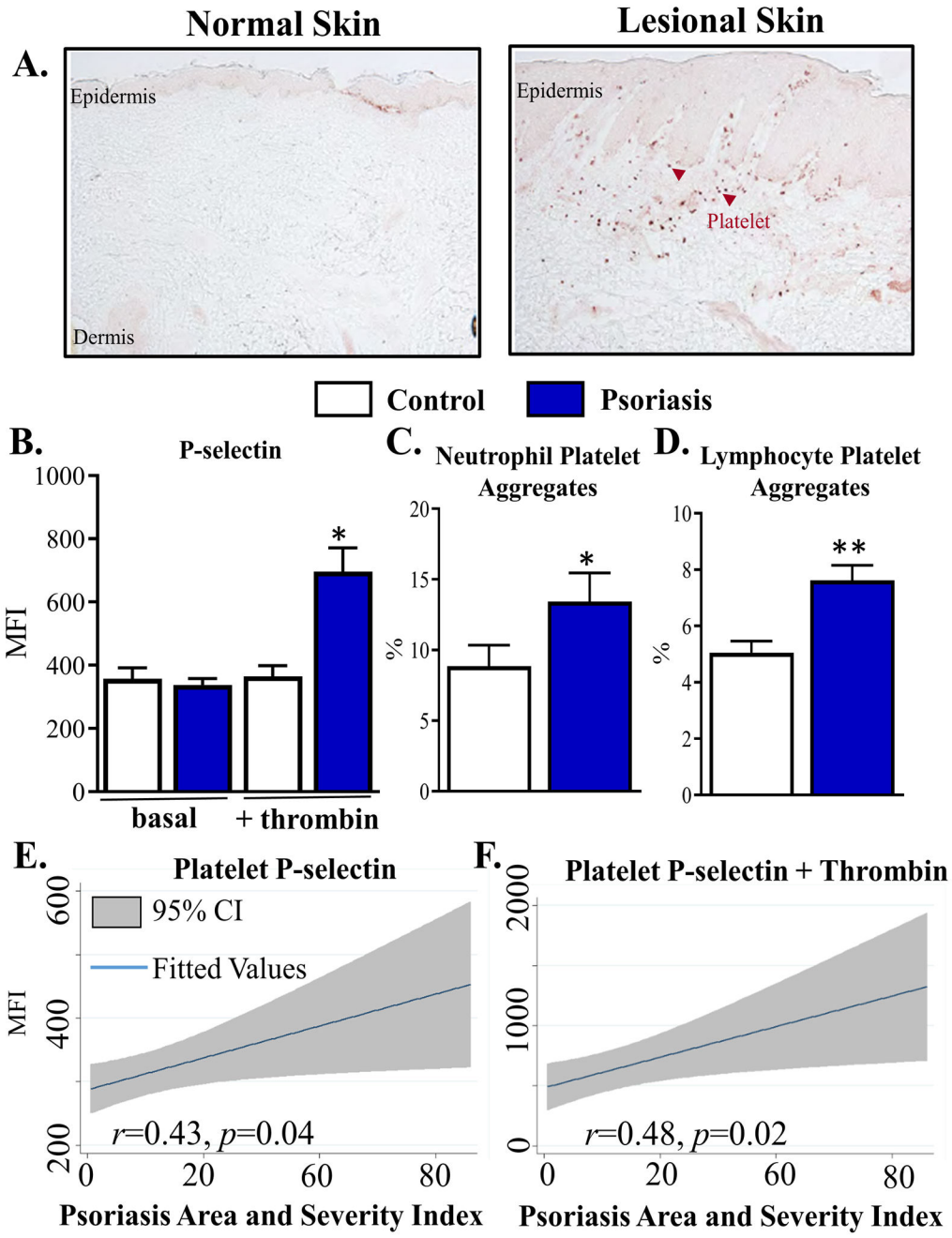

Figure 1.

Platelet activation is present in psoriasis and associated with disease severity. **A**, Immunohistochemistry of platelets in normal skin from a healthy control and lesional (psoriasis) skin (arrows indicate platelet staining with anti-ITGA2B). **B**, Flow cytometry quantification of platelet P-selectin (CD62L) expression in basal or activated (thrombin stimulated) condition between psoriasis vs. control. Flow cytometry quantification of (**C**) Neutrophil $CD45^{+}CD61^{+}$ and (**D**) lymphocyte platelet aggregate $CD45^{+}CD61^{+}$ subtypes in psoriasis vs. control. **E**, Regression plot and the correlation coefficient between psoriasis

area and severity index (PASI) and platelet P-selectin expression in basal or (**F)** activated (thrombin stimulated) condition. MFI, mean fluorescence units. N≈10 control/25 psoriasis patients per group. Bar graphs in Mean ± SEM unless otherwise specified. P<0.05*, p<0.01**

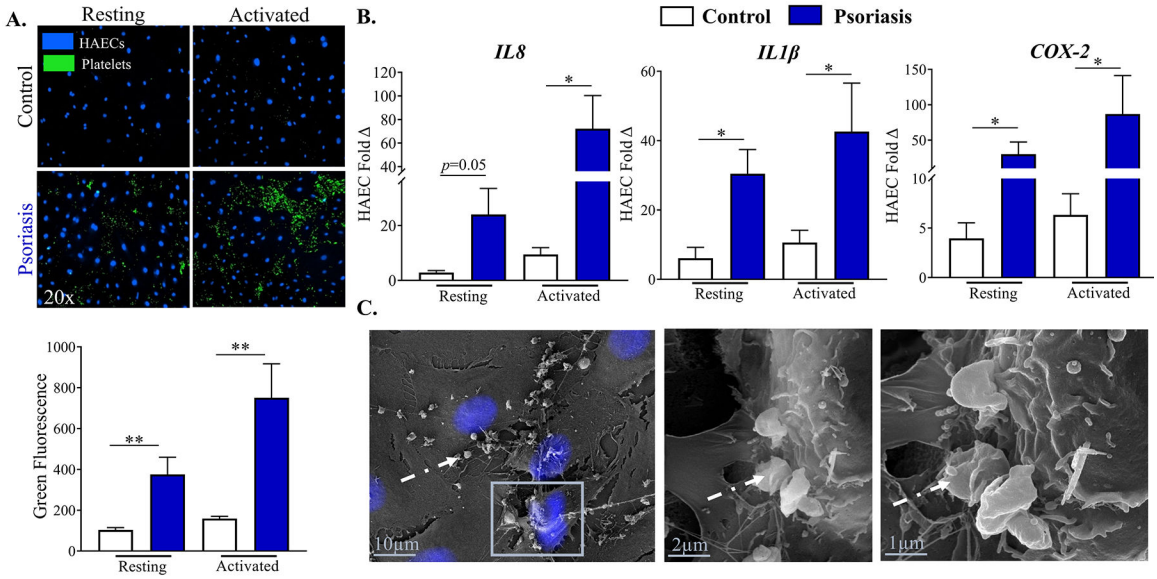

Figure 2.

Psoriasis platelets preferentially adhere to and promote endothelial inflammation. **A**, Isolated psoriasis vs. control platelets in basal and activated (thrombin stimulated) conditions (green) co-incubated with human aortic endothelial cells (HAECs, blue - DAPI stained, n≈6/group). **B**, Endothelial transcript expression after platelet co-incubation with HAECs in both basal and platelet activated conditions (n≈6/group, 4-hour co-incubation at 100:1 platelet to HAEC ratio). **C**, Scanning electron microscope images of a representative field depicting psoriasis platelets (dashed white arrow) interacting with HAECs (blue – superimposed immunofluorescence DAPI staining). DAPI, 4′,6-diamidino-2-phenylindole; IL, Interleukin. Bar graphs in Mean ± SEM unless otherwise specified. Green fluorescence in arbitrary units for quantification purposes. $p<0.05$*, $p<0.01$**

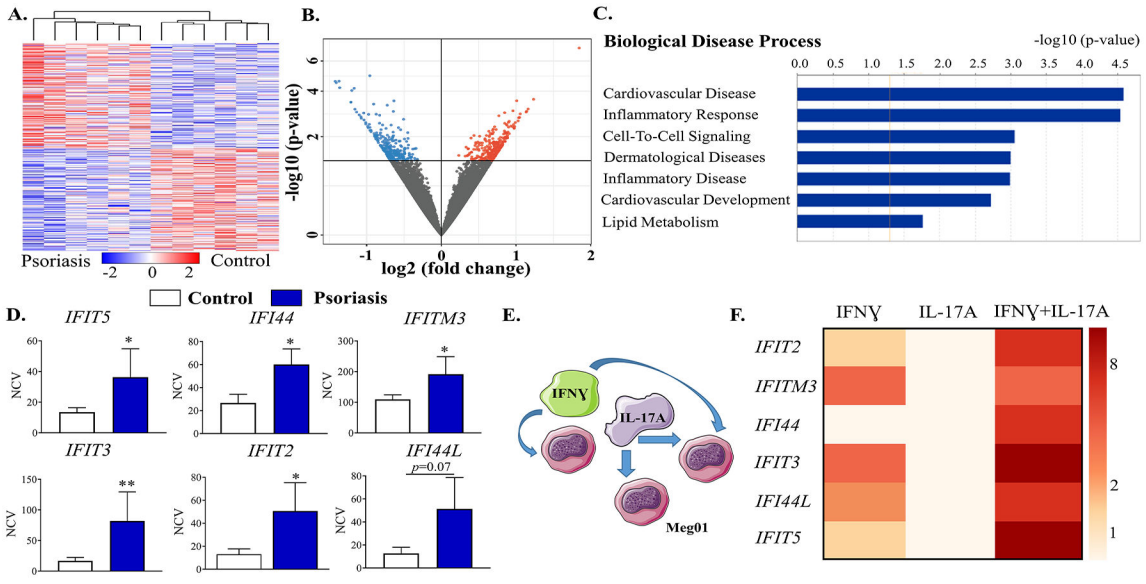

Figure 3.

Platelet RNA sequencing reveals biological pathways involved in cardiovascular disease. **A**, Heatmap and volcano plot (**B**) of platelet profiling by RNA sequencing in six psoriasis patients with active disease and six healthy age-, sex-matched controls ($p<0.05$). **C**, Biological pathway analysis (by Ingenuity Pathway Analysis) of platelet-derived pathways differentially expressed in psoriasis vs. control. **D**, Interferon-induced genes between psoriasis vs. controls. **E**, Experimental design of **(F)**, MEG-01 cell line stimulated with interferon (IFN) ɣ, interleukin (IL) – 17A or both (color indicates fold change, n=2–3 wells in 3 separate experiments). NCV, normalized count values. Bar graphs in Mean ± SEM. $p<0.05$*, $p<0.01$**

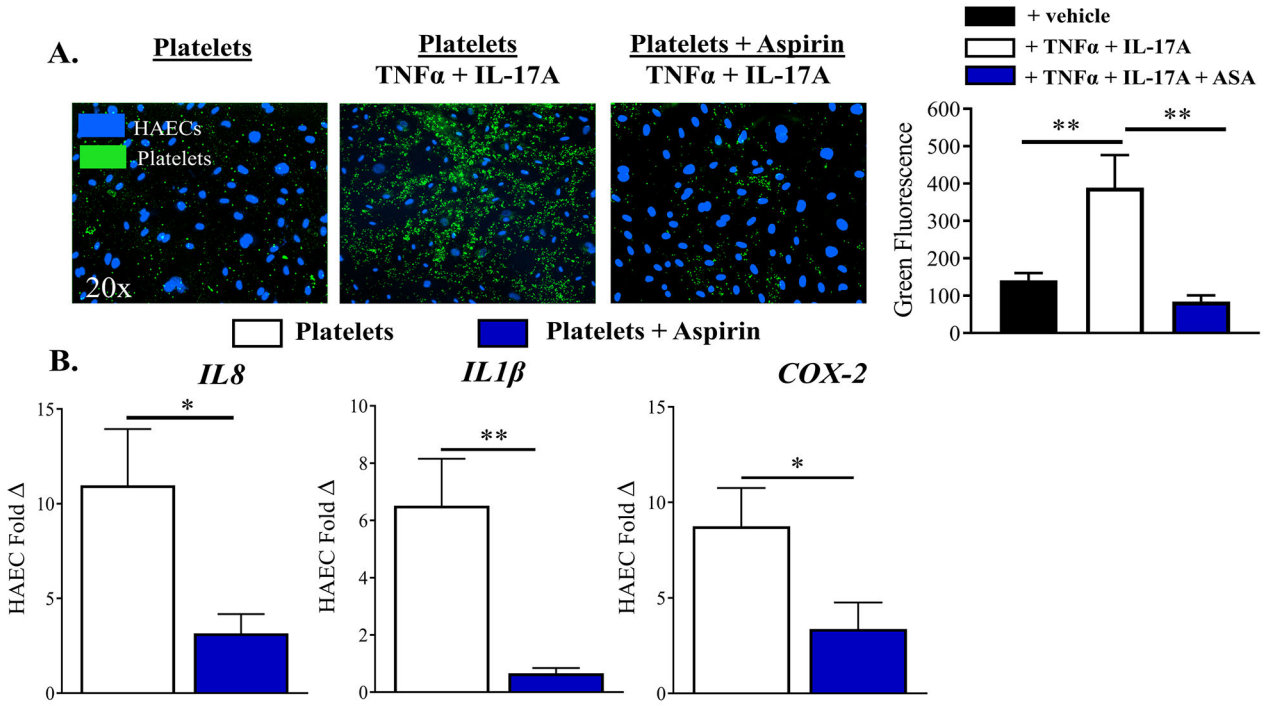

Figure 4.

Aspirin inhibition of platelets downregulates platelet-endothelial cell adhesion and activation. **A**, Resting or TNFα+IL-17A stimulated human aortic endothelial cells (HAECs, blue - DAPI stained) co-incubated with basal or aspirin pretreated platelets. **B**, Endothelial transcript expression after basal or aspirin pretreated platelets co-incubated with resting HAECs (n= 2–3 wells, 3 separate experiments). DAPI, 4′,6-diamidino-2-phenylindole; HAEC, human aortic endothelial cell; IL, Interleukin; TNF, tumor necrosis factor. Bar graphs in Mean ± SEM unless otherwise specified. $p<0.05$*, $p<0.01$**

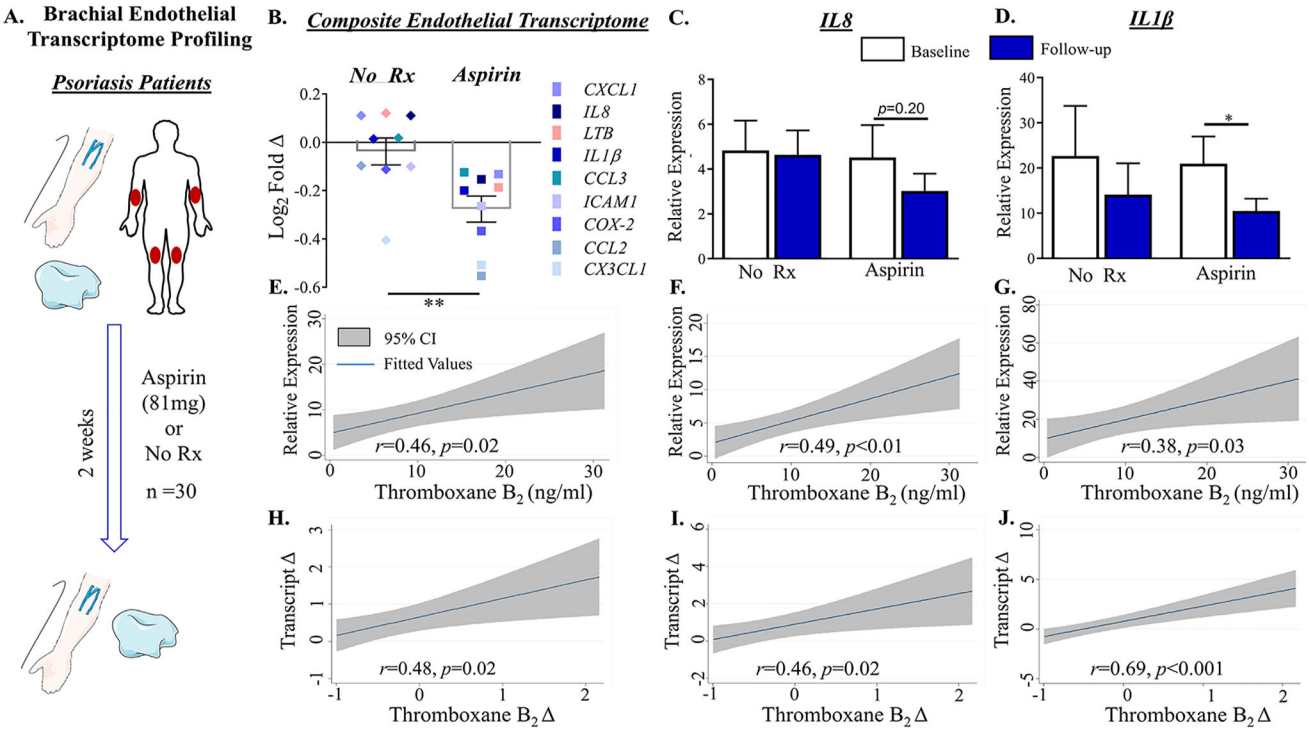

Figure 5.

Aspirin decreases endothelial pro-inflammatory activation. **A**, Randomized clinical trial in psoriasis evaluating the efficacy of aspirin 81mg (n=15) vs. no treatment (n=15) to reduce endothelial pro-inflammatory activation. **B - D**, Two-week change in the composite brachial vein endothelial transcriptome, *IL8* and *IL1β* after randomization to aspirin 81mg or no treatment. **E - G**, Regression plot and correlation coefficient between baseline serum thromboxane B2 (ng/ml) and brachial vein endothelial transcript expression. **H - J**, Regression plot and correlation coefficient between change in serum thromboxane B_2 and change in brachial vein endothelial transcript expression. Δ = relative change, (follow-up$_{2\ weeks}$ − baseline$_{time\ 0}$)/baseline$_{time\ 0}$; IL, interleukin; Relative expression = Gene expression/hARP. Rx = no treatment group. Bar graphs in Mean ± SEM. p<0.05*, p<0.01**

Table 1.

Clinical and Laboratory Characteristics

Characteristics	Psoriasis (n=45)	Control (n=18)	p-value
Age, y,	44.9 ± 14	40.5 ± 13	0.26
Male sex, %	22 (49)	10 (55)	0.63
Caucasian, %	33 (73)	10 (55)	0.49
Body mass index, kg/m^2	29 ± 8	26 ± 3	0.22
Hypertension, %	6 (13)	0	0.10
Systolic blood pressure, mm Hg	124 ± 16	123 ± 13	0.75
Diastolic blood pressure, mm Hg	75 ± 10	71 ± 8	0.14
Type 2 diabetes mellitus, %	1 (2)	0	0.52
Current tobacco use, %	1 (2)	0	0.52
ACC/AHA ASCVD Risk Score, %	4.0 ± 6.6	2.6 ± 4.7	0.41
Psoriasis			
Disease duration, y, median (IQR)	16 (10 – 24.5)		.
Psoriatic Arthritis, %	9 (20)		.
BSA, %, median (IQR)	4 (3 – 9.25)		.
PASI score, median (IQR)	4 (2.6 – 6.9)		.
Biologic therapy, %	15 (33)		.
Any other systemic therapy, %	10 (22)		.
Light therapy, %	18 (40)		.
Laboratory studies			
WBC, × 10^3 cells/mm^3	7.1 ± 2.5	5.7 ± 1.0	0.04
Hematocrit, %	39.4 ± 3.8	39.2 ± 3.8	0.86
Platelets, cells/L	245 ± 67	244 ± 67	0.96
Mean platelet volume, fL	9.8 ± 1.5	9.5 ± 1.5	0.41
Immature platelet fraction, %	4.0 ± 2.5	4.2 ± 3.3	0.89
Absolute neutrophils, × 10^3 cells/mm^3	4.4 ± 2.0	3.4 ± 1.0	0.07
Absolute monocytes, × 10^3 cells/mm^3	0.39 ± 0.16	0.70 ± 0.98	0.22
Absolute lymphocytes, × 10^3 cells/mm^3	1.9 ± 0.5	1.8 ± 0.6	0.42
IL-17A, NPX, median (IQR)	2.7 (1.8 – 5.1)	1.8 (0.5–1.5)	<0.01

Data are mean ± SD or N (%) unless otherwise stated. ACC indicates American College of Cardiology; AHA, American Heart Association; ASCVD, atherosclerotic cardiovascular disease; BSA indicates body surface area of psoriasis; IQR, interquartile range; NPX, normalized eXpression units; PASI, psoriasis area severity index; WBC, white blood cell.

Table 2A.

Correlation between Interferon Induced Platelet Function Transcripts and PASI

Platelet Transcript	*r*	p-value
IFIT3	0.88	0.02
IFIT2	0.75	0.09
IFI44	0.63	0.18
IFIT5	0.78	0.07
IFITM3	0.85	0.03
IFI16	0.58	0.23
IFI44L	0.79	0.06
IFIT1	0.84	0.04
OAS2	0.88	0.02
STAT1	0.91	0.01

Correlation coefficient of platelet normalized count values identified by canonical pathway analysis related to interferon signaling and PASI (psoriasis area and severity index, n=6).

Table 2B.

Correlation between Interferon Induced Platelet Function Transcripts and IL-17A

Platelet Transcript	*r*	p-value
IFIT3	0.58	0.04
IFIT2	0.67	0.02
IFI44	0.77	<0.01
IFIT5	0.69	0.01
IFITM3	0.59	0.04
IFI16	0.23	0.46
IFI44L	0.68	0.01
IFIT1	0.62	0.03
OAS2	0.71	0.01
STAT1	0.56	0.05

Correlation coefficient of platelet normalized count values identified by canonical pathway analysis related to interferon signaling and circulating IL-17A. IL, interleukin.(entire cohort n=12)