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PAI-1 augments mucosal damage in colitis

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Abstract

There is a major unmet clinical need to identify pathways in inflammatory bowel disease (IBD) to classify patient disease activity, stratify patients that will benefit from targeted therapies such as anti-TNF, and identify new therapeutic targets. In this study we conducted global transcriptome analysis to identify IBD-related pathways using colon biopsies, which highlighted the coagulation gene pathway as one of the most enriched gene sets in IBD subjects. Using this gene-network analysis across 14 independent cohorts and 1800 intestinal biopsies, we found that amongst the coagulation pathway genes, plasminogen activator inhibitor-1 (PAI-1) expression was highly enriched in active disease and in patients with IBD who did not respond to anti-TNF biologic therapy, and that PAI-1 is a key link between the epithelium and inflammation. Functionally, PAI-1

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Competing interests: DAL has a patent on MDI-2268 "Plasminogen activator inhibitor-1 inhibitors and methods of use thereof" # US 9,718,760 B2. DAL is on the scientific advisory board for, and has equity in, MDI Therapeutics which holds an option to licence MDI-2268 from the University of Michigan. GEK, TCL, and TSS have a patent pending on the use of PAI-1 in IBD, PCT Patent Application PCT/US2018/042761 "Methods and Uses of Inflammatory Bowel Disease Biomarkers". SK within the last 3 years has been an advisory board member for Anatara Lifesciences Ltd and Aerpio Therapeutics Ltd, and a consultant for Gossamer Bio Ltd (does not relate to this study). AS, JP, and KL are employees of Janssen R&D, LLC, a subsidiary of Johnson & Johnson and own Johnson & Johnson stock and options.

Data and materials availability: All data associated with this study are present in the paper or Supplementary Materials, and data deposition location is indicated in each of the relevant sections of the Methods.

and its direct target, the fibrinolytic protease tissue plasminogen activator (tPA), played an important role in regulating intestinal inflammation. Intestinal epithelial cells produced tPA, which was protective against chemical and mechanical- mediated colonic injury in mice. In contrast, PAI-1 exacerbated mucosal damage by blocking tPA-mediated cleavage and activation of anti-inflammatory TGF- β , whereas the inhibition of PAI-1 reduced both mucosal damage and inflammation. This study identifies an immune-coagulation gene axis in IBD where elevated PAI-1 may contribute to more aggressive disease.

One sentence summary:

SERPINE1/PAI-1 is elevated in the colon tissue of the most difficult to treat patients with IBD and leads to worsening of experimental colitis

Introduction

Complex autoimmune diseases typically involve interactions between genes and environmental factors that can influence disease severity, prognosis, and response to therapy (1). The interplay of genes and environment is well appreciated for both major forms of inflammatory bowel disease (IBD); ulcerative colitis (UC) and Crohn's disease (CD) (2). Both forms of IBD are heterogeneous in their clinical presentation and pathophysiology and subjects with UC and CD show variable response to specific therapies (3, 4). The clinical heterogeneity can be explained in part through pathways identified and linked to IBD through data obtained in human subjects including genetic, gene expression, morphologic, and serologic studies. Functional disease pathways in IBD, including altered activity of key genes, have emerged including microbial sensing (*NOD2*), immune activation (*IL23RJAK/STAT/TNF/IL10*), autophagy (*ATG16LI*), endoplasmic reticulum (*XBPI*) and oxidative stress (*CARD9*), Paneth cell defects, microbial dysbiosis, and saccharomyces cerevisiae antibody (anti-ASCA)/Perinuclear *anti*-neutrophil cytoplasmic antibody (anti-pANCA) circulating reactivity (5–9). However, despite these advances, much of the disease complexity remains unexplained. Furthermore, specific clinical unmet needs still exist, including patients that do not respond or maintain a response to drug therapies, especially in moderate-severe patients with IBD (3, 4). Thus, identification of novel disease pathways associated with IBD is of high importance.

One approach to understanding how gene-environmental interactions can converge to drive disease is to study commonly altered transcriptional pathways across multiple IBD cohorts. This type of analysis can identify as yet unexplored pathways in IBD to categorize subsets of patients and develop functional targets for new therapies. Given the number of public datasets now available it is possible to mine existing gene expression and metadata across multiple independent cohorts to identify new candidate pathways in IBD. The advantages of this approach are that it minimizes cross-cohort, technical, sampling, and inter-individual variation to inform on the confluence of genetic predisposition, heterogeneous disease progression, and environmental disease modulators. Hypotheses that emerge from the analysis of transcriptional networks produced from these data can then be tested using relevant *in vitro* and *in vivo* models of pathway function.

IBD is not limited to alterations in any one cellular compartment; rather, it is a complex disease that involves dynamic alterations in numerous cell types including epithelial, mesenchymal and immune cells. How diverse cellular networks interact during disease to regulate temporal activity remains unclear. Hematopoietic-derived immune cells (i.e. lymphocytes and myeloid derived cells) and their inflammatory mediators have received a great deal of attention to date (10). However, fewer studies have focused on transcriptional pathways linking the epithelium and the underlying stromal compartment and inflammatory cascade. Thus, this area is relatively underrepresented in our understanding of IBD and available therapeutic options. Identifying novel epithelial-immune linked gene networks from transcriptional studies may provide greater mechanistic insight into how IBD develops and progresses. An additional opportunity in transcriptional analysis of these large cohorts is to use gene-network and machine learning analysis to prioritize targets that sit at the regulatory interface of multiple cellular compartments, e.g. the epithelium and immune system. To develop candidate targets, Bayesian network analysis can be used to impute gene-gene interactions (11) and the relationship of gene pathways between the different cellular compartments of a whole organ. Here, we used the following criteria to discover novel candidate IBD pathways for gene target input into Bayesian networks: the pathways must be 1) highly enriched across multiple cohorts by multiple pathway algorithms and 2) linked in part to epithelial cell populations rather than solely to immune cells.

Using this approach, we found that the coagulation gene pathway is dysregulated in a subset of IBD subjects with active disease including those that are most difficult to treat, non-responders to anti-TNF therapy. Clinical studies have established that patients with IBD are at substantially increased risk for thrombotic events and those with active disease have abnormal blood coagulation parameters (12, 13), but the function and mechanism remains unclear. In this study, we show that a prominent member of this pathway, *SERPINE1*/PAI-1, is expressed in the epithelium and appears to act as a link between epithelial cells and inflammatory activity. Functionally, we show that PAI-1 plays an important role in worsening the severity of mouse models of colonic damage and repair by controlling key inflammatory modulators.

Results

Transcriptional analysis identifies coagulation as a highly enriched pathway in IBD and *SERPINE1*/PAI-1 as a link between the epithelium and inflammation

We utilized an unsupervised approach to highlight as yet unrecognized pathways that showed dysregulation in IBD. We first analysed datasets of global gene expression derived from 91 colon biopsies from both active UC and CD subjects versus controls (3 cohorts from a Multi-Institute Retrospective Cohort (MIRC), table S1) and found RNAs from 1773 genes that were commonly altered across cohorts (Fig. 1A). To this dataset, we applied multiple analytic tools that mine a variety of curated databases to identify over-represented pathways (Fig. 1A and B and table S2). Coagulation/haemostasis was consistently one of the top 10 most enriched gene pathways. This analysis indicates that by using multiple different algorithms, we have identified coagulation as a pathway linked to IBD.

Based on these findings, we next quantitatively assessed the association of the gene expression list with the coagulation pathway using Gene Set Enrichment Analysis (GSEA). The method confirmed the robust enrichment of coagulation in IBD datasets, (normalized enrichment score of 1.7, nominal $p=0.014$) (Fig. 1C). Amongst the individual coagulation enriched genes identified by the GSEA, we identified a subset of genes for potential further investigation based on the highest p-value and fold difference of expression (Fig. S1A and B).

One challenge in translating bioinformatics results into mechanistic insights in disease biology is identifying gene level targets for functional validation from a list of candidates. Here, we utilized Bayesian network analysis as one approach to further refine targets amongst the highest and most significantly dysregulated coagulation genes. Gene network analyses can provide a data-driven framework to predict gene-gene and gene-cell interactions and dependences within disease-relevant tissues. Cell interactions are important in IBD, as clusters of enriched genes occur in multiple cell types. Thus, in contrast to a canonical pathway gene list, the predicted gene associations from network analyses are tissue- and disease context-specific. For the most upregulated genes in Fig. S1B, we used a candidate gene approach to explore the three-step gene neighbourhood within a predictive Bayesian network model recently generated from transcriptional and genetic data from intestinal biopsy samples from the Crohn's disease CERTIFI trial (847 IBD biopsies, 28 non-IBD control biopsies; see table S3) (11, 14). For each candidate gene neighbourhood, we ran biological pathway and cell type enrichment analysis to determine the candidate gene's disease context-specific associations. Candidate gene neighbourhood analysis revealed a number of genes with enrichment in a specific cell type, including associations with immune cell genes (e.g. *C2*, Fig. S1C) or fibroblast/mesenchymal genes (e.g. *VWF*, Fig. S1D). This suggests that the genes in the neighbourhood of *C2* or *VWF* are predominantly related to immune cells or mesenchymal cells, respectively. In contrast, *SERPINE1* occupied a position at an interface between gene clusters associated with myeloid-driven inflammation (known involvement in IBD) and intestinal epithelial genes (Fig. 1D). These *SERPINE1* gene neighbourhoods were enriched in both genes expressed in intestinal epithelial cells and also showed biological enrichment in immune response (TNF and IL-17 signaling pathways) (Fig. S2A and B). Although this analysis suggests that *SERPINE1* may provide a link between these cellular compartments, it does not discount that other genes also sit in a similar position and link myeloid inflammation and epithelial clusters. However, the hypothesis that *SERPINE1* could serve as a node bridging inflammatory and epithelial-related biology, coupled with its strong upregulation in disease, strongly supports the selection of this candidate gene for functional validation.

Based on the hypothesis-generating findings of this suite of analytic tools, we validated that *SERPINE1* (protein name plasminogen activator-1; PAI-1) expression was specifically increased in IBD colon biopsies from active inflamed/involved areas of disease as compared to uninfamed/uninvolved regions, patients in remission, or non-IBD colon biopsies (Fig. 1E and F). We analysed the protein expression pattern of PAI-1 using immunofluorescence localization in colon tissue from a Washington University in St Louis (WashU) cohort (table S1). This showed increased numbers of PAI-1 positive cells in active/involved UC specimens, primarily within epithelial cells (Fig. 1G and H and Fig. S2C). These data

confirmed that *SERPINE1*/PAI-1 is reproducibly elevated in active IBD colon tissue and support the hypothesis that *SERPINE1*/PAI-1 plays a role in the inflammation/epithelium interface in this disease.

We next identified suites of genes with a highly correlated expression pattern to gain insight into the possible biological function of *SERPINE1* in IBD. We used whole transcriptome regression analysis on the MIRC samples to identify a group of genes whose expression was significantly correlated with *SERPINE1* ($r^2 > 0.5$ = 380 genes). This 380 gene set is herein referred to as the *SERPINE1* cluster (Fig. S3A and table S4). Over-representation pathway and gene ontology (GO) term analysis revealed *SERPINE1* cluster members were enriched for cytokine/chemokine inflammation as well as extracellular matrix (ECM)-related genes (Fig. S3B). The *SERPINE1* cluster was then verified by an analysis of 10 independent cohorts in the Expanded-MIRC to include biopsies with an increased diversity of disease severity, disease activity, and sampling site (total of 831 patient biopsies; see table S1). This expanded analysis verified strong correlations between *SERPINE1* and ECM genes, such as *ITGA5* ($r^2 = 0.72$) (Fig. S3C).

***Serpine1*/PAI-1 regulates experimental colitis**

Based on the clinical data suggesting a strong correlation of *SERPINE1*/PAI-1 with IBD, we hypothesized that high expression of PAI-1 may be detrimental in IBD. Therefore, we tested the role of PAI-1 in experimental colitis. Further rationale is that previous studies have shown that *Plasminogen*^{-/-} mice develop spontaneous rectal prolapse (15), suggesting that this pathway is broadly important in colon homeostasis through an unknown mechanism. We found that *Serpine1* colonic expression was increased ~6-fold in wild-type mice with colonic injury and inflammation secondary to treatment with dextran sodium sulphate (DSS) as compared to untreated controls. (Fig. S4A). To test whether PAI-1 had functional activity *in vivo*, we used gene-deficient mice and littermate controls (16, 17). We found that PAI-1 exacerbated features of DSS-induced colitis, as *Serpine1*^{-/-} mice showed reduced weight loss, mucosal damage, and crypt hyperplasia along with increased colon length compared to both *Serpine1*-heterozygote and wild-type littermates (Fig. 2A–C and Fig. S4B and C). Importantly, the improved recovery to DSS damage of *Serpine1*^{-/-} mice was associated with reduced inflammation as evidenced by lower IL-6 and numbers of infiltrating neutrophils in ulcerated regions (Fig. 2D and E and Fig. S4D). These data support a role of PAI-1 in promoting intestinal damage and inflammation.

IL-17A and Th17 cells drive expression of the PAI-1 binding target *Plat*

In the classical coagulation/fibrinolysis cascade, PAI-1 binds and inhibits the activity of the protein tissue plasminogen activator (tPA, encoded by the gene *Plat*, (18)). When active (not bound and inactivated by PAI-1), tPA cleaves the zymogen plasminogen, to its active form plasmin, which leads to fibrinolysis (diagrammed in Fig. S4E). As PAI-1 played a functional role in experimental colitis, we sought regulators of PAI-1 and its downstream targets (*Plat*/tPA) during inflammation in patient samples. We used Ingenuity Pathway Analysis (IPA) to predict canonical pathways that might drive the *SERPINE1* correlation cluster (Fig. S3 and table S4). This analysis revealed an over-representation of cytokine pathways as potential regulators (Fig. 3A; circled in red). We therefore screened an array of 15 IBD-associated

cytokines (TNF- α , IFN- γ , IL-1 β , IL-22, IL-6, IL-33, IL-12/23p40, Oncostatin M, IL-12, IL-23, IL-13, IL-17A, IL-17C, IL-17E, IL-17F) for their impact on *Serpine1* gene expression in primary mouse colon epithelial spheroids and surprisingly found that none of these cytokines alone elevated *Serpine1* expression >2-fold (Fig. S5A). However, using this same cytokine panel, we discovered that IL-17A potently increased *Plat* expression (Fig. 3B). None of the other cytokines increased *Plat* expression (>2-fold) including the closely related IL-17F. Interestingly, *CEBPB*, another gene that linked myeloid and epithelial clusters in the Bayesian analysis (Fig. 1D), is a known downstream regulator of IL-17A (19).

The increased expression of *Plat* in response to IL17A is of potential importance as this could counteract the effects of PAI-1. We next confirmed the effect of IL-17A on primary mouse colon epithelial spheroids using three differentiation states. Cells were grown as enriched for stem cells, terminally differentiated colonocytes, and an intermediate state producing mid-crypt-like differentiating colonocytes (Diff) (20, 21). All three states expressed detectable mRNA encoding IL-17A receptor subunits, with the terminally differentiated colonocytes expressing the highest amount (Fig. S5B). Treatment of epithelial cell spheroids with or without recombinant IL-17A followed by global transcriptomic analysis identified only 112 genes whose expression was induced by IL-17A treatment in any of the 3 cell states (FDR-adjusted $p < 0.05$ and fold change >2), and of these, only 5 genes were commonly induced in all 3 cell states; *Plat*, *Il1f6*, *Prl2a1*, *Duoxa2*, and *Plet1* (Fig. 3C and table S5). We confirmed that *Plat* was induced by IL-17A across all 3 states as verified by quantitative PCR (Fig. 3D). Pharmacological inhibition experiments showed that the increase in *Plat* expression was dependent on the transcription factor CBP/p300 (Fig. S5C), known to be activated by IL-17A (22).

To determine whether these changes in *Plat* could be induced not just by recombinant IL-17A but re-produced by interaction with Th17 immune cells, we utilized co-culture experiments. Th17 cells were generated from naïve CD4 T cells from *IL-17-eGFP* $\times$ *IL-22-tdTomato* $\times$ *OT-II* reporter mice (23). These Th17 cells were then FACS-purified, re-stimulated, and co-cultured with colon epithelial spheroids (Fig. S5D). Transcriptional analysis of the epithelial cells, after removal of CD4 T cells, demonstrated that *Plat* could be induced by co-culture of colon spheroids with Th17 cells, but not unstimulated T cells or Th1 cells (Fig. 3E). This Th17-induced *Plat* was IL-17RA-dependent as it was prevented by blocking the receptor with a monoclonal antibody (Fig. 3E).

***Plat*/tPA protects against colitis and mucosal damage**

We next investigated the function of *Plat*/tPA in response to colonic damage and the interaction of PAI-1 and tPA activation in the colon. PAI-1 is known to bind and inhibit the activity of tPA (Fig. S4E) (18). We found that as expected, the ratio of active to total tPA in the colon was increased in *Serpine1*-deficient mice compared to *Serpine1*-sufficient mice (Fig. S6A). We used *Plat*^{-/-} mice to determine whether *Plat*/tPA plays a role in DSS-induced colitis (24). *Plat*^{-/-} mice showed increased weight loss, mucosal damage, and crypt hyperplasia along with shorter colon length compared to littermate controls in the DSS model (Fig. 4A–C and Fig. S6B and C). The increased severity of colitis in *Plat*^{-/-} mice was

associated with greater inflammation through IL-6 and a small but non-significant increase in infiltrating neutrophils (Fig. 4D and E).

The repair process of colonic mucosal ulcers and wounds is initiated by a layer of epithelial cells migrating across the damaged area, termed wound-associated epithelial (WAE) cells, that protects the underlying immune compartment from excessive activation (25, 26). By analyzing RNA from a previous dataset of laser capture microdissection WAE cells (27) (25), we identified that *Plat* was up-regulated ~20-fold in WAE compared to normal crypts in mice (Fig. S6D). Protein immunofluorescent staining confirmed higher tPA in WAE cells overlying the wound, in sections of colonic biopsy-induced wounds (Fig. 4F and Fig S6E). Given the expression of *Plat*/tPA in WAE cells, we then used a focal colonic biopsy injury as a second model of colonic mucosal damage to investigate tPA function (28). In the early phase of repair that depends on WAE cells (25, 29), we found a significant defect in wound healing in *Plat*^{-/-} mice compared to littermate controls, both in terms of percentage unhealed wound area and neutrophil/fibrin caps overlying the wounds, further supporting a role for tPA in repair to colonic damage (p<0.05, Fig. 4G–I).

The WAE cell defect in the early phase of repair of *Plat*^{-/-} mice suggested a possible defect in migration of these cells across the wound bed (29). To test the effect of tPA on epithelial cell migration, we then used an *in vitro* scratch wound assay with a colonic epithelial cell line (T84). Direct addition of exogenous tPA, but not PAI-1, significantly increased the rate of wound closure over 24 hours *in vitro* (p<0.05, Fig. S6F). These data suggest that the tPA/PAI-1 interaction plays an important role in mucosal damage/repair.

As PAI-1 is elevated in IBD biopsies and functionally PAI-1 appears to be detrimental, while tPA appears to be protective against colonic damage, we sought to determine *PLAT* gene expression (which is independent of PAI-1) and total tPA protein (both active and bound to PAI-1) in human colon biopsies. We observed no differences in colonic specimens from patients with UC versus non-IBD controls either in the number of tPA positive cells or the expression of *PLAT* (Fig. S6G–J). These data suggest that in sites of inflammation and damage of patients with UC possess an imbalance in the ratio of PAI-1 to total tPA, leading to lower active tPA. Therefore, the potentially protective mechanism of elevation of tPA does not occur properly in IBD subjects.

PAI-1 inhibitor therapy reduces severity of colitis

To assess whether a small molecule inhibitor targeting PAI-1 could mimic the protection in *Serpine1*-deficient mice, we used the newly developed inhibitor MDI-2268 (30), which increased the ratio of active to total tPA *in vivo* in both plasma and colon tissue (Fig. 5A and Fig. S7A)(30). We administered this compound or a vehicle control to mice therapeutically on day 6, once mice began to lose weight from DSS-induced colitis. This treatment led to reduced weight change and mucosal damage as well as reduced signs of inflammation (IL-6 and neutrophils) compared to vehicle controls (Fig. 5B–E and Fig. S7B). To determine whether the effects of MDI-2268 were specific to tPA, and whether the inhibition of PAI-1 mediated its effects through increased active tPA, we administered MDI-2268 or vehicle control to *Plat*-deficient mice. In these experiments, *Plat*-deficient mice treated with MDI-2268 had similar weight loss, mucosal damage, and inflammation compared to

vehicle-treated controls (Fig. 5F and G and Fig. S7C–E). To confirm these results of PAI-1 inhibition by MDI-2268 in a second model of acute colitis we used infection with *Citrobacter rodentium*, which induces colonic damage consisting of aberrant crypt pathology. Using this model we found that treatment with MDI-2268 reduced crypt loss compared to vehicle controls (Fig. 5H and I). Together, these data show that therapeutic PAI-1 inhibition via MDI-2268 exerts a protective effect in colitis through increased amounts of active tPA.

PAI-1/tPA axis regulates TGF- β activation to augment epithelial hyper-proliferation and inflammation in colitis

We found that the *SERPINE1* gene was most highly correlated with molecules related to ECM and cytokine signaling (Fig. S3 and table S4), suggesting that these processes were potential targets of this pathway. Furthermore, as tPA/PAI-1 form a protease/anti-protease coupling and are both made and secreted into the ECM, we hypothesized that tPA/PAI-1 mediate at least some of their effect in colitis through cleavage and activation of latent cytokines that reside in the ECM. As TGF- β is made and secreted into the ECM in a latent form, and given its established role as anti-inflammatory in IBD and anti-proliferative on epithelial cells (27, 31–33), we examined the interaction between TGF- β and tPA/PAI-1. We used a cell-free system to test the capacity of tPA to enhance, and PAI-1 to suppress activation of latent TGF- β (Fig. 6A). We found that tPA could not directly activate TGF- β , but instead was required to first cleave plasminogen to plasmin. Plasmin then cleaved and activated TGF- β through a protease-dependent mechanism. This activation of TGF- β was completely reversed by PAI-1 at 2 hours of assay incubation and remained inhibited at 5 hours. The activation of TGF- β could be rescued through the use of the small molecule PAI-1 inhibitor (MDI-2268). To ascertain whether tPA proteolytic activation of TGF- β was specific or a ubiquitous mechanism of cleavage-mediated activation of cytokines, we examined the interaction between pro-IL-1 β (a cleavable and activatable cytokine) and tPA. These assays demonstrated that although pro-IL-1 β was proteolytically degraded by tPA there was no detectable activation of the short active form of IL-1 β (Fig. S8A). In contrast a generic protease chymotrypsin was able to cleave and activate large amounts of IL-1 β , suggesting that the action of tPA was at least partially specific to TGF- β cleavage sites.

TGF- β is well known to affect intestinal epithelial proliferation, a process that is elevated in active IBD. In our knockout mouse experiments, we had also observed effects of tPA and PAI-1 on epithelial crypt hyperplasia in mice subjected to DSS-induced damage (Fig. S4C and S6C). Therefore, we tested the effects of tPA/PAI-1 activation of TGF- β on the epithelium using an *in vitro* spheroid proliferation assay. Cell proliferation was measured by luminescence using primary colon epithelial spheroids derived from *Cdc25a-luciferase*-expressing mice (20). tPA-mediated cleavage of plasminogen led to activation of mature TGF- β , which potently suppressed epithelial hyper-proliferation through the TGF β RI (Fig. 6B and Fig. S8B). This process was completely inhibited by PAI-1, suggesting that elevated PAI-1 can contribute to epithelial hyper-proliferation by blocking tPA-mediated activation of TGF- β .

We then examined this axis *in vivo* using colon homogenates to test the ratio of active to latent TGF- β with a flow cytometric bead assay. Colons from *Plat*-deficient mice had reduced ratios of active to total TGF- β , and the converse was observed in *Serpine1*-deficient mice where ratios of active to total TGF- β were increased (Fig 6C and D). These findings suggested that similar to our observations *in vitro*, tPA plays a role in the activation of TGF- β , and PAI-1 inhibits this activation process *in vivo*. Based on these findings, we sought to determine whether the suppression of colitis from loss of PAI-1 *in vivo* was at least partially mediated by the increased activation of TGF- β in these mice. Antibody neutralization of TGF- β reversed the protective effects in *Serpine1*-deficient mice leading to increased weight loss, mucosal damage, hyperplasia, and IL-6 (Fig. 6E–G and Fig. S8C). These data suggest that TGF- β has a key role in mediating the effects of the tPA/PAI-1 pathway in colitis.

***SERPINE1* is elevated in active, more severe disease and patients that do not respond to anti-TNF therapy**

We next investigated *SERPINE1* for its expression in clinically difficult to treat subsets of patients with IBD. Receiver operator characteristic (ROC) curves demonstrated that *SERPINE1* expression showed discriminatory power to distinguish between active inflamed IBD biopsies versus uninfamed/non-IBD biopsies (Area under the curve; AUC 0.97, specificity 97%, sensitivity 91%) (Fig. 7A). Longitudinal analysis of a separate independent cohort with MAYO severity scores from the PURSUIT trial (table S1) demonstrated that *SERPINE1* expression also correlated with disease severity, both prior to induction therapy ($r=0.32$, $p=0.0025$) and 6 weeks after treatment ($r=0.37$, $p=0.0012$), with the anti-TNF biologic golimumab for patients with moderate-to-severe disease (Fig. 7B). Finally, unsupervised hierarchical clustering using the *SERPINE1* correlation cluster (table S4) robustly segregated colon biopsies into those derived from inflamed tissue versus uninfamed/non-IBD tissue with up to 97.5% accuracy (Fig. S9A and B). Therefore, *SERPINE1* and its correlation cluster can distinguish between active and inactive disease.

SERPINE1 was not only elevated in severe disease; its expression was also consistently elevated in patients who failed to respond to anti-TNF biologics. Standard therapy for patients with IBD with moderate-to-severe disease, who are uncontrolled on conventional treatments, involves an anti-TNF biologic, such as infliximab. However, ~40% of patients fail to respond or maintain a response to these biologics for unknown reasons (34, 35). There is a major unmet clinical need for a molecular definition of patients who will likely not respond to biologic therapy. We analysed transcriptomic data from colon biopsies taken prior to infliximab treatment in patients with moderate-to-severe disease (Infliximab Response Discovery Cohorts; IRDC; Supplementary table 1). Patient response to infliximab was determined by longitudinal follow-up (see Materials and Methods). The IRDC only included studies that took biopsies before initiation of infliximab so that pre-existing transcriptional differences in these patients could be analysed. There were only 18 genes with differential expression between infliximab responders and non-responders prior to treatment ($p<0.05$, fold change >2) when comparing gene expression across all IRDC datasets (Fig. S9C and table S6). Remarkably, *SERPINE1* was one of these 18 genes. We tested individual ROC curves for all of these 18 genes and discovered that *SERPINE1* was one of the top 4 AUC scoring genes, and it was consistently differentially expressed between responders and non-

responders (AUC=0.92; Fig. 7C). Finally, we confirmed these findings using a separate validation cohort of patients biopsied prior to treatment with the anti-TNF biologic golimumab in the PURSUIT trial. As observed for infliximab, expression of *SERPINE1* was higher in pre-treatment biopsies of patients who did not respond to golimumab (Fig. S9D). Overall, these data suggest that *SERPINE1* is elevated in the TNF-refractory subset of patients with IBD.

Discussion

IBD is a complex disease with divergent inputs from genetic, environmental, microbial, and epigenetic factors converging on stromal and immune compartments to drive disease progression. In this study, we have described an effect of coagulation genes in IBD that is conserved across patient cohorts. This finding may help to explain the clinical observation that patients with IBD have an approximately 3-fold increased risk of thrombotic events (12, 13), as thrombotic factors induced in the damaged colon tissue may leak into the systemic circulation. A prominent member of this pathway, *SERPINE1*/PAI-1, appears to be highly elevated in patients with active disease and correlates with the severity of disease. The expression of this molecule is higher still in the subset of patients with moderate-severe disease who are resistant to anti-TNF biologic therapy. PAI-1 is expressed in the inflamed epithelium and is predicted to exist at the interface between immune inflammation and epithelial cell involvement in IBD. Furthermore, the PAI-1 binding target tPA is induced by IL-17-driven immune signalling. Analysis of the function of PAI-1 and tPA shows these molecules have reciprocal effects in models of colonic damage and inflammation. Our data suggest that elevated PAI-1 leads to more severe injury and inflammation by inhibiting the ability of tPA to activate TGF- β to reduce ongoing damage.

The role of the epithelium in IBD has received less attention than that of immune cells, although these two compartments are intrinsically linked in intestinal homeostasis. Defects in the epithelium can lead to microbial dysbiosis and inflammation in the mucosa (5, 36). Uncovering key interactions between the stromal compartments and immune-mediated inflammation is of fundamental importance but also of tremendous complexity and cannot be easily modelled using *in vitro* systems. In depth analysis of Bayesian networks using IBD mucosal transcriptional datasets as input removes some of these layers of complexity. In this study, *SERPINE1* was shown to be a gene that occupies a unique space between myeloid and stromal cells. Our functional analysis also suggests that its over-expression may contribute to ongoing inflammation. The concept of PAI-1 adversely contributing to inflammatory disease pathology has been frequently observed in other organ systems, such as the lung, skin, liver, cardiac, and kidney (37–42). In these other organs various disease models have consistently shown that PAI-1 leads to increased inflammatory tissue damage, altered healing, and elevated fibrosis.

Recently, seminal work by *West et al.* used a global transcriptional analysis approach to identify that Oncostatin M (*OSM*) is elevated in the inflamed intestinal tissue of patients with IBD across multiple cohorts (43). *OSM* also correlated with response to anti-TNF therapy amongst a subset of patients. *OSM* is an IL-6 cytokine family member and drives inflammatory responses by signalling through a variety of non-epithelial, non-hematopoietic

stromal cells and leads to worsening severity in mouse models of colitis. Similar to PAI-1, the findings from *West et al.*, implicates *OSM* as a potential bridge between the immune and stromal compartments in IBD.

One of the mechanisms by which PAI-1/tPA may affect colonic injury models appears to be through the cleavage and activation of TGF- β which is known to be a potent anti-inflammatory and pro-repair cytokine during colitis and colonic wounding, respectively (27, 32, 33). The overall effect of elevated PAI-1 in patients with active IBD and still higher expression in the subset that is anti-TNF resistant may be mediated via a negative feedback loop with tPA/TGF β . Elevated PAI-1 may lead to ongoing chronic inflammation by dampening down this anti-inflammatory axis. Elevated PAI-1 may also signify a more severe level of colonic mucosal damage or fibrosis, which may correlate both with active disease and resistance to anti-TNF. It is probable that tPA/PAI-1 as a protease/anti-protease coupling have pleiotropic effects and there are likely other mechanisms by which these molecules may impact colonic injury and inflammatory phenotypes. Indeed, studies of PAI-1 deficiency in other organ systems have shown that the PAI-1/tPA axis cleaves and activates other tissue factors, including growth factors unrelated to coagulation or fibrinolysis. For example, in the liver tPA increases (and PAI-1 inhibits) activation of hepatocyte growth factor to protect against liver damage (44). Furthermore, studies in the gastric system have even shown a role for PAI-1 in both obesity and gastric mucosal healing (45, 46). In our study we also found that *SERPINE1* had strong associations with *CEBPB* (Bayesian network linking between the immune and epithelial compartments) and *ITGA5* (by gene correlation analysis) in the colon of patients with IBD. A previous functional study using glioma cells has shown that *SERPINE1* is directly regulated by *CEBPB* (47). *SERPINE1*/PAI-1 expression has also been shown to regulate the activity of integrins including the fibronectin receptor subunit alpha 5 (encoded by *ITGA5*) (48, 49). These studies suggest that a *CEBPB-PAI-1-ITGA5* axis may also be active in IBD.

Another unmet clinical need in IBD is to develop objective measures of disease activity to supplement patient-reported symptoms and endoscopic scoring. Currently this can be partly achieved through histological analysis of tissue biopsies, C-reactive protein (CRP), or fecal calprotectin (3, 4); potentially *SERPINE1*/PAI-1 and other molecular markers may have added utility and increase discriminatory power. Moreover, prediction of response to biologic therapy is needed to prevent unnecessary treatment, cost, and risk. Although biologic therapies with anti-TNF are now a mainstay for IBD therapy, up to 40% of patients are non-responsive and patients lose responsiveness over time (34, 35). This is an issue across many diseases, where a large proportion of patients are resistant to therapy but appear to have no obvious clinically distinguishing features. Furthermore, as more therapeutic options become available in IBD, a predictive biomarker is needed for personalized treatment. This would require analysis with multiple different therapies including anti-TNFs, anti-integrins, and anti-IL23 modalities.

We found that IL-17 was linked to our *SERPINE1* gene correlation cluster and that IL-17A was able to drive the expression of *Plat*. However, the role of IL-17A in IBD and mouse models of disease has produced somewhat conflicting results. Initial studies of IL-17A and Th17 cells linked these factors to worsening severity of colitis and deleterious pro-

inflammatory responses, as observed for many autoimmune diseases (50, 51). However, a decade ago there were also reports suggesting that IL-17A played a protective role (52), perhaps indicating that the effect of IL-17 was context and model-dependent. Monoclonal antibody-mediated blockade of IL-17A or its receptor in Crohn's disease patients led to a worsening of disease or increased adverse events in a subset of patients, strongly suggesting that IL-17A can in fact be protective in IBD (53, 54). More recent studies across multiple different mouse models have confirmed this and identified that one of the mechanisms underlying the protective effect of IL-17A is its ability to enhance the epithelial barrier and resist excessive responses to intestinal epithelial damage (55–57). Our data further support the latter paradigm suggesting that IL-17A may trigger expression of tPA to activate anti-inflammatory and pro-repair TGF- β . In both animal models and humans with rare TGF- β -deficiencies, active TGF- β has been demonstrated to be a critical molecule in suppression of IBD (31–33). Although TGF- β was originally linked to Th17 cell induction/autoimmunity, it is now well established that TGF- β actually leads to the development of a non-pathogenic form of IL-17-producing T cells (58), suggesting that tPA/TGF- β could be involved in a beneficial feedback loop in tissue homeostasis.

There are a couple of limitations to this study. Firstly, to study the function of any gene/s identified in human IBD cohorts in regard to disease development or activity is difficult given the absence of any truly complete mouse models of human IBD. Mouse models can mimic specific aspects of the pathology of human IBD such as ulceration or inflammation but no mouse model recapitulates the chronicity, complete pathology, or human microbial diversity of IBD. As an example of the limitations of cross-species comparisons, we found that in mouse colon tPA was expressed by WAE cells during tissue damage, however we are unable to capture these WAE cells in most human colon sections. This is likely because we can only get a single snapshot of human tissue during the acute damage/repair phase because longitudinal sampling of human colon is not feasible. In contrast, we found that the primary cells expressing tPA in human colon tissue were VIMENTIN+ fibroblasts. Secondly, although *SERPINE1*/PAI-1 was a marker of disease activity and poor responsiveness to anti-TNF therapy across multiple independent IBD cohorts, to accurately assess the utility of markers of disease activity and response to therapy requires large prospective clinical trials. Another limitation of markers such as *SERPINE1* is the narrow magnitude of differential expression between responders and non-responders to therapy, which makes variation amongst the population a major issue. This is especially the case in retrospective samples and may require a much larger sample size than the 124 patient biopsies across 4 cohorts we examined in this study. Quantitative PCR assays may partially mitigate this issue and permit distinction between subsets, as baseline array or RNA-seq data may lack sufficient normalization to be applicable across populations as a diagnostic.

This study identifies the coagulation pathway as functionally implicated in IBD. *SERPINE1*/PAI-1 are elevated during the active cycles of disease in the inflamed colon, and correlate with more severe disease and resistance to anti-TNF biologic therapy. This molecule appears at the interface between the epithelium and immune system suggesting that it may provide a link between the two compartments. Finally, elevated PAI-1 in IBD may inhibit the tPA-mediated cleavage and activation of anti-inflammatory TGF- β , thus leading to worsening severity of disease.

Materials and Methods

Study Design

Research objectives (pre-specified) were to identify previously unknown enriched pathways in IBD, highly dysregulated genes conserved across IBD cohorts, and genes differentially expressed between responders and non-responders to anti-TNF therapy prior to treatment. Research objectives (hypotheses specified after initiation of data analyses) were then to test whether *SERPINE1*/PAI-1 played a functional role in colitis and whether the mechanism underlying a function was related to protease/anti-protease activity and predicted cytokine/ECM gene cluster. Research subjects (described in detail in table S1 and S2) included ulcerative colitis and Crohn's disease patients and non-IBD controls. The mechanistic and functional studies use inbred mouse strains (listed below), and primary cell cultures from mice including primary colon epithelial spheroids and primary splenic-derived T cells. The overall design of this work includes controlled laboratory experiments and bioinformatics analysis of retrospectively collected patient samples. Randomization and blinding (by de-identified numbers) were used where appropriate both in the retrospective human cohorts and in assigning mice to experimental groups. For the human datasets a power analysis was used to calculate the necessary samples size to achieve reliable measurement of *SERPINE1* in active disease or anti-TNF responsiveness (38 patients; calculation using the T statistic and non-centrality parameter) but in all cases the sample sizes used (see figures and methods) were well in excess of what was calculated to be required. Data collection was all retrospective. Criteria for inclusion and exclusion of data is indicated in each of the relevant results, tables and methods section and established prospectively. Replicates are indicated in each figure legend with the corresponding panel, each experiment was performed 2–4 independent times. Primary data for panels or groups where $n < 20$ are reported in data file S1.

Human specimens

Formalin-fixed paraffin embedded sections from resections were obtained from patients with UC undergoing surgery for moderate-to-severe disease, or chronically active disease. Some non-IBD (non-inflamed) control specimens were obtained from normal regions of colon in patients with stomach tumors, colonic inertia, or prolapse. Inflammation severity of human colonic mucosa was classified by histopathological criteria. Classification was either inflamed/involved or uninfamed/uninvolved on the basis of assessment by a gastrointestinal pathologist (TLC). Where possible, specimens were collected from both inflamed/involved areas and macroscopically normal tissue at a distance from ulcers in the same patient. Human protocols were approved by the Washington University in St Louis research ethics committee and approved by the Institutional Review Board (protocol# 201209047).

Human GEO dataset analysis of transcriptomic data

Whole-transcriptome data from mucosal biopsies were downloaded from the Gene Expression Omnibus (GEO) website (<http://www.ncbi.nlm.nih.gov/geo/>). Before analysis, all data was pre-processed with Robust Multi-array Average (RMA), quantile normalized, and log2 transformed. Publically available data sets were used for analysis see Supplementary table 1 (GSE38713, GSE16879, GSE36807, GSE48634, GSE59071,

GSE65114, GSE47908, GSE73661, GSE9452, GSE23597, and GSE12251) (59–68). Analysis was performed with Partek Genomics Suite software (Partek). In addition to the publically available data sets, we analyzed SERPINE1 expression using transcriptomic data from the PURSUIT ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT00487539) identifier NCT00487539) anti-TNF clinical trial of patients with moderate-to-severe UC (69). PURSUIT was a Phase 2/3 multicenter, randomized, placebo-controlled, double-blind study to evaluate the safety and efficacy of golimumab induction therapy in patients with moderately to severe active UC. 200/100 mg or 400/200 mg of golimumab was administered subcutaneously at Weeks 0 and 2, respectively. Patients from PURSUIT were used as a validation dataset for response to anti-TNF and also for correlation of *SERPINE1* with MAYO endoscopic severity score using *SERPINE1* Affymetrix probe set 202628_s_at. Mucosal colonic biopsies were collected at weeks 0 and 6 during endoscopy from a subgroup of PURSUIT patients at 15–20 cm from the anal verge (n=87). Informed consent was obtained from individuals to undergo colonic biopsies for research purposes during colonoscopic procedures performed as part of the clinical trial. Following collection, the samples were preserved in RNeasy (Applied Biosystems). All biopsies were stored at –80 °C until RNA isolation was performed. RNA was isolated and hybridized to the GeneChip HT HG-U133+ PM Array (Affymetrix). The microarray data were pre-processed and normalized by RMA using Array Studio software version 10 (OmicSoft, a QIAGEN company) with quantile normalization and log₂ transformation.

Bayesian network analysis utilized biopsies from the CERTIFI cohort. Patient details for the biopsies from the CERTIFI cohort are described in (14) ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT00771667) identifier NCT00771667), and can be found summarized in Supplementary table 3.

Bayesian network analysis and SERPINE1 neighborhood enrichments

The Bayesian network model generated using 875 intestinal biopsies from CERTIFI study was downloaded from the Synapse repository at Sage Bionetworks (<https://www.synapse.org/#!Synapse:syn10792659>) and visualized using Cytoscape (70). The network node corresponding to *SERPINE1* gene was identified and a subnetwork containing all nodes three or fewer (undirected) steps away from *SERPINE1* was extracted. Two clusters, each containing 31 genes, were identified through observation of the topology of the induced subnetwork and characterized using enrichment analysis. Enrichment was performed using the command-line version of SaddleSum tool (71), version 1.5.1, with the 7796 genes represented as nodes in CERTIFI network as the statistical background. Two enrichment databases were used separately to avoid biases in multiple hypothesis correction arising from their unequal sizes. The first (9692 terms) consisted of human Gene Ontology (72) terms and KEGG pathways (73) downloaded from the NCBI FTP site (ftp://ftp.ncbi.nlm.nih.gov/pub/qmbpmn/SaddleSum/term_datasets/ETD-20170301/). The second database (327 terms) was a collection of human cell types and tissues derived from Gene Expression Barcode database, version 3.0 (74). A database term was considered significant if its one-sided Fisher's Exact test E-value (Bonferroni corrected p-value) was less than 0.05.

Pathway analysis

The over-representation pathway analysis was performed using WebGestalt (75), ConsensusPathDB (76) and Enrichr (77, 78) as pathway analysis web portals to access multiple online enrichment analysis tools. Ingenuity Pathway Analysis (IPA) software (QIAGEN Bioinformatics) was used to identify enriched canonical pathways and upstream regulators of our 380 gene *SERPINE1* correlations cluster (identified by global gene regression analysis).

Small molecule PAI-1 inhibitor

MDI-2268 was identified from structure activity relationship studies performed on a molecule first identified in a high-throughput screen of over 152,000 purified compounds at the University of Michigan Center for Chemical Genomics (30). MDI-2268 is highly specific for PAI-1 and has been shown to be active against human, murine, rat, and porcine PAI-1. MDI-2268 is an efficient inhibitor of PAI-1 in *ex vivo* plasma and *in vivo* following intraperitoneal injection in transgenic mice over-expressing PAI-1 (see Fig. S7 for compound structure).

In vitro TGF- β and IL-1 β activation reactions

tPA reactions were set up with: recombinant latent TGF- β (final concentration 2.7 μ g/ml latent TGF- β ; R&D), recombinant tPA (final concentration 48 μ g/ml tPA; EMD Millipore), recombinant plasminogen (final concentration 65 μ g/ml plasminogen; R&D), 1xprotease inhibitor (Pierce), recombinant mature TGF- β (final concentration 1.3 μ g/ml mature TGF- β ; Peprotech). Reaction constituents were mixed in DMEM buffer + 0.1% BSA and incubated at 37°C. An aliquot of the reaction mixtures were collected immediately at 0 hours and then again at 2 hours. A second set of reactions to test PAI-1 were set up with: recombinant PAI-1 (final concentration 2.8 μ g/ml PAI-1; Sigma), MDI-2268 (final concentration 1.6mM MDI-2268), recombinant latent TGF- β (final concentration 2.7 μ g/ml latent TGF- β), recombinant tPA (final concentration 0.8 μ g/ml tPA), recombinant plasminogen (final concentration 65 μ g/ml plasminogen), 1xprotease inhibitor; recombinant mature TGF- β (final concentration 6.7 μ g/ml mature TGF- β). Reaction constituents were mixed in a buffer containing 40 mM HEPES, 100 mM NaCl, 0.005% Tween-20, 1% DMSO. tPA/PAI-1 and MDI-2268/PAI-1 were each pre-incubated for an hour before other proteins were added. The reactions were incubated at 37°C and aliquots were collected at 0 hours, 2 hours, and 5 hours. A third set of reactions to test IL-1 β were set up with: recombinant tPA (final concentration 12 μ g/ml tPA), recombinant plasminogen (final concentration 16 μ g/ml plasminogen), pro-IL1 β (final concentration 1.3 μ g/ml pro-IL1 β ; Sino Biological), mature IL1 β (final concentration 6.7 μ g/ml mature IL1 β ; Peprotech) 1xprotease inhibitor, and chymotrypsin (final concentration 67 ng/ml chymotrypsin; Sigma). Reaction constituents were mixed in a buffer with DMEM, Tween-20 (0.005%), 1% DMSO and incubated at 37°C. An aliquot of the reaction mixtures was collected immediately at 0 hours and then again at 2 hours. For all samples the reaction was immediately quenched with protease inhibitor (Thermo Fischer Scientific) and Laemmli sample buffer, then the samples were heated for 80°C (TGF- β) or 98°C (IL1P) for 5 minutes.

Cytokine screen on primary colon epithelial cell spheroids

Primary epithelial cell spheroids derived from wild-type C57BL/6 mice were maintained in L-WRN media. As described above, epithelial cell spheroids were grown for 24 hours in DM with and without the following cytokines: TNF- α , IFN- γ , IL-1 β , IL-22, IL-6, IL-33, IL-12, IL-13, IL-17A, and IL-17F (all from Peprotech), and IL-12/23p40, Oncostatin M, IL-23, IL-17C, and IL-17E (from R&D Systems). Cytokines were added at either 20ng/ml or 100ng/ml doses. RNA was extracted and reverse transcription and quantitative PCR conducted as described below to measure the expression of *Serpine1* and *Plat* relative to *Gapdh* housekeeping gene.

Immunoblots

Supernatants were subjected to electrophoresis using Any-KD Mini-Protean gels (Bio-Rad) and separated by SDS-PAGE. Gels were transferred onto nitrocellulose membrane (Bio-Rad) by semi-dry transfer (24 V, 0.5 A, 1 h). Membranes were blocked with Blocking One (Nacalai) for 30 minutes and incubated with primary antibodies ((anti-TGFP β antibody (Abcam; clone 9016) anti-IL-1 β antibody (Novus biological; NB600–633)) in Blocking One at 4°C. Membranes were washed in TBS-Tween20 (0.05%) and then incubated with HRP-conjugated secondary antibodies (Thermo Fisher Scientific) for 1 hour at room temperature. Membranes were washed in TBS-T again before signal was developed using the SuperSignal West Femto Maximum Sensitivity Substrate chemiluminescence kit (Thermo Fisher Scientific).

Statistical analysis

A significant difference was considered $p < 0.05$, and tests were two-tailed with 95% CI, t tests or ANOVA were used and indicated in figure legends. Specific adjustments made to alpha levels or corrections for multiple testing (e.g. false discovery rate control) are indicated in the relevant results and figure legends. Parametric and nonparametric analyses were used where appropriate on the basis of testing for a normal distribution.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. The coagulation pathway and *SERPINE1* are enriched in IBD and link the epithelium to inflammation.

(A) Commonly dysregulated genes in colon biopsies between IBD and non-IBD controls ($p < 0.05$, 2-fold change) from MIRC (3 independent cohorts of ulcerative colitis patients, Crohn's disease and non-IBD controls; table S1). **(B)** Pathway over-representation analysis of the commonly dysregulated genes in (A) across databases, and identification of the coagulation/hemostasis pathway with FDR adjusted p-value scores. Number on bar indicates ranking position in the top 10 pathways for each specific database. **(C)** Gene set enrichment

analysis (GSEA) quantitatively identifying enrichment amongst the 1773 commonly dysregulated IBD probes from (A). **(D)** Bayesian network analysis of a three-step *SERPINE1*-induced subnetwork amongst intestinal epithelial and myeloid inflammation-associated gene clusters; *SERPINE1* and *CEBPB* (red dashed circles) are highlighted as genes linking these two clusters. **(E and F)** Box and whiskers plot of Log2 fold expression of *SERPINE1* in colon and ileal biopsies from ulcerative colitis (GSE38713) and Crohn's disease (GSE16879), using median and interquartile range. Whiskers represent 5% and 95% range. P-value based on one way ANOVA with Tukey's multiple comparison test. **(G)** Immunofluorescent protein staining and PAI-1-positive cell quantification in colon resection cases (Non-IBD colon n=11, UC uninvolved colon n=9, UC involved colon n=14), ****p<0.0001, one-way ANOVA with Tukey's multiple comparison test) **(H)** Immunofluorescent images of PAI-1 (red) and VIMENTIN (green) expression in resection cases from (G), images taken at 10x top panels (scale bars=100µm), and 20x bottom panels (scale bars=50µm).

Fig. 2. PAI-1 exacerbates DSS-induced colitis.

(A-E) *Serpine1*^{+/+}, *Serpine1*^{+/-} or *Serpine1*^{-/-} mice were administered DSS for 6 days followed by 6 days of recovery. (A) Weight loss compared to day 0, (B and C) colon damage and healing (% length of healed colon), (D) Ly6G+ neutrophils (immunofluorescence with a minimum of 10 high powered (20x) fields per mouse), and (E) concentration of IL-6 by ELISA were quantified in colon tissue on day 12. n=8–10 mice/group from 2 independent experiments, **p<0.01, ***p<0.001 compared to either *Serpine1*^{+/+} or *Serpine1*^{+/-} using two-way ANOVA, and *p<0.05, #p<0.01 using one-way ANOVA. Data represent means±SEM

Fig. 3. *SERPINE1* /PAI-1 correlation cluster is linked to cytokine signaling and IL-17A drives expression of the binding target *Plat*. (A) Ingenuity pathway analysis of canonical pathways regulating the *SERPINE1* correlation cluster (380 genes). Cytokine-associated pathways are circled in red. (B) Two doses (a, 20ng/ml; and b, 100ng/ml) of indicated cytokines were screened on primary mouse colon epithelial spheroids and expression of *Plat* measured by quantitative PCR, >2-fold increase indicated by red dotted line, n=3 independent experiments. (C) Commonly altered genes (FDR<0.05, 2-fold change) across 3 mouse colon epithelial states (stem cells, Diff=mid-

crypt/differentiating cells, terminally differentiated colonocytes) following treatment with recombinant IL-17A *in vitro*. Listed are the 5 genes commonly altered by IL-17A across all 3 states as determined by microarray analysis **(D)** *Plat* expression measured in mouse colon epithelial cells *in vitro* by quantitative PCR, * $p < 0.05$, ** $p < 0.01$ using one-way ANOVA, $n = 3-5$ independent experiments. **(E)** *Plat* expression measured by quantitative PCR in Diff cells after co-culture with Th17 cells +/- anti-IL-17RA, Th1 cells, or unstimulated T cells. $a/c = p < 0.01$ compared to Diff epithelial cells alone, $b/d = p < 0.05$ and $e = p < 0.01$ compared to epithelial cells + Th17 cells and epithelial cells + IL-17A, using one-way ANOVA with Tukey's multiple comparison test, $n = 4$ independent experiments. Data represent means $\pm$ SEM.

Fig. 4. *Plat*/tPA suppresses colitis and mucosal biopsy colon damage.

(A-E) *Plat*^{+/-} or *Plat*^{-/-} mice were administered DSS for 6 days followed by 6 days of recovery. (A) Weight loss compared to day 0, (B and C) colon damage and healing (% healed colon), scale bars=0.5mm, (D) Ly6G⁺ neutrophils, and (E) concentration of IL-6 by ELISA were quantified in colon tissue on day 12. n= 10–14 mice/group from 2 independent experiments, ***p<0.001 compared to *Plat*^{+/-} mice using two-way ANOVA, *p<0.05, **p<0.01 using a two-tailed unpaired t-test with 95% confidence intervals. (F) tPA protein expression in a colonic wound from a mucosal colonic pinch biopsy model showing

expression in WAE cells on top of wound (upper panel) and in normal uninjured crypts (bottom panel). **(G-I)** Mucosal colonic pinch biopsy was used to create wounds in *Plat*^{+/+} or *Plat*^{-/-} mice and colonic damage and repair was measured 4 days later by **(G-H)** percentage total unhealed wound area (dotted line indicates total wound area and solid line indicates unhealed area), and **(I)** percentage presence (red) or absence (blue) of a neutrophil/fibrin cap on top of the wounds. n=8–9 mice/group from 2 independent experiments, ***p<0.001 using a two-tailed unpaired t-test with 95% confidence intervals, scale bars=100µm . Data represent means±SEM.

Fig. 5. A small molecule PAI-1 inhibitor partially suppresses DSS- and *Citrobacter*-induced colitis in a tPA-dependent manner.

(A) Ratio of active tPA to total tPA measured in colon homogenates and plasma, $n = 7-11$ mice/group from 2 independent experiments, $*p < 0.05$ using a two-tailed unpaired t-test with 95% confidence intervals. (B-G) Mice were administered DSS for 6 days followed by 6 days of recovery; at day 6 mice received daily intraperitoneal injections of vehicle or a small molecule PAI-1 inhibitor, MDI-2268. (B) Weight loss compared to day 0, $n = 20$ mice/group from 2 independent experiments, $**p < 0.01$ using a two-way ANOVA, (C) colon damage and

healing (% healed colon), **(D)** Ly6G+ neutrophils, and **(E)** IL-6 in colon tissue were quantified, n=9–10 mice/group from 2 independent experiments, **p<0.01 using two-tailed unpaired t-test with 95% confidence intervals. **(F)** *Plat*^{-/-} mice weight loss compared to day 0, and **(G)** colon damage and healing (% healed colon) were quantified. n=8 mice/group from 2 independent experiments. **(H-I)** Mice were infected with *Citrobacter rodentium* for 12 days to induce colitis pathology and received daily intraperitoneal injections of vehicle or a small molecule PAI-1 inhibitor, MDI-2268, from day 0. **(H)** Percentage of colon with crypt loss/dropout and **(I)** representative H&E image of the transverse colon, scale bars=0.5mm, n=12–13 mice/group from 2 independent experiments, ***p<0.001 using a two-tailed unpaired t-test with 95% confidence intervals, ns; not statistically significant. Data represent means±SEM.

Fig. 6. PAI-1 inhibits the tPA-dependent cleavage and activation of TGF- β suppressing epithelial hyperproliferation and colitis.

(A) Immunoblot analysis of a cell free *in vitro* reaction for the conversion of latent/inactive TGF- β to mature/active TGF- β . (B) Primary colon epithelial spheroids were cultured with the indicated protein combinations and proliferation measured by luminescence, n=3 independent experiments, ***p<0.001 compared to either mature TGF- β or tPA + latent TGF- β + plasminogen using two-way ANOVA with Tukey's multiple comparison test. (C and D) Ratio of active TGF- β to total TGF- β in colon homogenates of DSS-treated mice

measured using a flow cytometric bead assay, n=4–5 mice/group is shown from 1 experiment that is representative of 2 independent experiments, *p<0.05 using a two-tailed unpaired t-test with 95% confidence intervals. **(E-G)** Mice were administered DSS for 6 days followed by 6 days of recovery; treatment with anti-TGF β (α TGF β) or isotype IgG control was initiated on day -2. **(E)** Weight loss compared to day 0, **(F)** colon damage and healing (% healed colon), and **(G)** IL-6 in colon tissue were quantified. n=7–10 mice/group from 2 independent experiments, *p<0.05 using one-way ANOVA with Tukey's multiple comparison test, **p<0.01 compared to either *Serpine1*^{-/-} or *Serpine1*^{-/-} + isotype IgG control using a two-way ANOVA. Data represent means $\pm$ SEM.

Fig. 7. *SERPINE1* is elevated in active disease and in non-responders to anti-TNF α biologic therapy

(A) Receiver operating characteristic (ROC) curve analysis on genesets (GSE38713, GSE16879, GSE36807, and GSE23597) defining specificity and sensitivity of *SERPINE1* discrimination between active and non-active disease in colon biopsies (n=133, area under the curve; AUC=0.97). (B) Spearman correlation of *SERPINE1* expression and MAYO score (n=87) from the PURSUIT cohort (NCT00487539) at week 0 before treatment and week 6 after golimumab treatment. (C) ROC curve analysis on colon biopsies from geneset GSE16879 defining *SERPINE1* discrimination between responders and non-responders to infliximab before treatment (AUC=0.92).