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Inflammatory monocytes expressing tissue factor drive SIV and HIV-coagulopathy

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Abstract

In human immunodeficiency virus (HIV) infection, persistent inflammation despite effective antiretroviral therapy (ART) is linked to increased risk of non-infectious chronic complications such as cardiovascular and thromboembolic disease. A better understanding of inflammatory and coagulation pathways in HIV infection is needed to optimize clinical care. Markers of monocyte activation and coagulation independently predict morbidity and mortality associated with non-AIDS events. In this study, we identified a specific subset of monocytes that express tissue factor (TF), persist after virological suppression and trigger the coagulation cascade by activating factor X. This subset of monocytes expressing TF had a distinct gene signature with upregulated innate immune markers as well as evidence of robust production of multiple proinflammatory cytokines including IL-1β, TNF-α, and IL-6 ex vivo and in vitro upon LPS stimulation. We validated our findings in a nonhuman primate model, showing that TF-expressing inflammatory monocytes were associated with SIV-related coagulopathy in the progressive (pigtail macaques) but not the non-pathogenic (African Green Monkeys) SIV infection model. Lastly, Ixolaris, an anti-coagulant that inhibits the TF pathway, was tested and potently blocked functional TF activity in vitro in HIV and SIV infection without affecting monocyte responses to toll-like receptor (TLR) stimulation. Strikingly, in vivo treatment of chronically infected PTMs with Ixolaris was associated with significant decreases in D-dimer and immune activation. These data suggest that TF expressing monocytes are at the epicenter of inflammation and coagulation in chronic HIV and SIV infection and may represent a potential therapeutic target.

Introduction

Monocytes are key mediators of innate immunity and have been closely associated with pathogenesis of chronic viral infections, including HIV (1, 2). Heightened circulating levels of monocyte activation markers, such as soluble (s) TF, sCD14 and sCD163 have been

associated with increased risk for death (3), non-infectious complications (4, 5), subclinical atherosclerosis (6), and immune reconstitution inflammatory syndrome (IRIS) in HIV-infected individuals (7). Moreover, differential activation of monocyte subsets has recently been described as a predictor of tuberculosis (TB)-associated IRIS in patients with HIV-TB co-infection (7). One important feature of monocytes in HIV pathogenesis is their capacity to produce TF (8-10). TF is expressed in response to inflammatory stimuli such as toll-like receptors (TLR) (11-13) and cytokine-driven signals (14, 15) and initiates the extrinsic coagulation cascade by cleaving coagulation factors leading to formation of Factor Xa, thrombin and fibrin, which when degraded forms the coagulation biomarker D-dimer (16, 17). For these reasons, augmented TF expression is associated with increased levels of D-dimer (18) and thus may be associated with an increased risk for cardiovascular complications in HIV-infected individuals (19). These findings support a direct role of activated monocytes in the persistent inflammatory milieu observed in chronic HIV infection.

The need to investigate the link between coagulation and inflammation in chronic viral infections is pressing. Inflammatory and coagulation markers are both independent predictors of morbidity and mortality in treated HIV individuals (20-23) and are clearly associated with non-infectious complications of HIV such as cardiovascular and thromboembolic disease (19) which are rising due to the aging of treated HIV-infected persons (24). In an experimental model of nonhuman primates (NHP) infected with SIVsab, we previously demonstrated that increases in D-dimer as well as monocyte activation markers (sCD14) predict disease progression (25). These findings highlighted monocyte activation as a key event driving persistent coagulation in SIV/HIV chronic infection, suggesting a need to delineate the role of monocyte-derived TF in SIV/HIV-driven systemic inflammation and coagulopathy.

In the present study, we evaluated the role of TF-expressing monocytes in HIV and SIV pathogenesis and related coagulopathy. We examined the links between inflammation and coagulation with the aim to identify potential targets for therapeutic interventions in HIV-infected persons.

Results

A specific monocyte subset is the major source of TF amongst peripheral blood mononuclear cells

To determine the potential of monocytes to express TF, peripheral blood mononuclear cells (PBMCs) from healthy blood donors were stimulated with LPS in vitro and TF expression was assessed by flow cytometry (monocyte gating strategy shown in Figure S1). The frequency of monocytes expressing TF (TF^{pos}) in unstimulated cultures was very low (median and interquartile range (IQR); median, 0.49% of total monocytes, IQR: 0.24-0.69). Upon LPS stimulation, the expression of TF by monocytes was significantly increased ($P<0.0001$, Figure 1A). We next tested whether TF expression reflects a state of cellular activation by quantifying the frequency of TF^{pos} monocytes following stimulation with increasing doses of LPS. A robust expression of TF by monocytes was observed only when cells were stimulated with ≥10 ng/mL of LPS whereas lower doses triggered a minor

induction of the enzyme (Figure 1B). The frequency of TF^{pos} monocytes did not increase further with escalated doses of LPS (Figure 1B), suggesting that only a subset of cells is capable of producing the coagulation factor in vitro. We next performed a phenotypic analysis to better delineate the subpopulation of mononuclear cells from PBMC that produce TF upon LPS stimulation. We observed that TF expression is restricted to $HLADR^{+}$, $Dump^{-}$ ($CD2^{-}CD3^{-}CD19^{-}CD20^{-}CD56^{-}$) cells, which were further characterized as $CD14^{+}$, $CD16^{-}$ and $CCR2^{+}$ monocytes (Figure S2). A more detailed analysis revealed that TF^{pos} cells exhibit differential expression of activation markers and co-stimulatory molecules, such as CD36, CD40, CD86, CD62L, CD163, TLR4 and IL-6R (Figure S2). To further investigate these populations, we sorted TF^{pos} and TF^{neg} monocytes from six healthy donors after LPS stimulation and examined the expression profile of a customized assortment of genes associated with monocyte activation and coagulation using a multiplex qPCR assay. Hierarchical clustering analysis of the overall gene expression profile of monocytes revealed that TF^{pos} cells exhibit a unique signature highlighted by increased expression of TLRs, proinflammatory cytokine receptors, and signaling molecules such as MyD88 and TRIF (Figure 1C-D) whereas TF^{neg} cells exhibited increased relative expression of CX3CR1 (Figure 1C-D). Principal Component Analysis (PCA) with vector analysis of the expression profile of all genes confirmed the observation that TF^{pos} and TF^{neg} monocytes have distinct gene expression profiles associated with inflammation and coagulation (Figure 1D). These findings indicate that rather than representing a general state of cell activation, TF expression by circulating mononuclear cells may be restricted to a specific monocyte subset.

It is known that expression of surface markers on monocytes is dynamic and can change depending on the stimulation and microenvironment conditions (26). To address whether this dynamic expression of surface markers was confounding our interpretation of which monocyte subset expresses TF in response to LPS, we sorted the three major monocyte subsets based on the dichotomous surface expression of CD14 and CD16 ($CD14^{++}CD16^{-}$, $CD14^{+}CD16^{+}$ and $CD14^{dim}CD16^{+}$), stimulated each subset with LPS and then examined TF expression in cell lysates and measured TF functional activity by a colorimetric assay which quantifies the formation of the coagulation factor Xa (27) (Figure 1E). In unstimulated conditions, TF protein expression and functional activity by each monocyte subset was uniformly low in cultures (Figure 1E). LPS stimulation induced an increase in TF protein expression and activity in $CD14^{++}CD16^{-}$ monocytes, a marginal increase of TF functional activity (by means of factor Xa formation) in $CD14^{+}CD16^{+}$ cells and no effect on $CD14^{dim}CD16^{+}$ monocytes (Figure 1E). These data demonstrate an inherent capacity for the $CD14^{++}CD16^{-}$ monocyte subset to express TF after LPS-driven activation.

TF-expressing monocytes are expanded in chronic HIV infection independent of antiretroviral treatment

It has been previously observed that monocytes from HIV^{+} patients display higher levels of TF expression compared to healthy controls (18). In the present study, we extended these observations by comparing TF expression in HIV^{+} individuals before and after ART initiation (after virological suppression was achieved) and age and gender-matched healthy controls. In a cross-sectional comparison of ex vivo TF expression, meadured by flow cytometry, we observed that the frequency of TF^{pos} monocytes was significantly higher in

both ART-naïve and ART-treated HIV$^+$ patients compared to healthy controls (P<0.001 and P<0.01 respectively, Figure 2A), albeit with considerable variability in expression levels. The median frequency of TF^{pos} cells was not statistically different between treatment naïve HIV$^+$ patients and those with ART-induced suppression of HIV viremia (Figure 2A). This finding suggests that TF protein expression in HIV$^+$ patients may not be substantially affected by ART. We next compared the potential of monocytes to produce TF in response to LPS between the different study groups. We found that the frequencies of TF^{pos} monocytes were significantly higher in HIV$^+$ patients, pre and post-ART, compared to healthy individuals in unstimulated cultures as well as upon LPS stimulation (Figure 2B). There was no observed difference in response to LPS between treatment-naïve HIV$^+$ patients and those on ART with virological suppression (Figure 2B). These results mirrored the findings obtained by quantification of TF protein expression in PBMC lysates (Figure 2C). We further demonstrated that TF expressed on the cell surface of monocytes from HIV$^+$ individuals was able to trigger Factor Xa formation, demonstrating that TF was functionally active in vitro (Figure 2D). Again, no difference was detected in TF functional activity between the groups of HIV$^+$ patients (Figure 2C-D). The differences in TF expression on monocytes were independent of the total monocyte counts in PBMC amongst the HIV-infected groups, which were not significantly different (median 4451 cells/μL, IQR: 2383-6829 in ART-naïve vs. 4507 cells/μL, IQR: 3308-7771 in virologically suppressed individuals, P=0.34).

These primary observations indicated an increased in the capacity of monocytes to produce TF and promote factor Xa formation in vitro upon cellular activation in HIV infection which persists after ART-induced HIV suppression. To further test this hypothesis, we prospectively assessed ex vivo TF expression as well as plasma levels of D-dimer and C-reactive protein (CRP) in an additional set of HIV$^+$ patients examined longitudinally with paired samples available from pre-ART and post-ART (at virological suppression) timepoints. This longitudinal paired analysis confirmed that the frequency of TF^{pos} monocytes was not significantly altered by ART (Figure S3). Out of 12 HIV$^+$ patients prospectively evaluated, 6 (50%) exhibited increases whereas 4 (33.3%) displayed reduction in TF expression upon ART-induced HIV suppression. Mean fluorescent intensities (MFI) values for TF expression did not differ between the timepoints (MFI at pre-ART: 575 arbitrary units [AU] ± 49.5 vs. MFI at virologic suppression timepoint: 553 AU ± 89.6, P=0.785). However, D-dimer levels decreased whereas CRP values remained unchanged after ART initiation (Figure S3). These findings show sustained elevation of TF expression on circulating monocytes regardless of the reduction in D-dimer levels, which strongly argues that HIV-associated coagulopathy may persist even after ART-induced HIV suppression, as has been previously suggested (28). In ART-naïve patients, the ex vivo frequency of TF^{pos} monocytes was positively correlated with the levels of D-dimer (r=0.69, P=0.015; Figure S3) and did not associate with concentrations of CRP (r=0.04, P=0.908; Figure S3).

To further assess the role of TF on monocytes in HIV pathogenesis and coagulopathy, we next examined the expression and activity of this coagulation factor in PBMC from NHP before and after SIVsab infection in vivo. In this biological system, AGMs are the natural hosts of SIVsab and undergo infection but do not progress to SIV disease and coagulopathy

despite active chronic viral replication reflected by lifelong high plasma SIV viremia (29, 30). Contrastingly, SIVsab infection causes progressive disease in PTMs (29). We have previously shown that PTMs infected with SIVsab experience increased systemic inflammation and coagulopathy compared to naïve animals (25). Prior to SIVsab infection, both PTMs and AGMs exhibited similarly low frequency of TF^{pos} monocytes (Figure 2E). The frequency of monocytes expressing TF ex vivo was substantially increased in chronically infected PTMs compared to naïve animals ($P<0.001$), whereas there was no marked effect of SIV infection in AGMs (Figure 2E). Importantly, monocytes, but not myeloid dendritic cells, were the major source of TF ex vivo and after LPS stimulation in vitro (Figure S4). Notably, monocytes isolated from naïve PTMs and AGMs displayed indistinguishable TF expression in response to LPS stimulation in vitro (Figure 2F). During chronic SIVsab infection, the frequency of TF^{pos} monocytes was higher in PTMs compared to AGMs in unstimulated cells and this difference was further increased upon LPS stimulation (Figure 2F). Comparable results were obtained by quantification of TF functional activity (Figure 2G). In addition, we found in our cohort of NHP that concentrations of D-dimer and CRP in plasma were significantly higher in accordance with the augmented TF monocyte expression in chronically infected PTMs compared to naïve animals (Table S1). Neither TF expression nor levels of these plasma markers of inflammation and coagulation were significantly altered upon SIVsab infection in AGMs (Table S1). To further investigate the role of TF in SIV pathogenesis, we examined TF expression prior to infection and at different time points post-infection in the mucosal tissues sampled from PTMs. We observed continuous increase of TF expression in the gut with disease progression (Figure S5). Furthermore, few TF positive cells were present in the lamina propria prior to infection, but their numbers increased after infection, first at the tip of the villi and then more profoundly in the lamina propria. The same dynamics were also observed in Peyer's patches (Figure S5). The significant increase in TF expression of both mucosal tissues and peripheral monocytes of chronically SIV-infected PTM suggest that TF expression may contribute to the pathogenesis of SIV-driven persistent coagulation and inflammation.

Proinflammatory cytokines increase TF expression during chronic HIV infection

A potential explanation for the activation of circulating monocytes and subsequent persistent TF expression in HIV-infected persons could be a soluble factor. To test this hypothesis, we column-purified $CD14^+$ monocytes from healthy blood donors and incubated them overnight with medium supplemented with either heat inactivated serum from human AB plasma (HAB serum), filtered pooled sera from healthy controls, or sera from individuals chronically infected with HIV or hepatitis C virus (HCV). TF protein expression was then quantified in cell lysates. Samples incubated with HIV^+ or HCV^+ sera displayed augmented production of TF (Figure 3A). TF expression was indistinguishable between cultures of monocytes incubated with sera from ART-naïve HIV^+ patients and at different time points upon treatment initiation (Figure 3B). This observation indicates that the factors driving TF expression by monocytes persist after ART implementation. Moreover, we performed a series of blocking experiments trying to delineate the molecules that are potentially driving monocyte activation and TF production from the sera of HIV infected individuals. We found that IL-1RI blocking resulted in a slight but consistent reduction of TF production in

monocytes cultured in the presence of HIV^+ serum (Figure 3C). Neutralizing IL-6R and interferon-gamma receptor (IFNγR) did not alter TF expression in this experimental setting (Figure 3C). Notably, TF production exhibited a substantial drop in monocyte cultures incubated with blocking antibodies against type I interferon receptor (IFNAR) or tumor necrosis factor receptor 1 (TNFR1) (P=0.003 and P=0.005, respectively, Figure 3C). These experiments confirmed that inflammatory signals present in serum from HIV-infected persons are capable of inducing TF production in monocytes. The specific drivers of these inflammatory signals could be coming from inflamed tissues or the blood itself. Microbial translocation in the gut has been described as an important trigger of persistent systemic inflammation detected in HIV^+ persons (31). To test the possibility that monocyte activation in blood could be resulting from a residual leakage of microbial products from mucosal interfaces, we incubated HIV^+ serum with polymyxin B before culturing the monocytes. Serum treated with this bactericidal compound resulted in the lowest TF expression among all the experimental conditions tested (Figure 3C). These findings demonstrate that circulating microbial products are indeed relevant drivers of TF expression by activated monocytes.

TF-expressing monocytes produce multiple proinflammatory cytokines

Chronic HIV infection has been associated with persistent immune activation and elevated markers of coagulation (22, 32). We hypothesized that TF-expressing monocytes, aside from promoting coagulation, could contribute to systemic inflammation. Indeed, in non-HIV-infected healthy individuals, TF^{pos} monocytes more frequently produced multiple proinflammatory cytokines, such as TNF-α, IL-1β and IL-6, simultaneously upon LPS stimulation (Figure 4A-C). We further investigated the profile of intracellular cytokine expression in monocytes ex vivo from our longitudinal cohort of HIV^+ patients before ART initiation and after virological suppression. HIV^+ individuals, regardless of treatment status, exhibited high frequencies of monocytes spontaneously producing pro-inflammatory cytokines (Figure S6A). Furthermore, by testing the monocyte response to LPS stimulation in our prospective cohort of HIV^+ patients, we detected markedly different intracellular cytokine expression profiles between TF^{neg} and TF^{pos} monocytes in ART-naïve HIV^+ patients (chi-square P<0.001). In contrast to monocytes from healthy controls (Figure 4C), following LPS stimulation, the vast majority of TF^{neg} monocytes from HIV infected individuals produced IL-6 alone (median 48.7%, IQR: 33.5-52.8% of all the cytokine producing TF^{neg} cells) whereas TF^{pos} monocytes more frequently produced TNF-α, IL-1β and IL-6 simultaneously (53.1% of all the cytokine producing TF^{pos} cells, IQR: 49.5-56.7%) (Figure 4D). The intracellular cytokine expression profile of LPS-stimulated monocytes significantly changed after ART initiation (chi square P=0.015 for the expression profile in TF^{neg} cells and P<0.001 for TF^{pos} monocytes from pre vs. post-ART initiation; Figure 4D). In virologically suppressed HIV^+ individuals, TF^{neg} cells exhibited a mixed cytokine expression profile with monocytes producing one or various combinations of two cytokines whereas the majority of TF^{pos} monocytes remained polyfunctional, producing a combination of three cytokines (TNF-α, IL-1β and IL-6) simultaneously (chi-square P<0.001 profile of TF^{neg} vs. TF^{pos} cells; Figure 4D). In addition, the frequency of TF-expressing monocytes strongly correlated with the frequency of polyfunctional monocytes before ART initiation (r=0.89, P<0.001) and in patients with virological suppression (r=0.91, P<0.001).

We next examined the monocyte response to LPS in our NHP cohort by assessing the intracellular expression of TNF-α, IL-1β and IL-6 *in vitro* and found that monocytes isolated from both naïve and chronically SIVsab infected PTMs and AGMs were capable of producing these cytokines upon LPS stimulation (Figure S6B). The cytokine expression profile of stimulated monocytes was not different between uninfected PTMs and AGMs, with a majority of the cytokine-producing cells expressing IL-1β alone in both species (chi-square P=0.459; Figure 4E and Figure S6B). The overall frequency of monocytes simultaneously expressing multiple pro-inflammatory cytokines (TNF-α, IL-1β and IL-6) ex vivo was significantly higher in chronically infected PTMs than in AGMs (Figure 4F). Upon activation in vitro, frequency of cytokine producing monocytes was higher in PTMs than in AGMs (Figure S6B). SIVsab infection caused changes in the cytokine expression profile of stimulated monocytes from both NHP species (chi-square P=0.035 for AGM naïve vs. chronically infected, P<0.001 for PTM naïve vs. chronically infected; Figure 4E). During chronic SIVsab infection, the majority of the activated monocytes from PTMs produced TNF-α, IL-1β and IL-6 simultaneously (median 43.5% of all the cytokine producing monocytes, IQR: 35.2-55.8%) whereas the activated monocytes from AGMs had a mixed expression profile and a low frequency of triple cytokine producers (median 12.5% of all the cytokine producing monocytes, IQR: 8.5-18.9%, P<0.001 vs. frequency of triple producers from PTMs) (Figure 4E).

We next quantitatively compared the dynamics of monocyte polyfunctionality in HIV-infected patients as well as in SIVab-infected NHP. In HIV^+ patients, the polyfunctionality of TF^{pos} monocytes remained high after ART initiation and was significantly increased in TF^{neg} cells post-ART compared to TF^{neg} cells before ART initiation (P<0.001, Figure 4F). The polyfunctionality of TF^{pos} monocytes from chronically $SIVsab^+$ PTMs was significantly higher when compared to TF^{neg} monocytes from the same animals and also when compared to TF^{pos} monocytes isolated from chronically $SIVsab^+$ AGMs (P<0.001 Figure 4G). These findings from both HIV-infected patients and SIV-infected NHPs support the hypothesis that in monocytes, increased TF expression is associated with an enhanced potential to produce multiple proinflammatory cytokines.

Thrombin triggers TF expression on $CD14^{high}$ monocytes and induces production of multiple proinflammatory cytokines via PAR-1 signaling

Our results so far demonstrated that $CD14^{high}$ monocytes have increased capacity both to promote activation of coagulation factors and to produce multiple proinflammatory cytokines linked to HIV and SIV pathogenesis. Activation of the coagulation cascade by TF in vivo ultimately results in formation of thrombin, an essential protein leading to thrombus development (33). Thrombin has been shown to activate NF-κB via cleavage of the cytoplasmic tail of protease-activated receptors (PAR) (34). Amongst the various types of PARs, PAR-1 and PAR-3 are known to be expressed on monocytes (35). Interestingly, a recent study has demonstrated that thrombin triggers TF expression on human monocytes via activation of PAR-1 (36, 37). We postulated that activated $CD14^{high}TF^{pos}$ monocytes might also be able to sense thrombin generated from coagulation. To test this hypothesis, we performed a series of experiments and first confirmed that thrombin triggers TF expression on monocytes from healthy donors (Figure S7A). Subsequently we found that this effect was

associated with reduced expression of PAR-1 on the surface of these cells upon thrombin stimulation (Figure S7B). Importantly, we observed that TF expression triggered by thrombin was restricted to $CD14^{high}$ monocytes (Figure S7C) and we further established that $CD14^{++}CD16^{-}$ and $CD14^{+}CD16^{+}$ cells exhibited similarly high expression of PAR-1 (Figure S7C). Additional assays in HIV^{+} patients suggested that PAR-1 is preferentially expressed in $CD14^{high}$ cells and that it is shed in response to thrombin in monocytes, a phenomenon that was associated with induction of TF expression by these cells (Figure S7D). These results indicate that the subset of monocytes that produces TF upon LPS-driven activation is the same that can respond to thrombin stimulation in vitro possibly via PAR1.

These results led us to speculate that monocytes activated by thrombin also produce multiple pro-inflammatory cytokines. We tested this idea by examining cytokine production by PBMC directly stimulated with thrombin in vitro. We found that thrombin induced robust production of intracellular cytokines (TNF-α, IL-1β and IL-6) by $CD14^{high}$ monocytes from healthy individuals (Figure S7E). Pharmacologic inhibition of PAR-1 signaling by SCH79797 completely neutralized the effects of thrombin on TF expression and cytokine production (Figure S7E). These results delineate how thrombin triggers an inflammatory response by monocytes in a PAR-1 dependent manner.

Ixolaris blocks TF activity without suppressing monocyte immune function *in vitro*

The results at this point support the hypothesis that monocytes are highly responsive to thrombin and act as a critical link between TLR-driven persistent inflammation and coagulation in chronic viral infections. Interventional therapies focusing on TF inhibition and/or blockade could therefore be key to breaking this vicious cycle of coagulation and inflammation in HIV and SIV pathogenesis. Ixolaris is a small molecule isolated from the saliva of the tick *Ixodes scapularis* and acts as a potent TF pathway inhibitor (TFPI), by blocking Factor VIIa/TF-induced coagulation (38, 39). Although Ixolaris has been tested in thrombosis models (40), it has not been evaluated in the setting of HIV-driven coagulopathy.

In our in vitro model, Ixolaris inhibited TF functional activity in monocytes from healthy donors stimulated with LPS in a dose-dependent fashion (Figure 5A). Importantly, even at doses 10 times higher than the maximum inhibitory concentration (10 ng/mL), Ixolaris did not exhibit substantial cytotoxicity (Figure S8). We next examined if Ixolaris blocks TF activity in pathological settings such as HIV and SIV infection. Indeed, Ixolaris completely inhibited formation of factor Xa in unstimulated conditions and after LPS stimulation in vitro in PBMCs from both ART-naïve and ART-treated, virologically suppressed HIV^{+} persons (Figure 5B). Ixolaris also significantly inhibited TF activity in PBMCs from chronically SIVsab-infected PTMs and AGMs (Figure 5C). Ixolaris did not affect TF protein expression or cytokine production triggered by LPS stimulation in vitro (Figure 5D). These results demonstrate that, at low doses, Ixolaris can potently inhibit TF functional activity in cells from HIV^{+} patients and from SIVsab-infected NHP and suggest that inhibition of TF activity could be used to suppress monocyte-driven activation of coagulation in these settings without directly affecting the capacity of these cells to respond to TLR stimulation.

In vivo administration of Ixolaris is safe and decreases coagulation and immune activation in SIVsab infection in PTMs

To assess the efficacy of Ixolaris, we first measured its anticoagulant effect in vitro (Figure 6). Ixolaris resulted in dose-dependent prolongation of prothrombin time (PT) /international normalized ratio (INR) when added to human plasma collected from healthy individuals (Figure 6A). Changes in PT/INR were similar when Ixolaris was added to plasma from uninfected healthy PTMs (Figure 6B). Activated partial thromboplastin time (aPTT), an intrinsic pathway coagulation marker, was not affected by Ixolaris in either monkey or human plasma, indicating that Ixolaris has a specific inhibitory effect on the extrinsic pathway. Our results thus confirmed the high efficacy and specificity of Ixolaris in NHP in vitro.

Chronic progressive HIV/SIV infection is characterized by high levels of immune activation, inflammation and hypercoagulation, which are robust independent prognostic factors of progression to AIDS and comorbidities (23, 41, 42). To investigate the effect of the anticoagulant therapy on the outcome of a highly pathogenic SIVsab infection, we administered Ixolaris to five PTMs upon SIVsab infection (at day of infection) and compared the natural history of SIVsab infection in Ixolaris-treated PTMs and untreated controls. Therefore, we first assessed the effects of the anticoagulant treatment by comparing the levels of immune activation and inflammation markers in PTMs receiving Ixolaris and in controls. The animals treated with Ixolaris showed significantly reduced levels of the proinflammatory cytokine IL-17 during early chronic infection ($P=0.03$, Figure 6C). The anticoagulant treatment also impacted T cell immune activation, as demonstrated by a lower frequency of $CD4^+$ T cells expressing $HLA\text{-}DR^+$ and $CD38^+$ (Figure 6D), and by significantly lower frequency of $CD8^+$ T cells expressing $HLA\text{-}DR^+$ and $CD38^+$ during early chronic infection ($P<0.001$, Figure 6E).

Following treatment, TF expression on circulating $CD14^+$ monocytes decreased compared with untreated controls (Figure 6F). In addition, Ixolaris treatment significantly lowered both CD80 expression in chronically infected PTMs ($P=0.004$, Figure 6G) and CD86 expression in both acutely and chronically infected PTMs ($P=0.03$, Figure 6H), compared with the untreated controls. To further validate the reduction of monocyte activation induced by Ixolaris treatment, we measured Glut-1 expression on $CD14^+$ monocytes, an important monocyte activation marker (43-45). Ixolaris-treated animals showed a significantly reduced Glut-1 expression following SIV infection, compared to untreated controls ($P=0.001$, Figure 6I). Furthermore, we examined associations between expression of monocyte markers and lymphocyte activation using generalizing estimating equations, due to the nature of the data distribution and small sample size of the experimental groups. Using this approach, we found that the monocyte activation markers strongly associated with lymphocyte activation. CD80 and CD86 expression on $CD14^+$ monocytes were directly associated with $HLA\text{-}DR^+$ $CD38^+$ expression on $CD4^+$T cells ($p<0.001$ and $p<0.001$, respectively), while Glut-1 expression on $CD14^+$ monocytes was strongly associated with $HLA\text{-}DR^+$ $CD38^+$ expression on both $CD4^+$ and $CD8^+$ T cells ($P<0.001$ and $P<0.001$, respectively). Altogether, these results suggest that Ixolaris treatment had a beneficial effect in reducing immune activation and inflammation associated with SIV infection.

The hypercoagulable status exhibited in SIVsab-infected progressive NHPs and HIV-infected patients is marked by elevated D-dimer, which is associated with increased incidence of cardiovascular comorbidities and mortality (25, 46, 47). Therefore, we next compared the D-dimer levels in Ixolaris-treated PTMs and controls. Ixolaris administration significantly reduced plasma D-dimer levels in acute SIVsab-infection of PTMs (P=0.033) (Figure 6J), and resulted in lower D-dimer levels during early chronic infection, indicating a clear effect of Ixolaris in improving the coagulation status in the SIV-infected animals. To monitor the infection in SIVsab-infected PTMs, we measured plasma SIV viremia in all infected animals (48). Interestingly, the Ixolaris-treated group showed overall lower viral loads compared to controls (Figure 6K).

Most importantly, Ixolaris-treated animals did not develop disease during the first 100 days postinfection (Figure 6L). In accordance with our previous studies in which two out of five SIVsab-infected PTMs were rapid progressors (49, 50), one animal out of three progressed to AIDS in the first 100 days postinfection in the untreated group, however, no rapid progression was registered in the Ixolaris group. Statistical significance was not reached in the present study, possibly due to the small sample size. This result is of potential translational interest, as it suggests that Ixolaris treatment may abrogate rapid progression in treated animals, likely due to the combination effect of reduced immune activation and inflammation, reduced hypercoagulable status and small reduction in plasma viremia.

Discussion

Chronic HIV infection has been associated with elevated circulating levels of biomarkers of coagulation, in particular D-dimer, and systemic inflammation, such as IL-6, sCD14 and CRP (22, 23, 51) which have been independently linked to a higher risk of non-AIDS related death and mortality (23) even in persons treated with ART (52). Although ART induced suppression of HIV viremia is shown to significantly reduce plasma levels of D-dimer, plasma levels remain higher than in non-HIV infected populations (32).

Monocytes have been previously described as immune cells involved in cardiovascular disease in both HIV^- and HIV^+ populations (4, 52-54) and are an important source of TF (9). In the current study we performed a detailed immune profiling of monocyte subsets and delineated molecular signatures that characterize TF-expressing cells in the context of TLR activation. Our results demonstrated that TF positive monocytes exhibit elevated expression of several genes associated with innate immune activation. These findings argue that the subpopulation of monocytes able to upregulate TF expression upon TLR4 activation is the classical subset defined as $CD14^+CD16^-$. Activated monocytes not expressing TF in the same circumstances exhibit markers of patrolling monocytes ($CD14^{dim}CD16^+$) such as upregulation of CX3CR1 gene expression. In the setting of coagulopathy, it is possible that patrolling monocytes, expressing high levels of CX3CR1, a vascular homing receptor, might interact with thrombin clots. Our results indicate that the patrolling monocytes express the lowest level of PAR-1 and that PAR-1 expression is decreased by thrombin stimulation. Therefore, in vivo, it is possible that the interaction between CX3CR1-expressing patrolling monocytes and thrombin clots accounts for the lower expression of PAR-1. Furthermore, we demonstrated that the frequency of monocytes expressing TF upon activation could not be

further increased with augmenting doses of TLR activation, suggesting an inherent capacity of these circulating monocytes to express this coagulation factor. Importantly, we confirmed that non-monocytic cells from peripheral blood are unable to express TF upon LPS stimulation. Flow cytometry-based phenotypic analysis revealed that compared to TF^{neg} monocytes, TF^{pos} cells displayed increased expression of HLA-DR and co-stimulatory molecules such as CD40 and CD86 as well as IL-6R. These results indicate a unique monocyte phenotype capable of producing TF. Previous flow-cytometry based studies have indicated that $CD16^+$ monocytes are able to produce TF (55). Our results however indicate that the molecular signature associated with TF expression in monocytes is restricted to the classical proinflammatory subset that lacks expression of CX3CR1. It is possible that different experimental settings and/or gating strategies have resulted in discrepancies between the studies, and for this reason we performed TF expression assessment in sorted monocyte subsets to identify the main TF producers. In addition, molecular gene analysis was performed in healthy individuals and gene expression might differ in HIV^+ individuals due to the trained immunity phenomenon (56). Our findings clearly indicate that classical proinflammatory monocytes are the major subset capable of robustly expressing TF protein and also upregulating TF functional activity upon LPS stimulation.

Previous studies have indicated that TF expression on monocytes is increased in HIV-infected individuals compared to healthy controls and that frequency of TF^{pos} cells is associated with HIV disease progression (18). Here we found that the frequency of TF^{pos} monocytes as well as TF functional activity was similar between ART-naïve HIV^+ individuals and those who achieved HIV suppression after ART initiation. These findings strongly indicate that TF expression by monocytes remains high in HIV^+ individuals regardless of ART-induced virological suppression, which could be associated with increased potential to activate the coagulation cascade and cause cardiovascular disease. Consistent with this concept, previous work has demonstrated a positive correlation between frequency of TF^{pos} monocytes and D-dimer levels in HIV^+ patients (18). We validated these findings in our analysis of ART-naive HIV^+ patients. Intriguingly, while D-dimer plasma levels were significantly reduced after ART-induced suppression of HIV viremia, the frequency of TF^{pos} monocytes remained elevated. It is plausible that some degree of coagulopathy persists despite the decreases in D-dimer and supports a more complex relationship between pro- and anti-coagulant factors in untreated HIV due to poor synthetic liver function (20). This also suggests that assessing TF expression on monocytes would increase sensitivity for the detection of coagulopathy. Our findings further reveal TF as a potential therapeutic target in ART-treated patients with evidence of coagulopathy.

The pathogenic role of coagulation during progressive SIVsab infection in PTMs has been established previously (25), and NHPs serve as an important HIV model that can be controlled for key variables, such as time of infection. In the present study, we observed that compared to AGMs, which experience active viral replication but no disease progression, PTMs have significantly increased frequency of TF-expressing monocytes as well as TF functional activity upon chronic SIVsab infection. Importantly, no differences were observed in TF expression and activity in vitro between PTMs and AGMs prior to SIV infection, suggesting that monocyte subsets from these two NHP species diverge drastically in response to chronic viral infection. These results also link the occurrence of SIV progression

and systemic coagulopathy with increased capacity of monocytes to produce TF upon activation.

Aside from their role in coagulation, monocytes are also important in inflammatory processes due to their production of cytokines. There is a growing body of evidence indicating that persistent inflammation is associated with increased mortality in HIV (23, 58). Our experiments assessing polyfunctionality of monocytes by means of production of IL-1β, IL-6 and TNF-α upon TLR activation in vitro clearly demonstrate that TF^{pos} cells are more frequently triple cytokine producers when compared to TF^{neg} monocytes in healthy individuals and in HIV^+ patients, as well as in chronically SIV-infected PTMs. These results suggest that the same monocyte subset that expresses TF upon activation may also be implicated in persistent inflammation by producing multiple cytokines. This idea was reinforced by our findings demonstrating an increased frequency of polyfunctional monocytes in PTMs compared to AGMs after SIVsab infection, but not in naïve animals. Thus, it is reasonable to propose that TF^{pos} cells may be critically implicated in promotion of systemic inflammation and coagulation associated with disease progression in chronic HIV and SIVsab infection (Figure S9).

Having identified a role of TF-expressing monocytes at the intersection of inflammation and coagulation, we hypothesized that interfering with TF could serve as a therapeutic approach to target hypercoagulation. Administration of Ixolaris in vivo decreased T cell activation as well as plasma IL-17 and D-dimer levels. Ixolaris produced no evidence of toxicity and was not associated with significant $CD4^+$ or SIVsab viremia changes. The results of the in vivo study are significant for two reasons. First, they point to a causal relationship between coagulation and immune activation and inflammation. Thus, not only did the Ixolaris treatment reduce inflammation, treatment also lowered monocyte and lymphocyte activation in the treated animals. The reduction of lymphocyte activation may be the direct result of reduced expression of the costimulatory markers CD80 and CD86 (two potent T cell activators) on monocytes. Alternatively, Ixolaris treatment could have impacted the levels of immune activation by directly reducing T cell expression of protease activator receptor 1 (PAR-1). Such a scenario is supported by our finding that Ixolaris treatment appears to have a stronger impact on the activation of $CD8^+$ T cells activation, as these cells express higher levels of PAR-1. In vitro assays showed that Ixolaris treatment did not diminish TF expression on monocytes in response to LPS, however these assays did not have the capacity to generate thrombin. Importantly, the in vivo data show reduction of monocyte activation after Ixolaris treatment, highlighting the importance of TF-generated thrombin on monocyte activation.

Our study limitations included the small sample size of our longitudinal cohort and the cross-sectional nature of many experiments. In addition, the in vivo Ixolaris administration study included only a small number of ART-naïve acutely infected animals, prohibiting a more detailed evaluation of possible toxicity, drug interaction, complications and potential survival benefit in untreated or treated animals.

The impact of anticoagulant therapy on immune activation and inflammation in SIVsab-infected PTMs demonstrates that hypercoagulation is a significant source of persistent

immune activation and inflammation in this model and probably in HIV-infected patients as well. Anticoagulant therapy by itself reduced important measurements of immune activation and inflammation and therefore has potential to improve the clinical management of HIV-infected patients. Second, we show that anticoagulant treatment improved the natural history of highly pathogenic SIVsab infection even in the absence of any other intervention aimed at either controlling viral replication (ART) or improving the health of the gut. Therefore, our study suggests that targeting the coagulation pathway in HIV-infected patients may be effective in reducing the immune activation and inflammation that are linked to cardiovascular comorbidities in HIV infection. These findings indicate that targeting TF may be employed as a host-directed therapy in chronic HIV infection as well as other inflammatory diseases with similar immune pathology.

Materials and Methods

Study Design

Ethics statements are detailed in the Supplementary Materials and Methods. Participants were prospectively enrolled in a clinical observational study to evaluate impact of ART in ART-naïve HIV-infected persons. The study objectives included the evaluation of phenotype and function of monocytes at different stages of HIV infection. Participants were selected retrospectively from a completed trial for evaluation of the monocyte function based on PBMC availability, compliance with ART and lack of aberrant immune responses or clinical course that would entail administration of medications that may affect coagulation such as corticosteroids or chemotherapy or other immune therapies.

For the in vivo NHP studies, 8 pigtailed macaques were infected with SIVsab. In PTMs, Ixolaris was administered starting from the day of the infection to assess its impact on key parameters of SIV infection (survival, viral replication, $CD4^+$ T cell counts and activation status, systemic inflammation and coagulation status). The study design involved assessment of the impact of Ixolaris on key variables of acute SIV infection, as this stage of infection is when differences in these parameters can reach statistical significance even in small animal groups. Also, the study design permitted not only comparisons between the animals in the Ixolaris-treated group and controls, but also in the same animals before and after SIVsab infection and Ixolaris treatment. Multiple samples collected at well defined key time points of SIVsab infection enabled these comparisons. Primary data are located in table S6.

Description of HIV-infected patients

Cross-sectional analysis was performed in HIV-infected individuals enrolled in protocols at the National Institutes of Health. Cryopreserved PBMC from 10 HIV^+ ART-naïve donors and 10 HIV^+ virally suppressed patients (median: 128 weeks on ART, IQR: 112-128 weeks) were used. The characteristics of the participants from the cross-sectional analysis are shown in Table S2. Prospective analysis was performed in HIV infected ART-naïve patients over the age of 18 with $CD4^+$ T-cell counts <100 cells/μL prior to therapy initiation. Cryopreserved PBMC samples were used from 15 patients pre and post ART initiation after HIV suppression (median: 160 weeks on ART, IQR: 96-192 weeks). The characteristics of

the participants from the prospective analysis are shown in Table S3. Blood from age- and gender-matched healthy donors was collected from the NIH blood bank.

Description of the Non-human Primates samples used for *in vitro* and *in vivo* studies

Cryopreserved PBMCs from six pigtailed macaques (PTMs) and six age-matched African green monkeys (AGMs) were studied from pre-SIVsab infection (SIV^-, naïve) and 72 days post-SIVsab infection (SIV^+). The characteristics of the animals before and after SIVsab infection are shown in Table S1. For the pre-clinical study in vivo, eight PTMs were intravenously infected with plasma equivalent to 300 tissue culture infectious doses (TCID50) of SIVsab strain BH66 (49). Ixolaris therapy (20 μg/kg, subcutaneously, daily) was initiated in five PTMs at the time of infection and was maintained for 80 days. Blood was collected from all animals twice prior to infection, twice a week for the first two weeks postinfection (p.i.), weekly during early chronic infection (up to ten weeks p.i.) and then monthly. The Ixolaris-treated group was closely monitored for signs of bleeding. Plasma viral RNA loads were quantified using quantitative real-time PCR specifically developed for SIVsab, as described previously (60, 61).

LPS stimulation

Cryopreserved PBMCs were thawed and resuspended in RPMI-1640 media supplemented with 10% human AB serum at 10^6 cells/well in 96-well plates. Cells were washed and resuspended in complete media with 5μg/mL brefeldin-A (Sigma-Aldrich, St. Louis, MO) and stimulated with indicated doses of ultrapure LPS (Sigma-Aldrich) in the presence or absence of purified Ixolaris (10nM) for 6 hours at 37°C in 5% CO_2. Cells were then stained following the flow cytometry assay described above.

In vitro experiments with HIV^+ and HCV^+ serum

Column-purified $CD14^+$ monocytes were obtained from healthy blood donors and resuspended in RPMI-1640 media supplemented with 10% of indicated serum (commercialized human AB serum or pooled 0.22μm filtered sera from 12 healthy controls, 20 ART-naïve HIV^+ patients and at week 12 and 48 of ART, and 16 HCV^+ persons) at 10^6 cells/well in 96-well plates. Cells were left overnight (18h) at 37°C in 5% CO_2. Cells were then washed and lysed for measurement of TF protein expression in cell lysates. In additional experiments, cells were incubated with 10μg/mL of anti-IL1R1 (polyclonal, R&D Systems), anti-IL6Ra (clone 17506, R&D Systems), anti-IFNγR (clone 92101, R&D Systems), anti-IFNAR (polyclonal, ab10719, Abcam) and anti-TNFR1 (clone 16803R, R&D Systems) for 1h before incubation with HIV^+ serum and persisted in cultures for additional 18h. HIV^+ serum was also treated with Polymyxin (0.5μg/μL, Sigma-Aldrich) for 1h and filtered before incubating for 18h.

Testing the efficacy of Ixolaris in inhibiting coagulation in NHP in vitro

The in vitro efficacy of Ixolaris was assessed by measuring its impact on clotting time on citrate plasma from SIV-naïve healthy pigtail macaques (PTMs). Results were compared to the in vitro testing of Ixolaris-treated plasma from healthy human subjects. Plasma samples from PTMs and humans were incubated with Ixolaris (0.5, 1, 1.5, 2, or 2.5 μg/ml) for two

minutes at 37°C. PT/INR and aPTT were then immediately measured. Testing was performed by ITxM Diagnotics.

Statistical Analysis

Median and interquartile ranges were used as measures of central tendency. All statistical comparisons were pre-specified and two-tailed. Differences with P-values < 0.05 were considered statistically significant. Data from continuous variables comparing two groups were analyzed using Wilcoxon matched-pairs, Mann-Whitney and Spearman rank correlation tests. Comparisons between more than two groups were performed using the Kruskal-Wallis test with Dunn's multiple comparisons *ad hoc* test. Nominal variables and the expression profile of cytokine production by monocytes were compared using the Fisher's exact test (two groups) or Chi-square test (more than 2 groups). Hierarchical cluster analysis (Ward's method with bootstrap 100×) was used to test if TF^{pos} and TF^{neg} monocytes could be clustered separately based on expression profile of selected proinflammatory genes measured by qPCR (after data was z-score normalized). A model of principal component analysis with an associated vector analysis were employed to visualize the distinction between TF^{pos} and TF^{neg} cells and the direction in which each marker influences mathematically the distribution of the data. These data analyses were performed using GraphPad Prism 6.0 (GraphPad Software Inc.) and JMP 11.0 (SAS) softwares. Additional description of statistical analyses is in Supplementary Materials and Methods.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References and Notes

1. Lauvau G, Chorro L, Spaulding E, Soudja SM. Inflammatory monocyte effector mechanisms. Cell Immunol. 2014; 291:32–40. [PubMed: 25205002]
2. Ginhoux F, Jung S. Monocytes and macrophages: developmental pathways and tissue homeostasis. Nat Rev Immunol. 2014; 14:392–404. [PubMed: 24854589]
3. Sandler NG, Wand H, Roque A, Law M, Nason MC, Nixon DE, Pedersen C, Ruxrungtham K, Lewin SR, Emery S, Neaton JD, Brenchley JM, Deeks SG, Sereti I, Douek DC. I. S. S. Group. Plasma levels of soluble CD14 independently predict mortality in HIV infection. J Infect Dis. 2011; 203:780–790. [PubMed: 21252259]
4. Baker JV, Hullsiek KH, Singh A, Wilson E, Henry K, Lichtenstein K, Onen N, Kojic E, Patel P, Brooks JT, Hodis HN, Budoff M, Sereti I. C. S. S. Investigators. Immunologic predictors of

coronary artery calcium progression in a contemporary HIV cohort. AIDS. 2014; 28:831–840. [PubMed: 24370480]

5. Wilson EM, Singh A, Hullsiek KH, Gibson D, Henry WK, Lichtenstein K, Onen NF, Kojic E, Patel P, Brooks JT, Sereti I, Baker JV. H. I. V. A. i. t. E. o. E. T. I. Study to Understand the Natural History of, Monocyte-Activation Phenotypes Are Associated With Biomarkers of Inflammation and Coagulation in Chronic HIV Infection. J Infect Dis. 2014; 210:1396–1406. [PubMed: 24813472]
6. McKibben RA, Margolick JB, Grinspoon S, Li X, Palella FJ Jr, Kingsley LA, Witt MD, George RT, Jacobson LP, Budoff M, Tracy RP, Brown TT, Post WS. Elevated levels of monocyte activation markers are associated with subclinical atherosclerosis in men with and those without HIV infection. J Infect Dis. 2015; 211:1219–1228. [PubMed: 25362192]
7. Andrade BB, Singh A, Narendran G, Schechter ME, Nayak K, Subramanian S, Anbalagan S, Jensen SM, Porter BO, Antonelli LR, Wilkinson KA, Wilkinson RJ, Meintjes G, van der Plas H, Follmann D, Barber DL, Swaminathan S, Sher A, Sereti I. Mycobacterial Antigen Driven Activation of CD14++CD16- Monocytes Is a Predictor of Tuberculosis-Associated Immune Reconstitution Inflammatory Syndrome. PLoS Pathog. 2014; 10:e1004433. [PubMed: 25275318]
8. Broze GJ Jr. Binding of human factor VII and VIIa to monocytes. J Clin Invest. 1982; 70:526–535. [PubMed: 6980899]
9. Osterud B, Bjorklid E. Tissue factor in blood cells and endothelial cells. Front Biosci (Elite Ed). 2012; 4:289–299. [PubMed: 22201871]
10. Ruf W. Protease-activated receptor signaling in the regulation of inflammation. Crit Care Med. 2004; 32:S287–292. [PubMed: 15118532]
11. Kothari H, Rao LV, Vankayalapati R, Pendurthi UR. Mycobacterium tuberculosis infection and tissue factor expression in macrophages. PLoS One. 2012; 7:e45700. [PubMed: 23029190]
12. Tsao BP, Fair DS, Curtiss LK, Edgington TS. Monocytes can be induced by lipopolysaccharide-triggered T lymphocytes to express functional factor VII/VIIa protease activity. J Exp Med. 1984; 159:1042–1057. [PubMed: 6368733]
13. Opal SM, Esmon CT. Bench-to-bedside review: functional relationships between coagulation and the innate immune response and their respective roles in the pathogenesis of sepsis. Crit Care. 2003; 7:23–38. [PubMed: 12617738]
14. Esmon CT. Does inflammation contribute to thrombotic events? Haemostasis. 2000; 30 Suppl 2:34–40. [PubMed: 11251339]
15. Levi M, van der Poll T, Buller HR. Bidirectional relation between inflammation and coagulation. Circulation. 2004; 109:2698–2704. [PubMed: 15184294]
16. Owens AP 3rd, Mackman N. Role of tissue factor in atherothrombosis. Curr Atheroscler Rep. 2012; 14:394–401. [PubMed: 22886473]
17. Pawelski H, Lang D, Reuter S. Interactions of monocytes and platelets: implication for life. Front Biosci (Schol Ed). 2014; 6:75–91. [PubMed: 24389263]
18. Funderburg NT, Mayne E, Sieg SF, Asaad R, Jiang W, Kalinowska M, Luciano AA, Stevens W, Rodriguez B, Brenchley JM, Douek DC, Lederman MM. Increased tissue factor expression on circulating monocytes in chronic HIV infection: relationship to in vivo coagulation and immune activation. Blood. 2010; 115:161–167. [PubMed: 19828697]
19. Funderburg NT, Lederman MM. Coagulation and morbidity in treated HIV infection. Thromb Res. 2014; 133 Suppl 1:S21–24. [PubMed: 24759134]
20. Baker JV, Brummel-Ziedins K, Neuhaus J, Duprez D, Cummins N, Dalmau D, DeHovitz J, Lehmann C, Sullivan A, Woolley I, Kuller L, Neaton JD, Tracy RP. I. S. S. Team. HIV replication alters the composition of extrinsic pathway coagulation factors and increases thrombin generation. J Am Heart Assoc. 2013; 2:e000264. [PubMed: 23896681]
21. Boulware DR, Hullsiek KH, Puronen CE, Rupert A, Baker JV, French MA, Bohjanen PR, Novak RM, Neaton JD, Sereti I. I. S. Group. Higher levels of CRP, D-dimer, IL-6, and hyaluronic acid before initiation of antiretroviral therapy (ART) are associated with increased risk of AIDS or death. J Infect Dis. 2011; 203:1637–1646. [PubMed: 21592994]
22. Duprez DA, Neuhaus J, Kuller LH, Tracy R, Belloso W, De Wit S, Drummond F, Lane HC, Ledergerber B, Lundgren J, Nixon D, Paton NI, Prineas RJ, Neaton JD. I. S. S. Group.

Inflammation, coagulation and cardiovascular disease in HIV-infected individuals. PLoS One. 2012; 7:e44454. [PubMed: 22970224]

23. Kuller LH, Tracy R, Belloso W, De Wit S, Drummond F, Lane HC, Ledergerber B, Lundgren J, Neuhaus J, Nixon D, Paton NI, Neaton JD. I. S. S. Group. Inflammatory and coagulation biomarkers and mortality in patients with HIV infection. PLoS Med. 2008; 5:e203. [PubMed: 18942885]
24. High KP, Brennan-Ing M, Clifford DB, Cohen MH, Currier J, Deeks SG, Deren S, Effros RB, Gebo K, Goronzy JJ, Justice AC, Landay A, Levin J, Miotti PG, Munk RJ, Nass H, Rinaldo CR Jr, Shlipak MG, Tracy R, Valcour V, Vance DE, Walston JD, Volberding P. O. A. R. W. G. o. HIV, Aging. HIV and aging: state of knowledge and areas of critical need for research. A report to the NIH Office of AIDS Research by the HIV and Aging Working Group. J Acquir Immune Defic Syndr. 2012; 60 Suppl 1:S1–18. [PubMed: 22688010]
25. Pandrea I, Cornell E, Wilson C, Ribeiro RM, Ma D, Kristoff J, Xu C, Haret-Richter GS, Trichel A, Apetrei C, Landay A, Tracy R. Coagulation biomarkers predict disease progression in SIV-infected nonhuman primates. Blood. 2012; 120:1357–1366. [PubMed: 22653975]
26. Grage-Griebenow E, Flad HD, Ernst M. Heterogeneity of human peripheral blood monocyte subsets. J Leukoc Biol. 2001; 69:11–20. [PubMed: 11200054]
27. Francischetti IM, Gordon E, Bizzarro B, Gera N, Andrade BB, Oliveira F, Ma D, Assumpcao TC, Ribeiro JM, Pena M, Qi CF, Diouf A, Moretz SE, Long CA, Ackerman HC, Pierce SK, Sa-Nunes A, Waisberg M. Tempol, an intracellular antioxidant, inhibits tissue factor expression, attenuates dendritic cell function, and is partially protective in a murine model of cerebral malaria. PLoS One. 2014; 9:e87140. [PubMed: 24586264]
28. Funderburg NT. Markers of coagulation and inflammation often remain elevated in ART-treated HIV-infected patients. Curr Opin HIV AIDS. 2014; 9:80–86. [PubMed: 24275673]
29. Ma D, Jasinska A, Kristoff J, Grobler JP, Turner T, Jung Y, Schmitt C, Raehtz K, Feyertag F, Martinez Sosa N, Wijewardana V, Burke DS, Robertson DL, Tracy R, Pandrea I, Freimer N, Apetrei C. C. The International Vervet Research. SIVagm Infection in Wild African Green Monkeys from South Africa: Epidemiology, Natural History, and Evolutionary Considerations. PLoS Pathog. 2013; 9:e1003011. [PubMed: 23349627]
30. Ma D, Jasinska AJ, Feyertag F, Wijewardana V, Kristoff J, He T, Raehtz K, Schmitt CA, Jung Y, Cramer JD, Dione M, Antonio M, Tracy R, Turner T, Robertson DL, Pandrea I, Freimer N, Apetrei C. Factors Associated with Siman Immunodeficiency Virus Transmission in a Natural African Nonhuman Primate Host in the Wild. Journal of Virology. 2014; 88:5687–5705. [PubMed: 24623416]
31. Marchetti G, Tincati C, Silvestri G. Microbial translocation in the pathogenesis of HIV infection and AIDS. Clin Microbiol Rev. 2013; 26:2–18. [PubMed: 23297256]
32. Neuhaus J, Jacobs DR Jr, Baker JV, Calmy A, Duprez D, La Rosa A, Kuller LH, Pett SL, Ristola M, Ross MJ, Shlipak MG, Tracy R, Neaton JD. Markers of inflammation, coagulation, and renal function are elevated in adults with HIV infection. J Infect Dis. 2010; 201:1788–1795. [PubMed: 20446848]
33. Kalz J, ten Cate H, Spronk HM. Thrombin generation and atherosclerosis. J Thromb Thrombolysis. 2014; 37:45–55. [PubMed: 24241912]
34. Shpacovitch V, Feld M, Hollenberg MD, Luger TA, Steinhoff M. Role of protease-activated receptors in inflammatory responses, innate and adaptive immunity. J Leukoc Biol. 2008; 83:1309–1322. [PubMed: 18347074]
35. Colognato R, Slupsky JR, Jendrach M, Burysek L, Syrovets T, Simmet T. Differential expression and regulation of protease-activated receptors in human peripheral monocytes and monocyte-derived antigen-presenting cells. Blood. 2003; 102:2645–2652. [PubMed: 12805069]
36. Lopez ML, Bruges G, Crespo G, Salazar V, Deglesne PA, Schneider H, Cabrera-Fuentes H, Schmitz ML, Preissner KT. Thrombin selectively induces transcription of genes in human monocytes involved in inflammation and wound healing. Thromb Haemost. 2014; 112:992–1001. [PubMed: 25057055]
37. Coughlin SR. Protease-activated receptors in hemostasis, thrombosis and vascular biology. J Thromb Haemost. 2005; 3:1800–1814. [PubMed: 16102047]

38. Francischetti IM, Valenzuela JG, Andersen JF, Mather TN, Ribeiro JM. Ixolaris, a novel recombinant tissue factor pathway inhibitor (TFPI) from the salivary gland of the tick, Ixodes scapularis: identification of factor X and factor Xa as scaffolds for the inhibition of factor VIIa/tissue factor complex. Blood. 2002; 99:3602–3612. [PubMed: 11986214]
39. Carneiro-Lobo TC, Schaffner F, Disse J, Ostergaard H, Francischetti IM, Monteiro RQ, Ruf W. The tick-derived inhibitor Ixolaris prevents tissue factor signaling on tumor cells. J Thromb Haemost. 2012; 10:1849–1858. [PubMed: 22823596]
40. Nazareth RA, Tomaz LS, Ortiz-Costa S, Atella GC, Ribeiro JM, Francischetti IM, Monteiro RQ. Antithrombotic properties of Ixolaris, a potent inhibitor of the extrinsic pathway of the coagulation cascade. Thromb Haemost. 2006; 96:7–13. [PubMed: 16807644]
41. Fahey JL. Cytokines, plasma immune activation markers, and clinically relevant surrogate markers in human immunodeficiency virus infection. Clin Diagn Lab Immunol. 1998; 5:597–603. [PubMed: 9729522]
42. Hunt PW. HIV and inflammation: mechanisms and consequences. Curr HIV/AIDS Rep. 2012; 9:139–147. [PubMed: 22528766]
43. Butterfield TR, Hanna DB, Kaplan RC, Kizer JR, Durkin HG, Young MA, Nowicki MJ, Tien PC, Golub ET, Floris-Moore MA, Titanji K, Fischl MA, Heath SL, Martinson J, Crowe SM, Palmer CS, Landay AL, Anzinger JJ. Increased glucose transporter-1 expression on intermediate monocytes from HIV-infected women with subclinical cardiovascular disease. AIDS. 2017; 31:199–205. [PubMed: 27835618]
44. Freemerman AJ, Johnson AR, Sacks GN, Milner JJ, Kirk EL, Troester MA, Macintyre AN, Goraksha-Hicks P, Rathmell JC, Makowski L. Metabolic reprogramming of macrophages: glucose transporter 1 (GLUT1)-mediated glucose metabolism drives a proinflammatory phenotype. J Biol Chem. 2014; 289:7884–7896. [PubMed: 24492615]
45. Palmer CS, Anzinger JJ, Zhou J, Gouillou M, Landay A, Jaworowski A, McCune JM, Crowe SM. Glucose transporter 1-expressing proinflammatory monocytes are elevated in combination antiretroviral therapy-treated and untreated HIV+ subjects. J Immunol. 2014; 193:5595–5603. [PubMed: 25367121]
46. Boulware DR, Hullsiek KH, Puronen CE, Rupert A, Baker JV, French MA, Bohjanen PR, Novak RM, Neaton JD, Sereti I. Higher levels of CRP, D-dimer, IL-6, and hyaluronic acid before initiation of antiretroviral therapy (ART) are associated with increased risk of AIDS or death. J Infect Dis. 2011; 203:1637–1646. [PubMed: 21592994]
47. Pandrea I, Landay A, Wilson C, Stock J, Tracy R, Apetrei C. Using the pathogenic and nonpathogenic nonhuman primate model for studying non-AIDS comorbidities. Curr HIV/AIDS Rep. 2015; 12:54–67. [PubMed: 25604236]
48. Pandrea I, Apetrei C. Where the wild things are: pathogenesis of SIV infection in African nonhuman primate hosts. Curr HIV/AIDS Rep. 2010; 7:28–36. [PubMed: 20425055]
49. Mandell DT, Kristoff J, Gaufin T, Gautam R, Ma D, Sandler N, Haret-Richter G, Xu C, Aamer H, Dufour J, Trichel A, Douek DC, Keele BF, Apetrei C, Pandrea I. Pathogenic features associated with increased virulence upon Simian immunodeficiency virus cross-species transmission from natural hosts. J Virol. 2014; 88:6778–6792. [PubMed: 24696477]
50. Pandrea I, Xu C, Stock JL, Frank DN, Ma D, Policicchio BB, He T, Kristoff J, Cornell E, Haret-Richter GS, Trichel A, Ribeiro RM, Tracy R, Wilson C, Landay AL, Apetrei C. Antibiotic and Antiinflammatory Therapy Transiently Reduces Inflammation and Hypercoagulation in Acutely SIV-Infected Pigtailed Macaques. PLoS Pathog. 2016; 12:e1005384. [PubMed: 26764484]
51. Borges AH, Silverberg MJ, Wentworth D, Grulich AE, Fatkenheuer G, Mitsuyasu R, Tambussi G, Sabin CA, Neaton JD, Lundgren JD. Insight, Esprit, S. S. Groups. Predicting risk of cancer during HIV infection: the role of inflammatory and coagulation biomarkers. AIDS. 2013; 27:1433–1441. [PubMed: 23945504]
52. Baker JV, Neuhaus J, Duprez D, Kuller LH, Tracy R, Belloso WH, De Wit S, Drummond F, Lane HC, Ledergerber B, Lundgren J, Nixon DE, Paton NI, Neaton JD. I. S. S. Group. Changes in inflammatory and coagulation biomarkers: a randomized comparison of immediate versus deferred antiretroviral therapy in patients with HIV infection. J Acquir Immune Defic Syndr. 2011; 56:36–43. [PubMed: 20930640]

53. Ghattas A, Griffiths HR, Devitt A, Lip GY, Shantsila E. Monocytes in coronary artery disease and atherosclerosis: where are we now? J Am Coll Cardiol. 2013; 62:1541–1551. [PubMed: 23973684]
54. Rogacev KS, Cremers B, Zawada AM, Seiler S, Binder N, Ege P, Grosse-Dunker G, Heisel I, Hornof F, Jeken J, Rebling NM, Ulrich C, Scheller B, Bohm M, Fliser D, Heine GH. CD14+ +CD16+ monocytes independently predict cardiovascular events: a cohort study of 951 patients referred for elective coronary angiography. J Am Coll Cardiol. 2012; 60:1512–1520. [PubMed: 22999728]
55. Funderburg NT, Zidar DA, Shive C, Lioi A, Mudd J, Musselwhite LW, Simon DI, Costa MA, Rodriguez B, Sieg SF, Lederman MM. Shared monocyte subset phenotypes in HIV-1 infection and in uninfected subjects with acute coronary syndrome. Blood. 2012; 120:4599–4608. [PubMed: 23065151]
56. Netea MG, Joosten LA, Latz E, Mills KH, Natoli G, Stunnenberg HG, O'Neill LA, Xavier RJ. Trained immunity: A program of innate immune memory in health and disease. Science. 2016; 352:aaf1098. [PubMed: 27102489]
57. Kristoff J, Haret-Richter G, Ma D, Ribeiro RM, Xu C, Cornell E, Stock JL, He T, Mobley AD, Ross S, Trichel A, Wilson C, Tracy R, Landay A, Apetrei C, Pandrea I. Early microbial translocation blockade reduces SIV-mediated inflammation and viral replication. J Clin Invest. 2014; 124:2802–2806. [PubMed: 24837437]
58. Tenorio AR, Zheng Y, Bosch RJ, Krishnan S, Rodriguez B, Hunt PW, Plants J, Seth A, Wilson CC, Deeks SG, Lederman MM, Landay AL. Soluble markers of inflammation and coagulation but not T-cell activation predict non-AIDS-defining morbid events during suppressive antiretroviral treatment. J Infect Dis. 2014; 210:1248–1259. [PubMed: 24795473]
59. Monteiro RQ, Rezaie AR, Ribeiro JM, Francischetti IM. Ixolaris: a factor Xa heparin-binding exosite inhibitor. Biochem J. 2005; 387:871–877. [PubMed: 15617517]
60. Pandrea I, Apetrei C, Dufour J, Dillon N, Barbercheck J, Metzger M, Jacquelin B, Bohm R, Marx PA, Barre-Sinoussi F, Hirsch VM, Muller-Trutwin MC, Lackner AA, Veazey RS. Simian immunodeficiency virus SIVagm.sab infection of Caribbean African green monkeys: a new model for the study of SIV pathogenesis in natural hosts. J Virol. 2006; 80:4858–4867. [PubMed: 16641277]
61. Pandrea I, Gaufin T, Gautam R, Kristoff J, Mandell D, Montefiori D, Keele BF, Ribeiro RM, Veazey RS, Apetrei C. Functional cure of SIVagm infection in rhesus macaques results in complete recovery of CD4+ T cells and is reverted by CD8+ cell depletion. PLoS Pathog. 2011; 7:e1002170. [PubMed: 21829366]
62. Pinheiro, J., Bated, D. Mixed-effects models in S and S-plus. Springer-Verlag, Ed.; New York: 2002.
63. Shi X, Gangadharan B, Brass LF, Ruf W, Mueller BM. Protease-activated receptors (PAR1 and PAR2) contribute to tumor cell motility and metastasis. Mol Cancer Res. 2004; 2:395–402. [PubMed: 15280447]
64. Francischetti IM, Oliveira CJ, Ostera GR, Yager SB, Debierre-Grockiego F, Carregaro V, Jaramillo-Gutierrez G, Hume JC, Jiang L, Moretz SE, Lin CK, Ribeiro JM, Long CA, Vickers BK, Schwarz RT, Seydel KB, Iacobelli M, Ackerman HC, Srinivasan P, Gomes RB, Wang X, Monteiro RQ, Kotsyfakis M, Sa-Nunes A, Waisberg M. Defibrotide interferes with several steps of the coagulation-inflammation cycle and exhibits therapeutic potential to treat severe malaria. Arterioscler Thromb Vasc Biol. 2012; 32:786–798. [PubMed: 22116094]

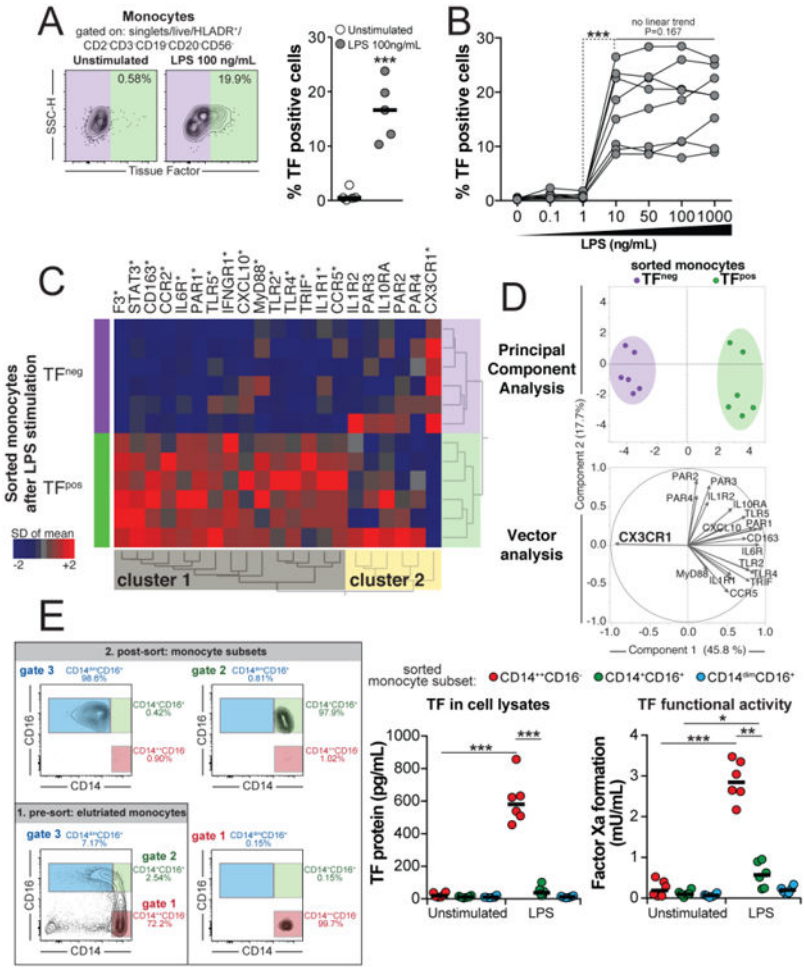

Fig. 1. LPS drives TF expression on human monocyte subsets

(A) Left panel shows representative FACS plots of tissue factor (TF) expression on human monocytes from healthy controls upon LPS stimulation in vitro. Right panel shows summary data (n=5) of frequency of TF positive monocytes. Lines represent median values. Data were analyzed using Mann-Whitney test. **(B)** Frequency of TF-expressing monocytes on PBMCs from healthy controls stimulated with increasing doses of LPS in vitro (n=8). Data were analyzed using Kruskal-Wallis test with Dunn's multiple comparisons and linear trend *ad hoc* test. **(C)** Hierarchical cluster analysis of the expression profile (z-score normalized) of indicated genes assessed by qPCR in monocytes (n=6 healthy donors) sorted after 6h of LPS stimulation (100ng/mL) as described in Methods. **(D)** Principal Component Analysis of the expression level of indicated genes was performed. **(E)** Different monocyte subsets were sorted based on surface expression of CD14 and CD16 (n=6 healthy donors). Representative plots show monocytes pre and post sorting (left panel). TF protein expression in cell lysates and TF functional activity measured by formation of Factor Xa *in vitro* were compared between the different monocyte subsets in vitro using Kruskal-Wallis test with Dunn's multiple comparisons post test. Lines represent median values * P<0.05, ** P<0.01, *** P<0.001.

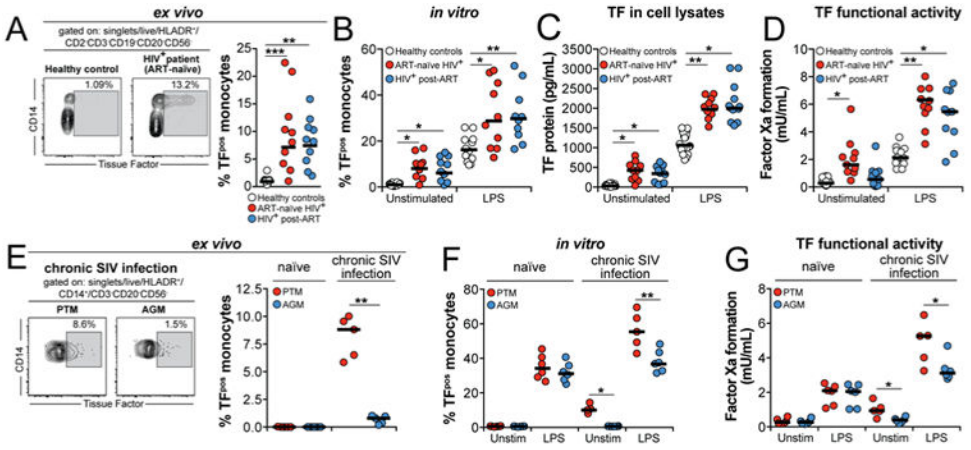

Fig. 2. Frequency of TF- expressing monocytes is increased in chronically HIV infected individuals and in SIV infected non-human primates despite virological suppression status

(A) Left panel shows representative plots of TF positive (TF^{pos}) monocytes ex vivo in healthy controls and HIV^+ patients. Right panel shows summary data of frequency of TF^{pos} monocytes from a cross-sectional analysis including healthy controls (n=12), ART-naïve HIV^+ patients (n=10) and HIV^+ individuals post-ART induced virological suppression (HIV^+ post-ART; n=10). PBMCs were stimulated with LPS in vitro and frequency of TF^{pos} monocytes **(B)** as well as TF protein expression in cell lysates **(C)** and TF functional activity **(D)** were compared between the cross-sectional study groups using Kruskal-Wallis test with Dunn's multiple comparisons post test. **(E)** Left panel shows representative plots of TF expression on monocytes ex vivo in chronically SIV-infected PTMs (n=6) and AGMs (n=6). Right panel shows summary data of frequency of TF^{pos} monocytes ex vivo from both naïve and chronically SIV-infected animals. PMBCs were stimulated with LPS in vitro and frequency of TF^{pos} monocytes **(F)** and TF functional activity **(G)** were compared between naïve or SIV-infected PTMs and AGMs. Lines represent median values. Data were analyzed using Mann-Whitney test. * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

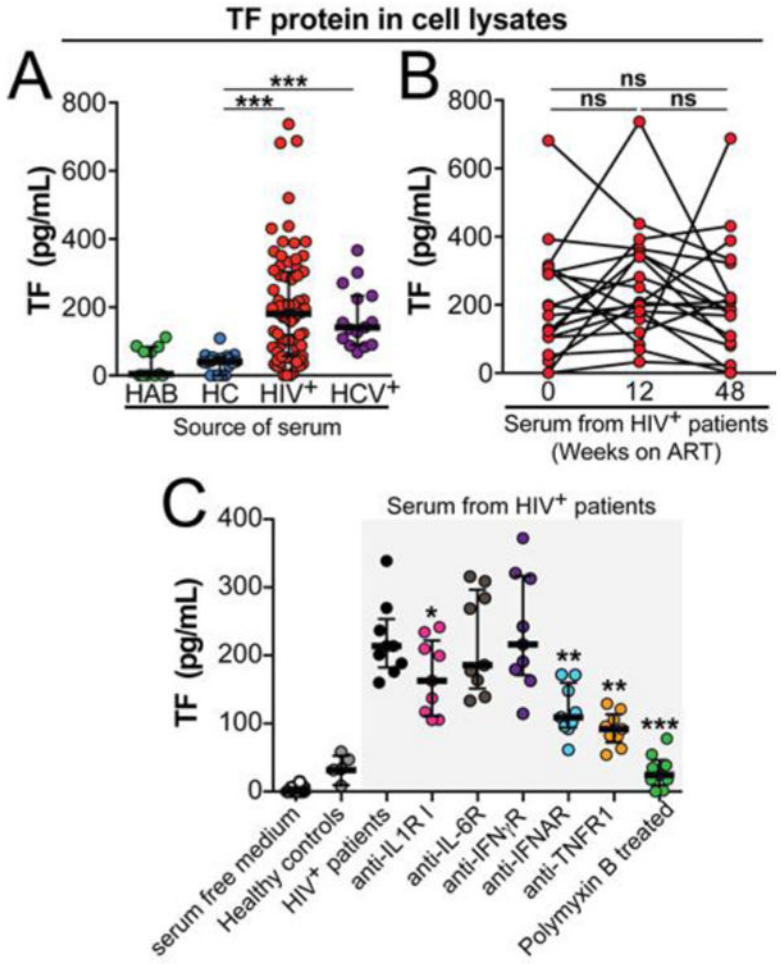

Fig. 3. Inflammatory mediators and microbial products may drive TF expression by circulating monocytes in the context of HIV infection (A)

Column-purified $CD14^+$ monocytes from 10 healthy controls were cultured for 18h in the presence of RPMI medium supplemented with 10% manufactured human AB serum, or serum isolated from healthy controls, ART-naïve HIV^+ patients or HCV^+ individuals as described in Methods. (**B**) Monocytes were also cultured in the presence of serum from ART-naïve HIV-infected patients at different time points after ART initiation. (**C**) Monocytes were cultured for 18h in the presence of indicated blocking antibodies (10μg/mL) or with serum previously treated with polymyxin B (0.5μg/μL). Cells were washed and lysed for assessment of TF protein expression using ELISA. Unmatched data were analyzed using Mann Whitney *U* test whereas matched pairs were compared using Wilcoxon matched pairs test. * P<0.05, ** P<0.01, *** P<0.001. ns, nonsignificant.

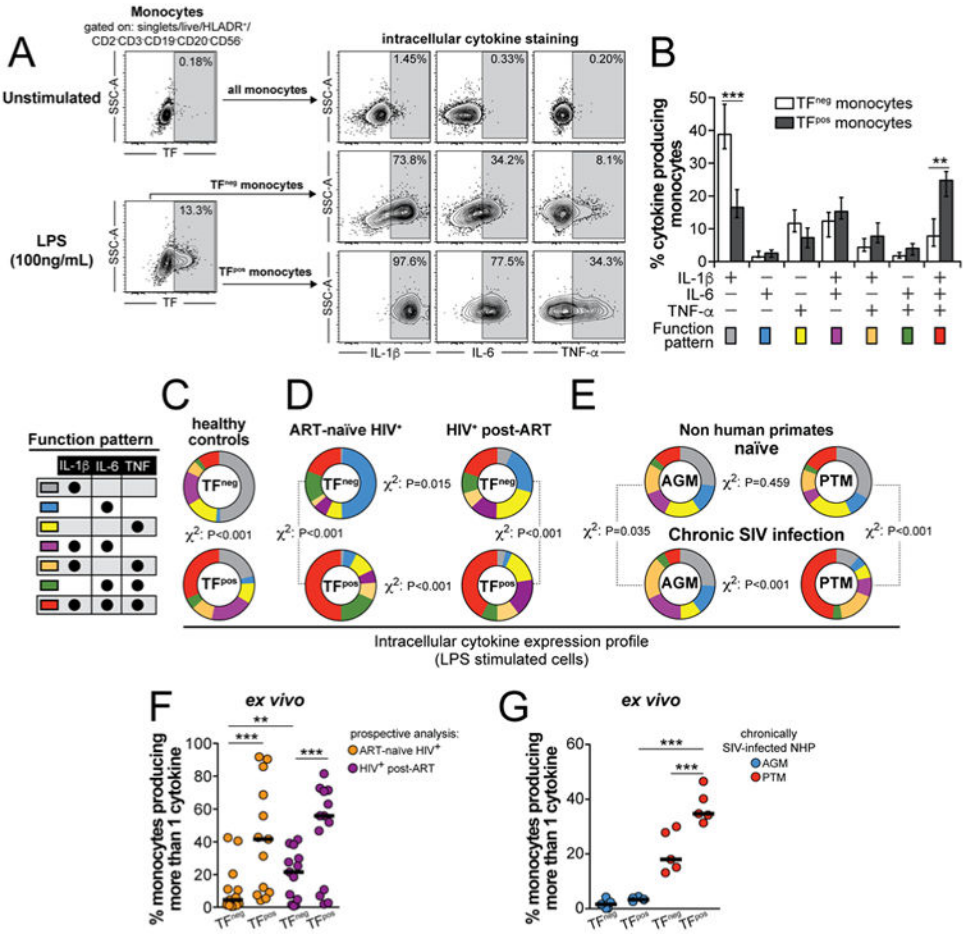

Fig. 4. TF-expressing monocytes produce multiple pro-inflammatory cytokines

(A) Representative plots show intracellular cytokine staining for IL-1β, IL-6 and TNF-α in monocytes from healthy donors (n=12) upon LPS stimulation in vitro. **(B)** Polyfunctional analysis of TF^{neg} and TF^{pos} monocytes upon LPS stimulation. **(C-E)** The cytokine expression profiles in TF^{neg} and TF^{pos} monocytes were compared using Chi-square tests. **(F)** Frequency of monocytes producing more than one cytokine ex vivo was compared between TF^{neg} and TF^{pos} monocytes in a prospective cohort of ART-naïve HIV^{+} patients (n=15) before therapy initiation and after ART-induced virological suppression (HIV^{+} post-ART). **(G)** Frequency of monocytes producing more than one cytokine ex vivo was compared between TF^{neg} and TF^{pos} monocytes in chronically SIV-infected PTMs (n=6) and AGMs (n=6). Unmatched data were compared using the Mann-Whitney test whereas matched comparisons were performed using the Wilcoxon matched pairs test. ** P<0.01, *** P<0.001.

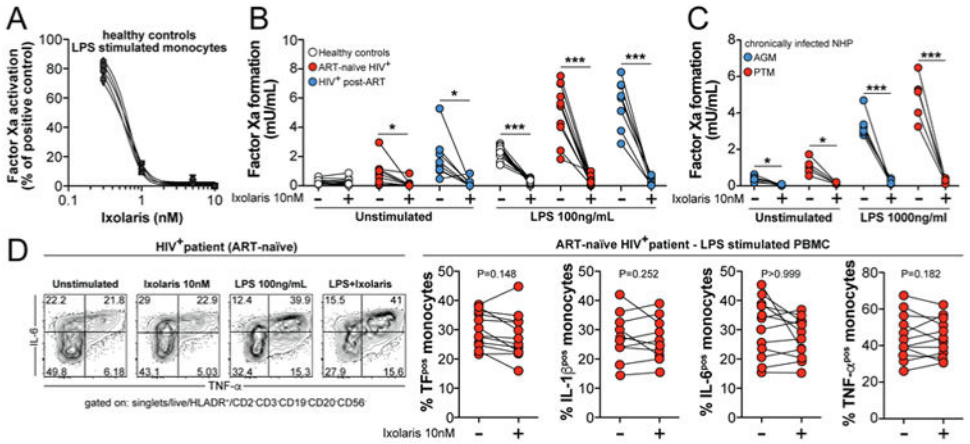

Fig. 5. Ixolaris potently blocks TF activity but not protein expression in activated monocytes from HIV$^+$ patients and chronically SIV-infected NHPs

(A) Tissue factor functional activity in vitro measured by Factor Xa formation in elutriated monocyte cultures from healthy donors stimulated with LPS and treated with indicated doses of Ixolaris (n=5). Data represent percentage of the positive control (LPS 1μg/ml without Ixolaris). TF activity upon treatment with Ixolaris was compared between **(B)** healthy controls (n=12) and unmatched ART-naïve HIV$^+$ (n=10) patients and those who achieved ART-induced virological suppression (post-ART; n=10) as well as between **(C)** chronically SIV-infected PTMs (n=6) and AGMs (n=6). **(D)** Representative plots of intracellular IL-6 and TNF-α production in monocytes from ART-naïve HIV$^+$ individuals stimulated in the presence or absence of Ixolaris (left panel). Summary data showing frequency of TF^{pos} monocytes as well as percent of cytokine producing monocytes in stimulated cultures treated or non-treated with Ixolaris (right panel). Data were analyzed using the Wilcoxon matched pairs test. * P<0.05, *** P<0.001.

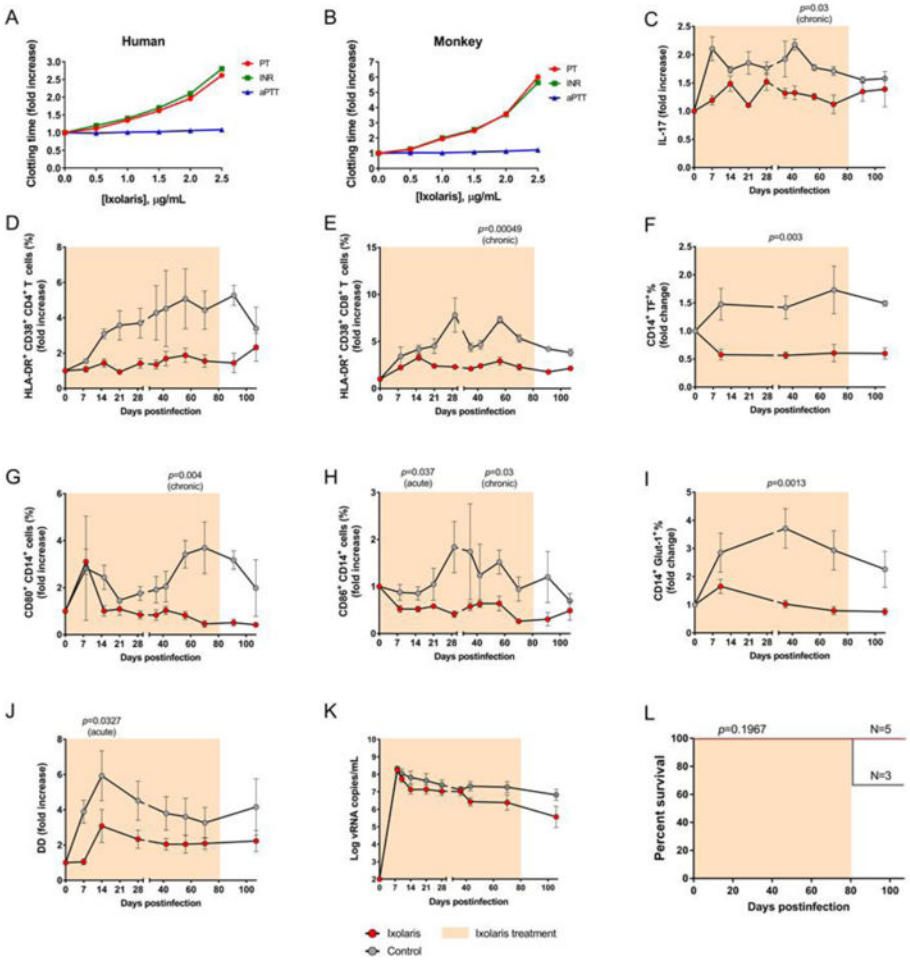

Fig. 6. Anticoagulant treatment positively impacts the immune activation and systemic inflammation of highly pathogenic SIVsab infection in PTMs

(A) Dose-dependent dynamics of PT/INR and aPTT after Ixolaris addition to plasma isolated from uninfected human subjects. **(B)** Dose-dependent dynamics of PT/INR and aPTT after Ixolaris addition to plasma isolated from uninfected PTMs. **(C)** Dynamics of the pro-inflammatory cytokine IL-17 in untreated vs Ixolaris-treated SIVsab-infected PTMs assessed by Luminex. Dynamics of activated double positive HLA-DR$^+$/CD38$^+$ CD4$^+$ **(D)** and CD8$^+$ T cells **(E)** in untreated vs Ixolaris-treated SIVsab-infected PTMs assessed by flow cytometry. Dynamics of TF expression **(F)** on CD14$^+$ monocytes in untreated vs Ixolaris-treated SIVsab-infected PTMs assessed by flow cytometry, as well as CD80 **(G)**, CD86 **(H)** and Glut-1 **(I)** expression on CD14$^+$ monocytes in untreated vs Ixolaris-treated SIVsab-infected PTMs assessed by flow cytometry. **(J)** Dynamics of D-Dimer in untreated vs Ixolaris-treated SIVsab-infected PTMs assessed by an immunoturbidimetric assay. **(K)** Dynamics of plasma SIVsab viremia in untreated vs Ixolaris-treated SIVsab-infected PTMs assessed by a real-time PCR assay. **(L)** Survival in untreated vs Ixolaris-treated SIVsab-infected PTMs. Statistical analyses were performed with grouping of acute vs chronic infection time points as described in Methods, except for TF and Glut-1 for which the

statistical analyses were done on whole dynamics, due to limited availability of samples for flow staining. Survival analysis was performed using the Mantel-Cox test.