# Infection by *Mycobacterium tuberculosis* promotes human macrophage immunothrombosis through tissue factor expression

Seán Donohue[1, 2], Anjali Yennemadi[1], Faye Murphy[1], Gina Leisching[1], Joseph Keane[1,3]
1. Trinity Translational Medicine Institute, St James's Hospital, Trinity College Dublin, 2. Department of Infectious Diseases, St James's Hospital, Dublin, 3. Department of Respiratory Medicine, St James's Hospital, Dublin
Correspondance: sdonohue@tcd.ie

## Background

Immunothrombosis drives microthrombi formation at the site of infection. This may help the host reponse by containing the pathogen, but in some infections can become dysregulated and contribute to disease severity and pathology[1]. Microthrombosis is evident in postmortem lung specimens of patients who have died from tuberculosis (TB)[2] but little else is known about immunothrombosis in TB. Tissue Factor (TF) is a key mediator of immunothrombosis by activating the extrinsic pathway of coagulation, and tissue factor pathway inhibitor (TFPI) is its natural inhibitor[1]. Type 1 interferons have been implicated as initiators of the TF pathway in other infections, leading to thrombosis[3].

## Aims

1. Assess if macrophage stimulation with *Mycobacterium tuberculosis* (Mtb) results in TF expression
2. Determine if type 1 interferons/IFN pathway mediates TF expression in Mtb-stimulated macrophages

## Methods

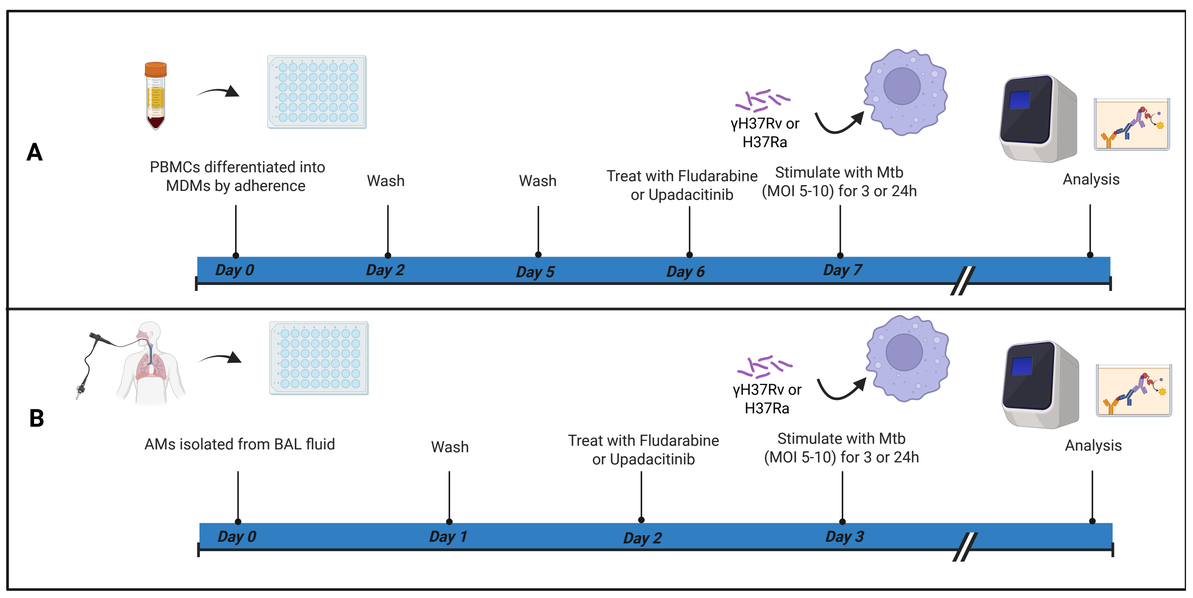


**Figure 1.** ***A,*** Human monocyte-derived macrophages (MDM) were isolated from buffy coats of whole blood from anonymous healthy donors using density gradient centrifugation and adherence to plastic.Cells were washed on days 2 and 5, and pretreated for 24h with either 50μM fludarabine (STAT1 inhibtor) or 45nM upadacitinib (JAK1 inhibitor). On day 7, MDMs were treated with irradiated (γH37Rv) or avirulent (H37Ra) Mtb (MOI 5-10) for 3 or 24 hours. qPCR was performed on cDNA synthesised from RNA extracted from the cells, FXa assay on whole cells, and ELISA was performed on cell lysates. ***B,*** human alveolar macrophages (AM) were retrieved from bronchoalveolar lavage fluid of patients undergoing clinically-indicated bronchoscopy. AMs were washed on day 1. Pretreatment with the same inhibitors took place on day 2 and stimulation as above on day 3. qPCR and ELISA were carried out in the same fashion as above.

## Results

**Mtb elaborates a prothrombotic phenotype in human macrophages**

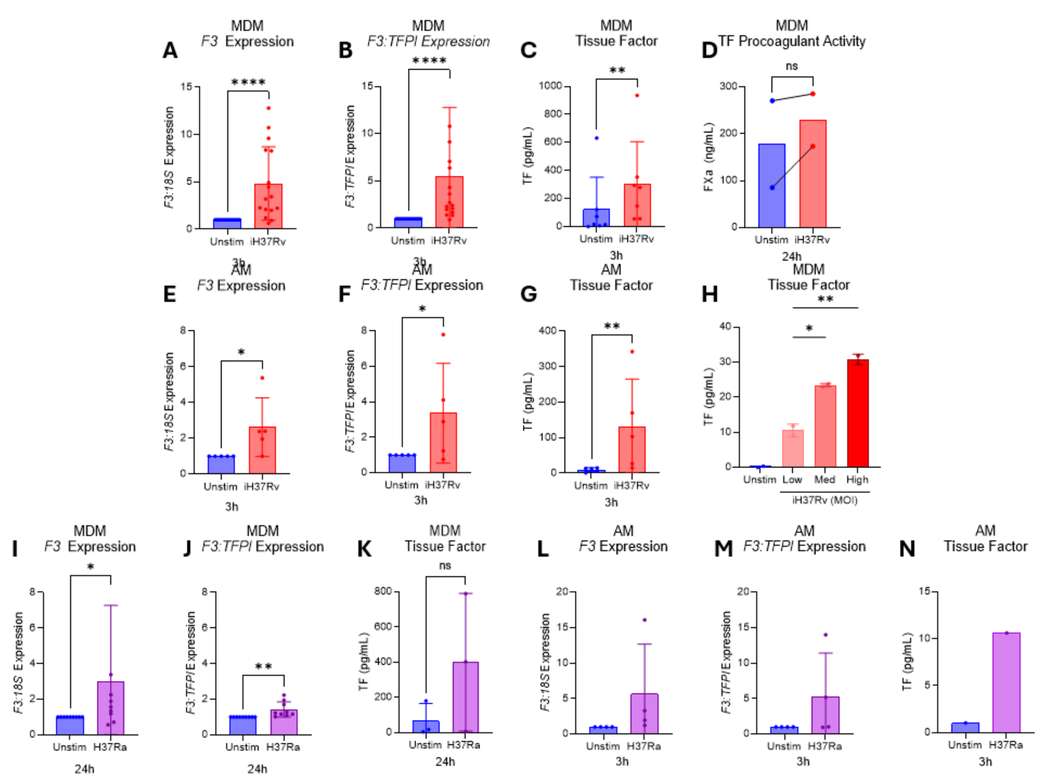


**Figure 2. Mtb stimulation increases Tissue Factor expression in human macrophages.** MDMs and AMs were left unstimulated or exposed to γ-irradiated *M. tuberculosis* strain H37Rv (A-H) or H37Ra (I-N). *F3* gene expression relative to 18S and *F3:TFPI* ratio were measured by qPCR (A-B, E-F, H, I-J, M-N). TF protein in cell lysates was measured by ELISA (C, G, H, K, N), and TF procoagulant activity was assessed by FXa generation assay (D). A dose response to increasing iH37Rv MOI was assessed in MDMs (H). Data are shown as individual donors with mean ± SD. Statistical significance was determined by paired t-test; * $p$ <0.05, ** $p$ <0.01, *** $p$ <0.001, **** $p$ <0.0001, ns not significant.

## Results

**Mtb-induced Tissue Factor expression is STAT1-dependent in MDMs but not in AMs**

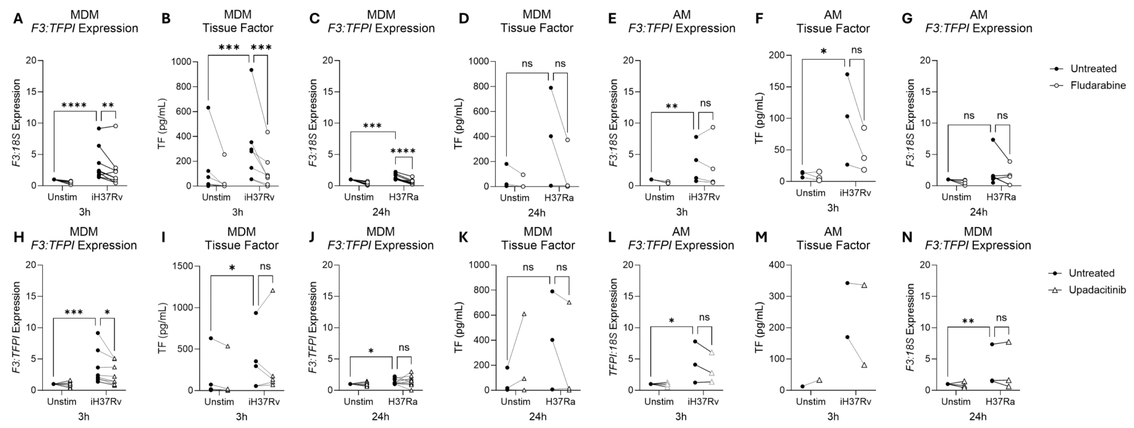


**Figure 3. Mtb-induced TF expression is STAT1-dependent in MDMs but not in AMs.** MDMs (A-D, H-K) and AMs (E-G, L-N) were left untreated or pre-treated with for 24 h with STAT1 inhibitor (50 μM fludarabine, A-G) or JAK1 inhibitor (45 nM upadacitinib, H-N) before stimulation with iH37Rv or H37Ra (MOI 5–10) for 3 or 24 h. *F3* gene expression relative to 18S and *F3:TFPI* ratio were measured by qPCR (A, C, E, G, H, J, K, N). TF protein in cell lysates was measured by ELISA (B, D, F, I, K, M). Data are shown as individual donors with mean ± SD. Statistical significance was determined by Two-Way ANOVA; * $p$ <0.05, ** $p$ <0.01, *** $p$ <0.001, **** $p$ <0.0001, ns not significant.

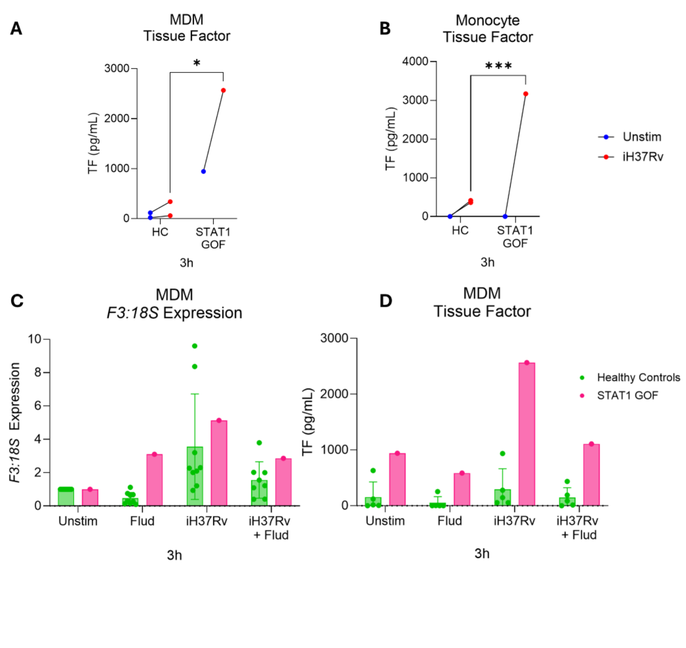


**Figure 4. STAT1 gain-of-function mutation enhances Mtb-induced TF expression.** MDMs derived from healthy controls (HC) or STAT1 gain-of-function (GOF) patients were left unstimulated or exposed to iH37Rv with or without fludarabine pre-treatment. *F3* gene expression relative to 18S was measured by qPCR (C). TF protein in cell lysates was measured by ELISA (A, B, D). TF production was also assessed in monocytes from HC and STAT1 GOF donors following stimulation with iH37Rv. Statistical significance was determined by Two-Way ANOVA; * $p$ <0.05, ** $p$ <0.01, *** $p$ <0.001, **** $p$ <0.0001.

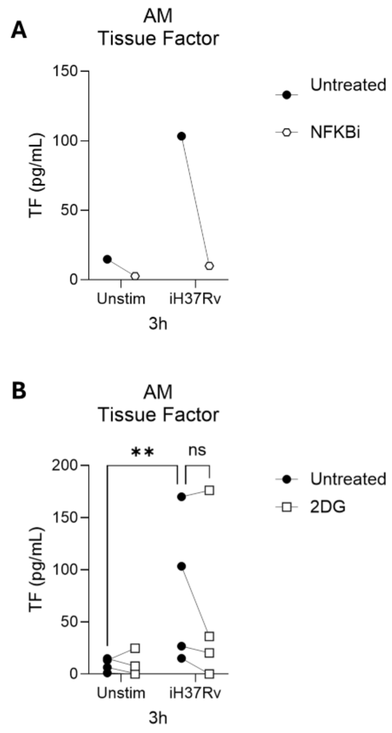


**Figure 5. Glycolitic and NFkB inhibition may reduce TF expression in AMs.** AMs were left untreated or pre-treated with an NFkB inhibitor (A) or 2-deoxyglucose (2DG)(B) prior to stimulation with iH37Rv. TF protein in cell lysates was measured by ELISA. Statistical significance was determined by Two-Way ANOVA; * $p$ <0.05, ** $p$ <0.01, ns not significant.

## Discussion and Conclusion

Mtb induces a prothrombotic phenotype in human macrophages, characterised by increased TF at a transcriptional, translational and functional level, supporting a role for immunothrombosis in TB. TF induction occured in both MDMs and AMs, but the underlying regulation appears to differ. In MDMs, TF production was STAT1-dependent, supported by inhibition with fludarabine and enhanced production in STAT1 GOF macrophages. In contrast, AM TF induction appeared independent of STAT1, suggesting alternative regulatory pathways. Preliminary data indicate NFkB-signalling and glycolysis may contribute to TF regulation in AMs. Immunothrombosis may have implications in the development of venous thromboembolism, post-TB lung disease, and given reduced antibiotic penetration could lead to drug resistance[4]. Further investigation may help identify targets for adjunctive host-directed therapies to improve TB treatment and ameliorate these complications.

## References and Acknowledgements

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