Tissue factor-expressing monocytes inhibit fibrinolysis through a TAFI-mediated mechanism, and make clots resistant to heparins

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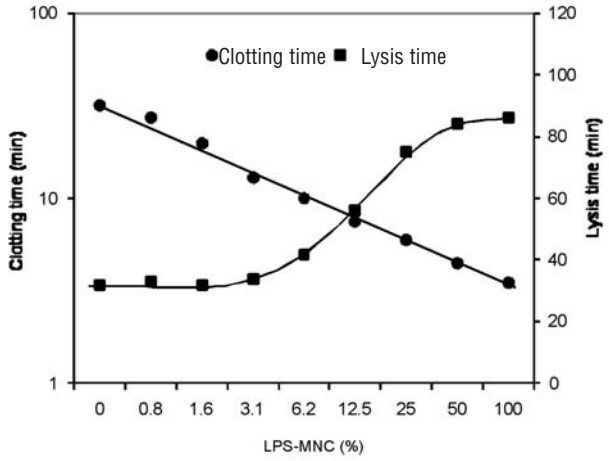
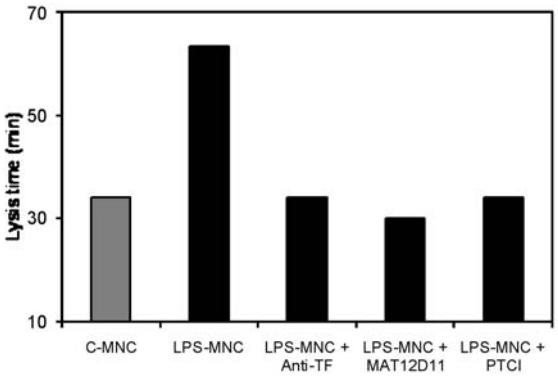
Effect of LPS-stimulated blood mononuclear cells (LPS-MNC) on fibrinolysis in CTI-inhibited plasma

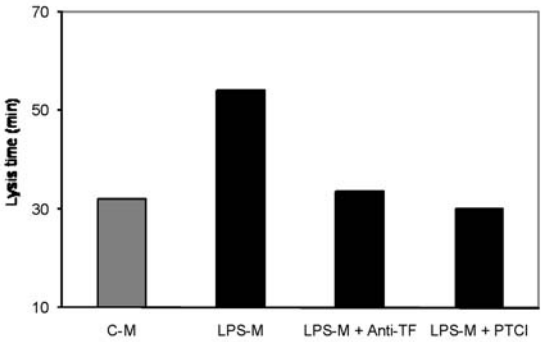
All data reported below were obtained using plasma derived from blood collected on citrate plus CTI (40 μg/mL), which is referred to as CTI-plasma.



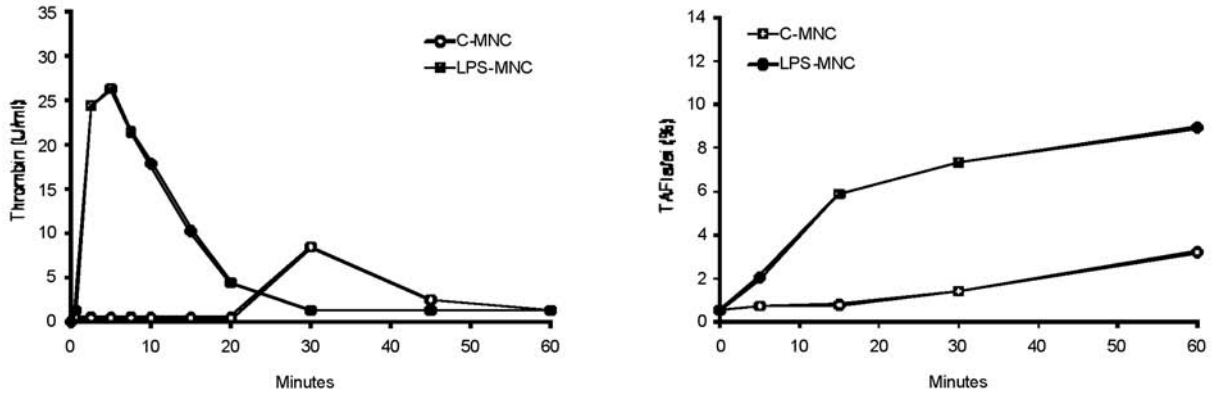
Online Supplementary Figure S1. Clotting time and lysis time changes as a function of the percentage of activated MNC. Mixtures of LPS-MNC and C-MNC were prepared in order to obtain the indicated percentage of LPS-MNC. Cell numbers were 3×10^6/mL in all preparations. Clot formation and t-PA-induced fibrinolysis of recalcified CTI-plasma were evaluated by a turbidimetric assay as reported in Methods. A single experiment is shown.



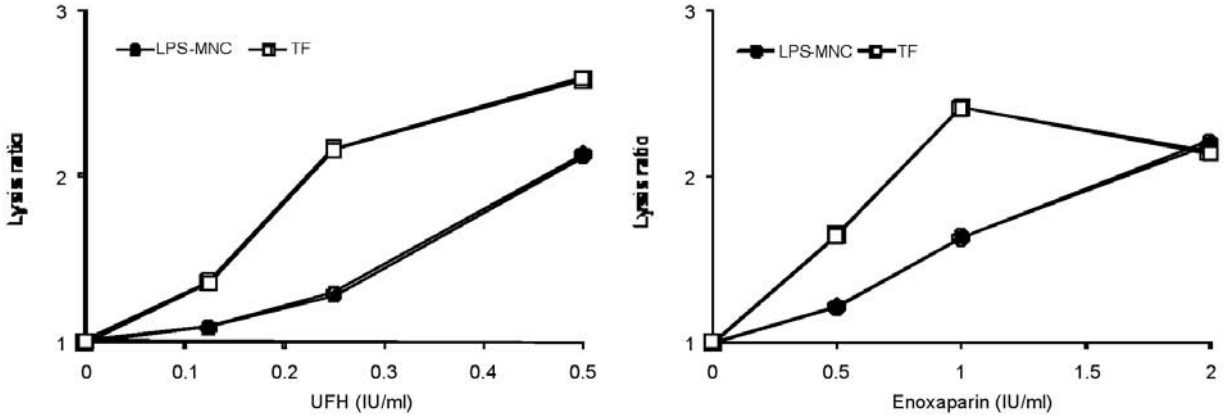
Online Supplementary Figure S2. Role of TF, thrombin and TAFI in the inhibition of fibrinolysis by LPS-MNC. t-PA-induced lysis of CTI-plasma clots was measured in the presence of C-MNC and LPS-MNC. The sample containing LPS-MNC was also tested after treatment of cells with an anti-TF monoclonal antibody (Anti-TF), after treatment of plasma with the anti-TAFI monoclonal MAT12D11, or in the presence of the TAFIa inhibitor PTCI. Results represent the mean of 2 experiments with cells from different donors.



Online Supplementary Figure S3. Inhibition of fibrinolysis by adherent monocytes. Control (C-M) and LPS-stimulated adherent monocytes (LPS-M) were prepared as reported in Methods. Clots were generated onto the surface of adherent cells by adding t-PA-containing recalcified CTI-plasma and the lysis time was measured by the changes in OD. LPS-M were also tested after treatment of adherent cells with anti-TF monoclonal antibody (LPS + Anti-TF), or in the presence of the TAFIa inhibitor PTCI (LPS-M + PTCI). A single experiment is shown. The anti-TAFI monoclonal antibody was not tested in this experiment.



Online Supplementary Figure S4. Thrombin generation (left) and TAFIa/ai accumulation (right) in CTI-inhibited plasma in the presence of control (C-MNC) and LPS-treated mononuclear cells (LPS-MNC). Results are the mean of two experiments with cell preparations from different donors.



Online Supplementary Figure S5. Influence of LPS-MNC and TF (Recombiplastin) on the profibrinolytic activity of unfractionated (UFH) and low molecular weight heparin (enoxaparin) in CTI-plasma. Recombiplastin was used at a dilution displaying the same TF activity as the LPS-MNC preparation tested in parallel. Results show the lysis ratio, calculated as the ratio between the lysis time in the absence of heparin and the lysis time in the presence of each heparin concentration. A single experiment is shown.