Organ-on-Chip Technology Recapitulates Thrombosis Induced by an anti-CD154 Therapeutic Monoclonal Antibody

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Abstract

Blocking of CD40L-mediated signaling represents a validated therapeutic strategy for treatment of several auto-immune disorders, however, development of therapies against this target was stalled for several years because of unexpected thrombotic and cardiovascular events during clinical development of the anti-CD40L mAb Hu5c8. These side effects were not detected during preclinical testing. Platelet activation assays have been used to test the hypothesis that thrombosis was caused by binding of Hu5c8IgG1 to FcγRIIa receptors on platelets. To provide additional confidence in the safety of new anti-CD40L mAbs that are designed not to bind FcγRIIa, a micovessel-chip (Vessel-Chip) was developed that could capture human relevant endpoints for detection of coagulopathy, providing a patient-specific platform for safety testing. The Vessel-Chip includes a vascular channel lined by human endothelial cells and perfused with human whole blood at a physiologically-relevant shear rate. Treatment with clinical-relevant concentrations of hu5c8IgG1 and sCD40L resulted in endothelial activation, platelet adhesion, platelet aggregation, fibrin clot formation, and increased secretion of thrombin anti-thrombin (TAT) complex. Conversely, these endpoints were attenuated following treatment with Hu5c8IgG2σ, a mAb that does not bind FcγRIIa receptors. Given lack of suitable preclinical models for detection of thrombosis, these data provide confidence in the potential safety of the newer generation anti-CD40L mAbs designed not to bind FcγRIIa receptors. Detection of TAT in the model confirms that important counter-regulatory mechanisms that occur during thrombosis, such as thrombin and anti-thrombin generation, occur de-novo in the model. This model provides a unique platform for preclinical assessment of thrombosis risk in a patient-specific manner, but can also be used for discovery of anti-thrombotic agents, and mechanism of action elucidation thus providing a useful tool for drug discovery and development.

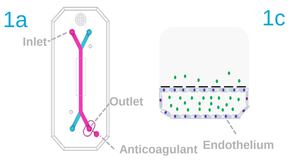
Introduction

Activation of T cells via binding of the CD40 ligand (CD40L/CD154) to the CD40 receptor is a critical step in the initiation of the adaptive immune response. Blocking of CD40L-mediated signaling has been proposed as a therapeutic strategy for the treatment of conditions associated with hyper-activation of adaptive immunity. Pre-clinical studies have demonstrated that monoclonal antibodies (mAbs) used against CD40L can suppress organ transplant rejection or autoimmunity. However, the development of anti-CD40L mAbs was halted because of serious incidents of thromboembolism and cardiovascular events during clinical trials with Hu5c8 and IDEC-131, two candidate drugs for treatment of lupus and Crohn's disease. We and others have recently reported on development of micro-engineered Organ-Chips that contain a human endothelium perfused with human whole blood at physiological relevant shear rates. These chips have been successful in recapitulating many of the key aspects of thrombosis. In the present work, we explored whether this modeling of human vasculature in an Organ-Chip can successfully predict the pro-thrombotic effects of Hu5c8 and can be applied for ex vivo preclinical predictive testing.

Results

Our micro-engineered blood Vessel-Chip is made of a transparent polymer and features two main fluidic channels separated by a thin, porous membrane (Fig. 1a). The geometry of the lower vascular microchannel incorporates an anticoagulant port (Fig.1a and Fig.2a), which is a critical element for blood sampling and for enabling downstream analysis of soluble biomarkers. The vascular compartment of the chip is entirely covered with HUVECs (Fig.1b). To test the thrombotic modulating activities of our Vessel-Chip, we perfused freshly collected human whole blood through the lumen of the chip. Under normal healthy conditions, the endothelium provided an antithrombotic surface where blood flowed smoothly, and indeed we detected minimal platelet adhesion or fibrin deposition under control conditions (Fig.1c). Treatment with either TNF-α or sCollagen led to significant stimulation of platelet aggregation and fibrin deposition on the surface of the endothelium, as demonstrated by increased areas of platelet coverage and fibrin deposition (Fig.1c). Scanning electron microscopic (SEM) analysis also revealed that TNF-α pre-treatment of the vascular endothelium induced formation of compact clots composed primarily of erythrocytes and platelets surrounded by a fibrin network, whereas blood treated with sCollagen formed a meshwork of complex fibrin-rich clots that contained mostly red blood cells with altered stellate morphology, which is known to be associated with the retraction of fibrin during later stages of blood clotting (Fig.1d).

1a



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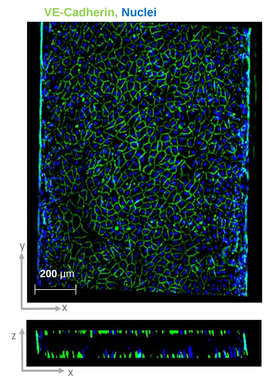

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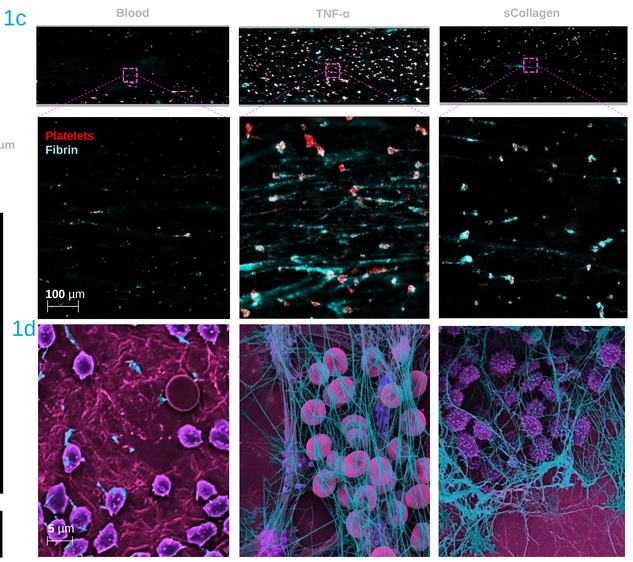

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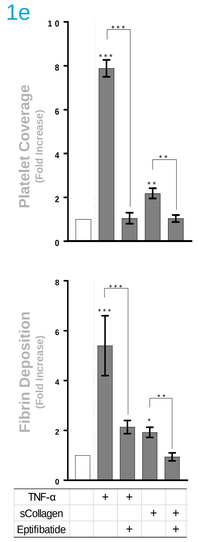
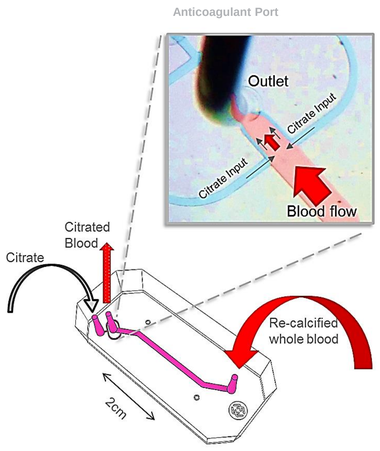


We confirmed that the ability of both of these pro-thrombotic treatments (TNF-α and sCollagen) to promote platelet aggregation and fibrin clot formation could be suppressed by co-administering a clinically relevant concentration of Eptifibatide (2 µg/ml) (Fig.1e) that inhibits the endogenous platelet integrin αIIb/βIII receptor, which mediates fibrinogen binding.

2a



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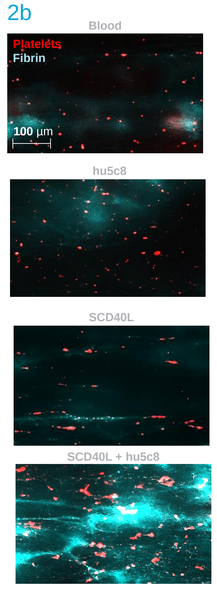

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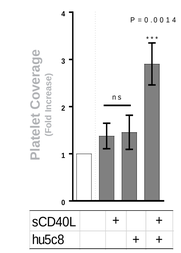

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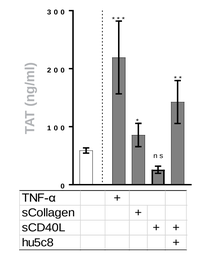
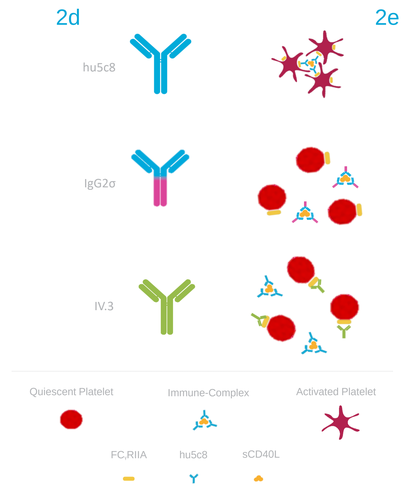


We next investigated whether the system can be used to recapitulate the thrombotic events associated with the anti-CD40L mAb Hu5c8, testing physiologically relevant concentrations of Hu5c8 (240 µg/ml) benchmarked to a dose of 20 mg/kg in cynomolgus monkeys, which is the same dose in man that caused thrombosis. We also used disease-relevant concentrations of sCD40L (10 ng/ml), which is a typical blood value reported in lupus patients (≈1.5-15ng/ml).

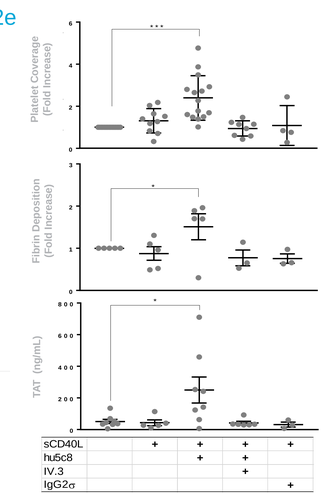
There were no significant treatment-related effects when the endothelium was exposed to either sCD40L or Hu5c8 alone compared to untreated blood, whereas treatment with Hu5c8/sCD40L induced a large increase in platelet aggregate formation and fibrin deposition on the endothelium (Fig.2b, 2c).

Blood sampled from the anticoagulant port (Fig.2a) of the chip was analyzed for thrombin anti-thrombin complex (TAT), an accepted clinical blood biomarker for clot formation. Levels of TAT (Fig.2d) were significantly increased following treatment with TNF-α or Hu5c8/sCD40L, and minimally increased with sCollagen. Formation of TAT confirms that local, intrinsic generation of thrombin, a potent platelet agonist, occurs in the chip vessel, and that counter regulatory mechanisms for coagulation are retained.

2d



2e



Mechanistic studies using platelet assays suggest that high-ordered immune complexes of Hu5c8 and sCD40L activate platelets via interaction of Hu5c8's IgG domain with platelet FcγRIIa receptors. To investigate whether a similar mechanism occurs in an Organ-Chip, experiments were conducted in the presence of the FcγRIIa blocking antibody IV.3 or with a variant of Hu5c8 (IgG2σ) that is designed not to bind to FcγRIIa receptors. These studies revealed up to ~2-fold increase in platelet coverage in some of the donors treated with sCD40L; however, there was no significant increase in the mean value compared to controls (Fig.2d). As expected, perfusion of the Vessel-Chip with the combination Hu5c8/sCD40L resulted in a statistically significant increase in platelet aggregation and fibrin clot formation, as well as increased levels of TAT. Both addition of the IV.3 blocking antibody and substitution of Hu5c8 with Hu5c8 (IgG2σ) were sufficient to prevent increases in platelet coverage, fibrin deposition and TAT release. This shows that the mechanism of thrombosis in an Organ-Chip relies on FcγRIIa binding, which is consistent with other previous studies.

Summary

- We developed an engineered Vessel-Chip that can be used to identify in a single assay multiple, in vivo-relevant end points of thrombosis, including platelet-endothelial interactions, platelet aggregation, fibrin clot formation, and TAT release — with some of these measured in real-time.
- Emulate's Vessel-Chip could be potentially applied as an ex vivo platform to detect thrombosis-related toxicity of drugs in preclinical studies, to de-risk thrombosis-associated unwanted effects during drug development, and to test for efficacy of novel anti-thrombotic compounds.

References

1. D. Huh, H. J. Kim, J. P. Fraser, D. E. Shea, M. Khan, A. Bahinski, G. A. Hamilton, D. E. Ingber, Microfabrication of human organs-on-chips, Nature Protocols 8, 2135–2157 (2013).

2. A. Jain, R. Barrile, A. D. van der Meer, A. Mammoto, T. Mammoto, K. De Ceunynck, O. Aisiku, M. A. Otieno, C. S. Louden, G. A. Hamilton, R. Flaumenhaft, D. E. Ingber, A primary human lung alveolus-on-a-chip model of intravascular thrombosis for assessment of therapeutics, Clin. Pharmacol. Ther. (2017), doi:10.1002/cpt.742.

3. A. Jain, A. D. van der Meer, A.-L. Papa, R. Barrile, A. Lai, B. L. Schlechter, M. A. Otieno, C. S. Louden, G. A. Hamilton, A. D. Michelson, A. L. Frelinger, D. E. Ingber, Assessment of whole blood thrombosis in a microfluidic device lined by fixed human endothelium, Biomedical Microdevices 18 (2016), doi:10.1007/s10544-016-0095-6.

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