USE OF ‘SAME DONOR’ ENDOTHELIAL CELLS AND PBMC CO-CULTURE TO DETECT CYTOKINE STORM REACTIONS TO A TGN1412-LIKE ANTI-CD28 ANTIBODY: A NOVEL ASSAY FOR BIOLOGIC DRUG SAFETY SCREENING

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INTRODUCTION

In 2006, the area of biologic drug development was heavily impacted, when six healthy male volunteers experienced a profound cytokine storm reaction after being given infusions of an antibody to CD28, TGN1412 (1). This trial highlighted a major unmet need for accurate in vitro human cell based bioassays to predict cytokine storm-inducing agents.

In current assays, peripheral blood mononuclear cells (PBMCs) are grown in co-culture with endothelial cells from a different donor or antibodies are air dried to plastic (2, 3). Here we have used endothelial cells derived from blood progenitors and PBMCs from the same donor to create an autologous assay for detection of cytokine storm.

AIMS:

• To characterize endothelial cells derived from blood in terms of response to shear stress and expression of archetypal pathways 

• To determine responses of endothelial cell-PBMC co-culture assays to biologics with different cytokine storm profiles (Figure 1).

HYPOTHESIS:

• Same donor endothelial cell:PBMC co-culture assays detect cytokine inducing biologics

METHODS

RESULTS: Characterisation of blood outgrowth endothelial cells

Figure 2. (A) BOEC expressed CD31 (green), VE-cadherin (red) and F-actin (green) (nuclei are stained with DAPI (blue) when cultured for 4 days under conditions of shear. Images were taken at the centre of the well where shear stress had no preferred direction and cells appear cobble stoned in morphology (top) and at the edge of the well, where shear stress is unidirectional and cells align (bottom) (4). (B) BOEC expressed the archetypal endothelial cell enzymes eNOS and COX-1 and (C) released the endothelial hormones ET-1 at comparable levels to endothelial cells from umbilical vein (HUVEC) and lung microvasculature (HMVEC). Data are mean ± SEM (BOEC n=8 from 3 donors) (HMVEC n=4 from 3 isolations) (HUVEC n=6 from 6 isolations).

RESULTS: Responses of same donor co-cultures to release 9-key cytokines associated with cytokine storm

Figure 3. (A) Cells were treated with inflammatory stimuli LPS (1µg/ml), PamiCSK4 (1µg/ml), C12-E-DAP (10µg/ml), TNFα (10ng/ml), and IL-1β (1ng/ml). (B) Concentration response curve of monocaco-cultures to TGN1412-like anti-CD28 (0.001-10µg/ml) (n=2 donors). (C) Cells were treated with the anti-CD28 (10µg/ml), Campath (10µg/ml), Herceptin (10µg/ml), Avastin (10µg/ml), or Arzerra (10µg/ml) for 24h. Data are mean ± SEM (n=5 from 6 matched donors). Data were analyzed by one-way ANOVA followed by Dunnett’s multiple comparison test vs. control (*p<0.05).

SUMMARY

These observations show that, our ‘same-donor’ cell bioassays, but not monocultures of either cell type alone, respond avidly to a TGN1412-like antibody, and modestly to Campath, but not to control antibodies such as Herceptin, Avastin and Arzerra.

Key cytokines released from co-cultures of same donor BOEC and PBMCs included those identified in the original trial of TGN1412, such as CXCL8, IL-2, IL-6, GM-CSF, IL-10 and IFNγ.

We suggest that this assay provides a co-culture system by which biologics can be tested. This assay employs same-donor co-culture, cells from individual patients can be tested. This assay thus paves the way for personalized bioassay approaches.

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