Proceedings of the British Pharmacological Society at http://www.pA2online.org/abstracts/Vol16Issue1abst210P.pdf

Safety screenings for cytokine storm-inducing biologics using cryopreserved BOECs and PBMCs

K. B. Tan, D. M. Reed, A. Zetterqvist, Z. Nikolakopoulou, Q. Toe, N. S. Kirkby, J. A. Mitchell. National Heart & Lung Institute, Imperial College London, London, UNITED KINGDOM.

Introduction

We have previously developed a co-culture assay using autologous blood outgrowth endothelial cells (BOECs) and peripheral blood mononuclear cells (PBMCs) that detects and delineates therapeutic antibodies associated with cytokine storm responses[1]. However, the lengthy outgrowth process of BOECs and compliance issues associated with multiple same donor blood donations limit the practical roll out of such an assay in a pharmaceutical testing setting. These issues could be overcome by using cryopreserved cells. Here we have investigated the effects of cryopreservation on the utility of BOECs and PBMCs as a platform to detect cytokine responses induced by therapeutic antibodies.

Method

PBMCs and BOECs were isolated and cultured as we have described before[1]. Cells were either used without (fresh) or after cryopreservation (Cryo). Flow cytometry analysis was performed on PBMCs to assess the expression of TLR4, CD28 and CD52, which are target receptors for LPS, NIB1412 and Campath respectively. Cell combinations and treatments were performed as described previously[1] and TNF measured by ELISA in conditioned media after 24 hours.

Results

Freshly isolated and cryopreserved PBMCs expressed identical levels of TLR4, CD28 and CD52 (Figure 1A). Similarly co-cultures of fresh and cryopreserved BOECs and PBMCs performed similarly for TNFα release from cells stimulated with NIB1412 and Campath or when stimulated with control antibodies; Herceptin, Avastin or Arzerra, therapeutic antibodies which are not associated with cytokine storm responses (Figure 1B).

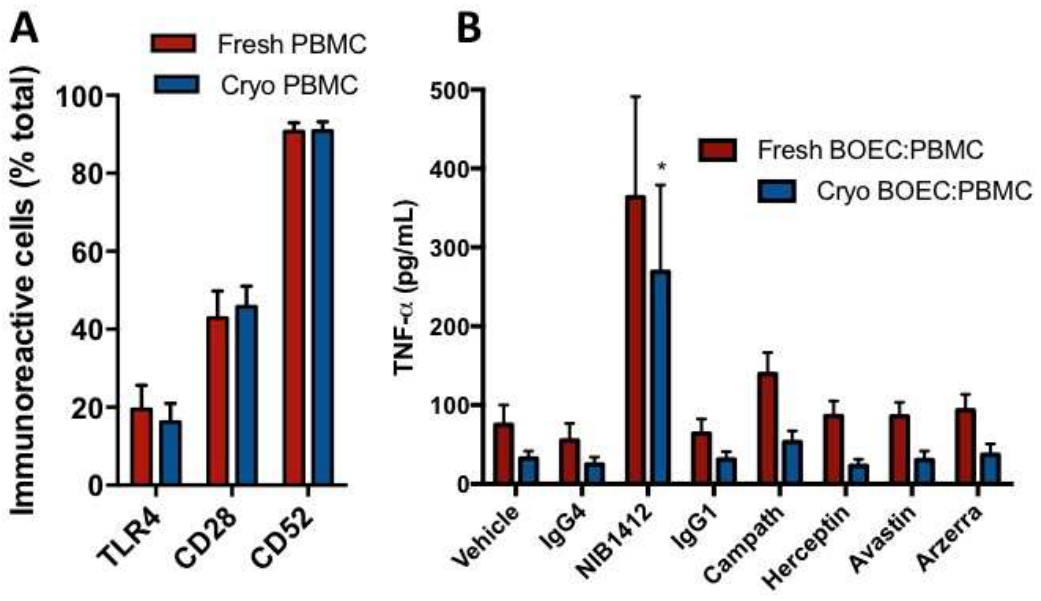

Figure 1: Effect of cryopreservation of cells on receptor expression and cytokine release. Cells were untreated or treated with antibodies at 1µg/ml for 24 hours. Data is the mean S.E.M. of n=3 analysed by respective one-way ANOVA followed by Tukey post test (*p≤0.05).

Conclusion

Cryopreservation of PBMCs and BOECs does not affect the ability of cells to express selected cell surface receptors or to respond to therapeutic antibodies. Whilst these results require validation, our study provides a potential solution to limitations of using autologous cultures of BOECs and PBMCs for bioassay platforms including those defined by us[1] to detect cytokine storm responses. Such an approach would allow for this assay to be optimised and upscaled for high-throughput screenings.

Reference

(1) Reed DM *et al.* (2015) FASEB Journal **29**(6):2595