Developing an assay to observe the effects of IL-1β on activation of endothelium and adhesion molecule expression

Ryan Lamb (r.lamb@newcastle.ac.uk), Tom Pither, Andrew Fisher
School of Biomedical Sciences, Faculty of Medical Sciences, Newcastle University

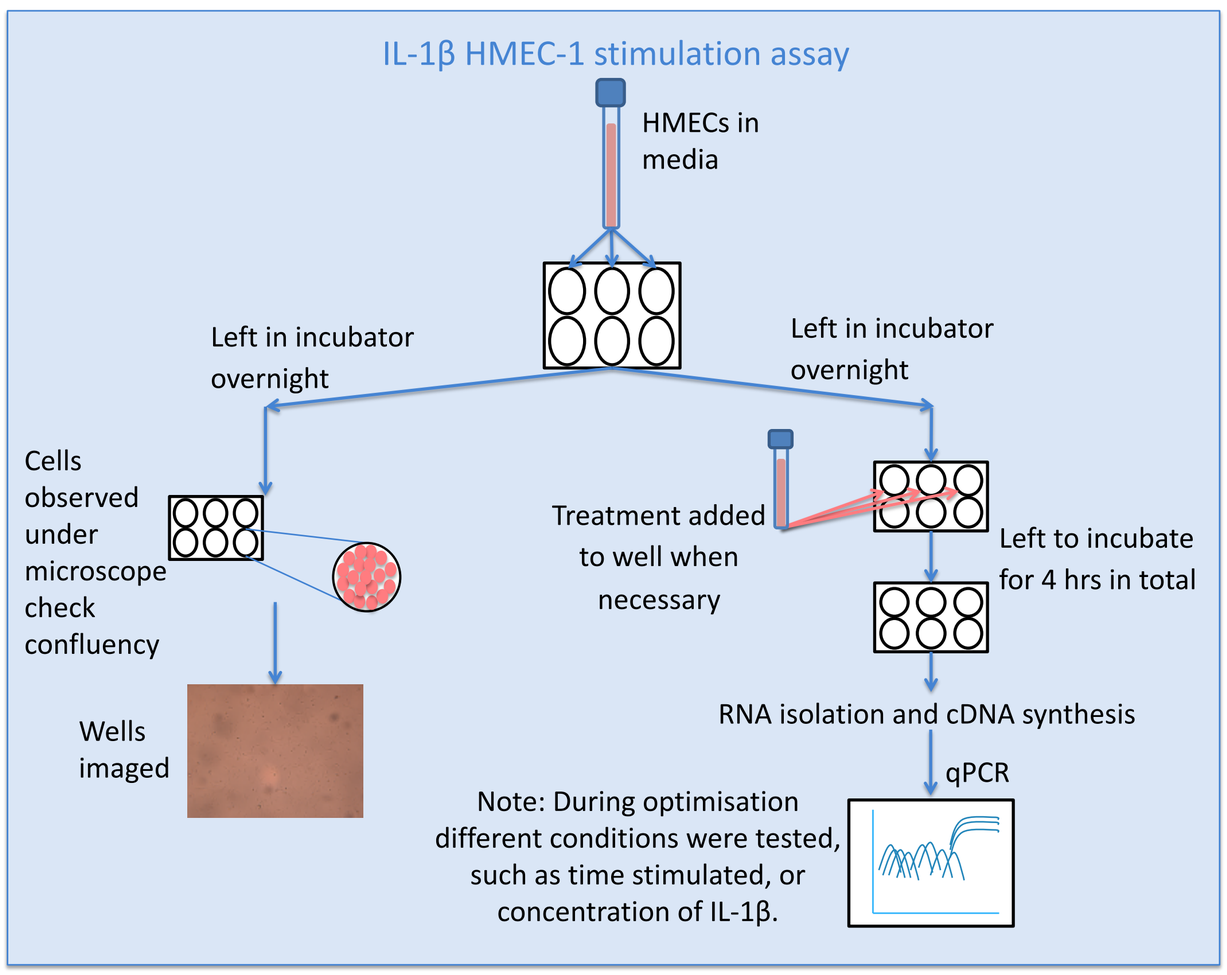
Introduction

- ≥ 14% of patients on lung transplant waiting lists die yearly in the UK, making donor organ shortage a key cause of death[1].
- Ex vivo lung perfusion allows assessment and possibly reconditioning of organs otherwise considered unsuitable for transplant[2,3].
- Previous work showed higher levels of IL-1β in perfusates of declined donor lungs compared with perfusates of transplanted lungs[2].
- It is thought that IL-1β causes upregulation of adhesion molecules on pulmonary microvascular endothelial cells which provokes subsequent neutrophils adherence and extravasation into the tissues.
- This pathway may be susceptible to therapeutic targeting.

Aims

- Optimise an assay to observe differences in gene expression of adhesion molecules ICAM1 and E-selectin (SELE) in HMEC-1 cells, when stimulated with IL-1β.
- Observe effects of IL-1β blockers on gene expression of ICAM1 and SELE.
- Project aims to aid future research in increasing the number of organs for transplant by furthering understanding of cytokine effects on endothelial cells, to aid further work on adhesion molecules and immune cells.

Methods



Seeding densities

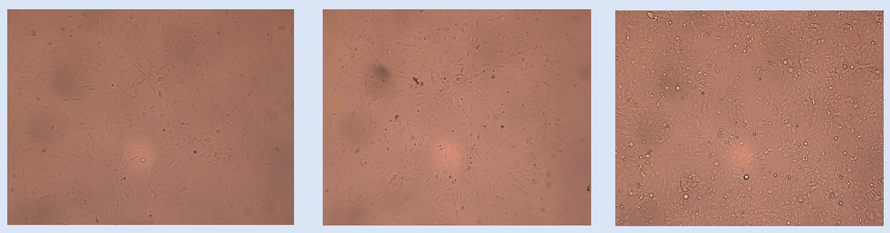


Figure 1: Images of different seeding densities in the 6 well plate taken the day after seeding.

At 1.5 x10^5 cells per ml, the cells have not formed a confluent monolayer. At 2 x10^5 cells per ml cells have mostly formed a confluent layer of cells, making it the optimum seeding density for HMEC-1 cells in a 6 well plate. At 3 x10^5 cells per ml, the cells have become over confluent.

Flow Cytometry of HMEC-1 cells

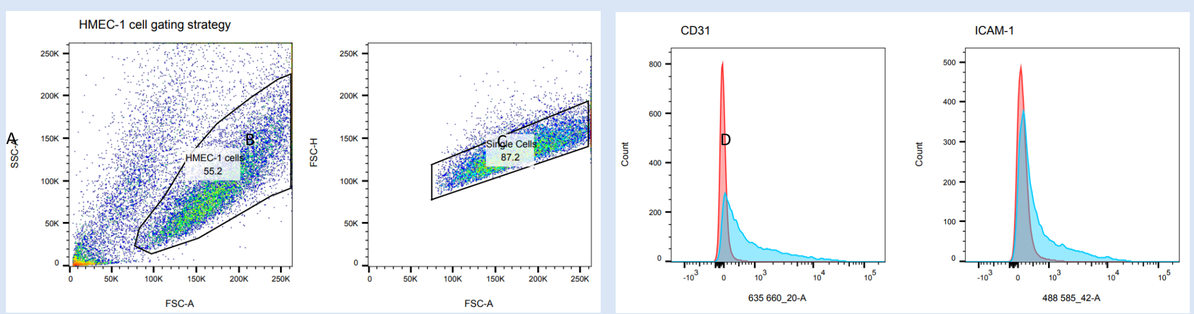


Figure 2:
Flow cytometry of HMEC-1 cells to identify their endothelial cell like features.
Red- Isotype
Blue – Antibody/cells basal expression

Cells gated to exclude any cells not of HMEC-s size, or dead cells (A). Cells then gated to exclude any duplicate cells, to ensure only single cells were observed (B). High levels of expression of CD31 and ICAM-1 in cells, at basal conditions. Therefore, cells demonstrating endothelial cell characteristics at basal level, and so form a suitable model for research.

Results

- The efficiency for the HPRT1 primer (housekeeping gene) was found to be 78.6%. The curve for the primer can be seen in figure 3.
- A large fold change was seen at 1 ng/ml of IL-1β for the ICAM1 gene (figure 4).
- A large fold change was observed in SELE expression after 2 hours of incubation with IL-1β (figure 5).
- As seen in figure 6, the largest increase in ICAM1 expression was observed at 4 hours of incubation with IL-1β.

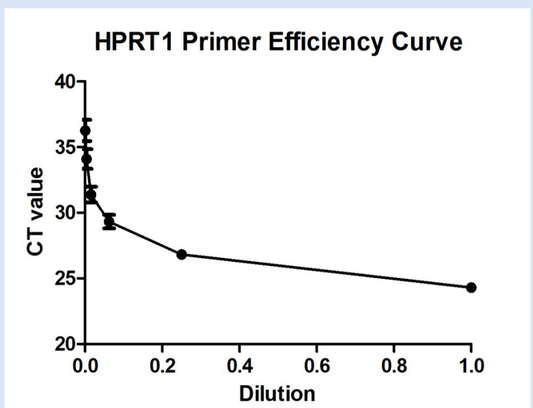


Figure 3: Primer Efficiency Curve, showing the CT value at each of the cDNA concentrations tested

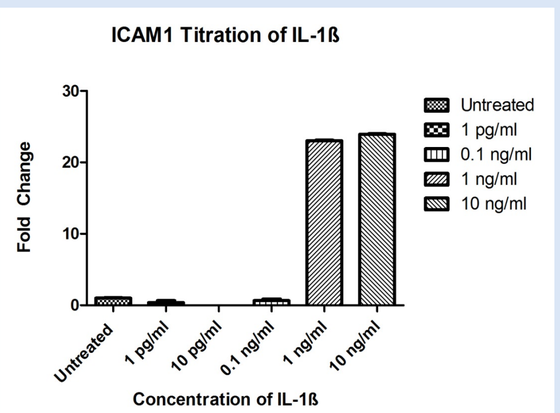


Figure 4: Graph for the IL-1β titration assay for ICAM1, showing the fold change at differing IL-1β concentrations.

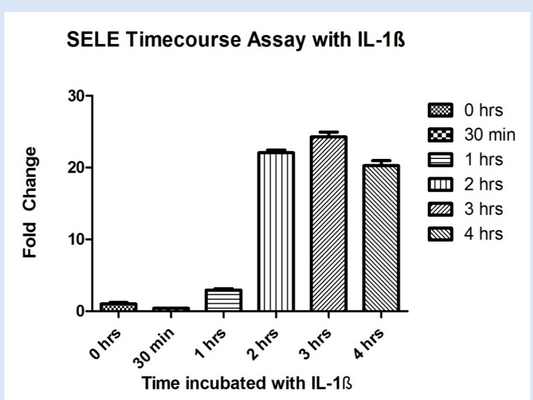


Figure 5: IL-1β Time course assay for SELE

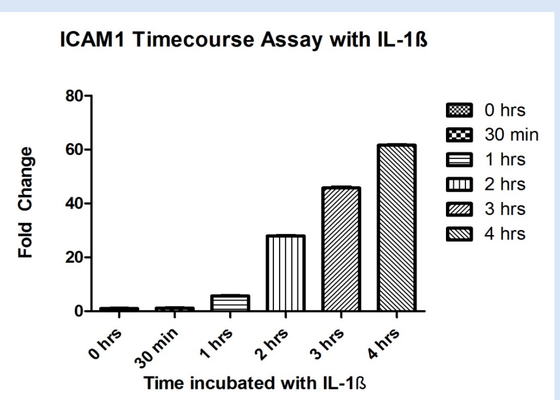


Figure 6: IL-1β Time course assay for ICAM1

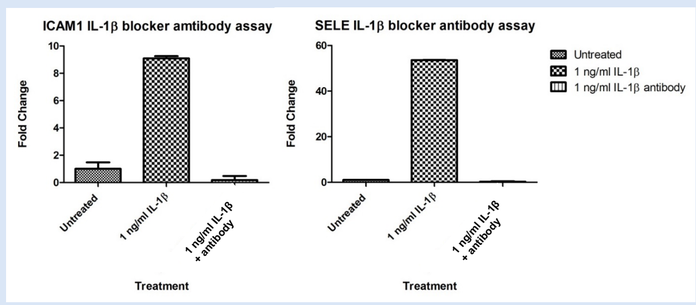


Figure 7: IL-1β blocker antibody assay for ICAM1 (left) and SELE (right)

The IL-1β blocker antibody assay showed that the presence of the antibody in the media of the wells reduced the levels of transcription of ICAM1 and SELE.

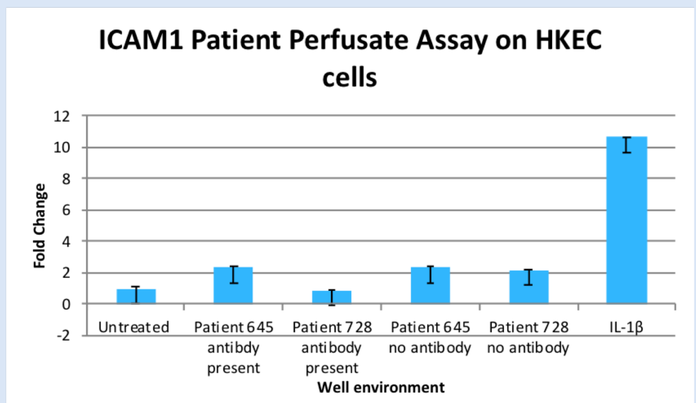


Figure 8: HKEC cells treated with Patient perfusate samples with and without IL-1β blocker antibody

From this data set, there is not a change in the expression of ICAM1 for Patient 645 when the antibody is present compared with without. There is a slight decrease in the expression of ICAM1 when the antibody is present for Patient 728.

Discussion

- Only the primer efficiency of HPRT1 was calculated, because when unstimulated, the levels of SELE and ICAM1 expression were too low to reliably calculate their primer efficiency. The primer efficiency for HPRT1 was found to be 78.60%. This is within what was within the limits expected/desired.
- Since there is not much difference between the fold change of ICAM1 expression in patient samples with and without IL-1β blocker antibody, it is possible that the levels of IL-1β in the samples were very low. If there was time, it would have been good to quantify the IL-1β levels in the samples. This could have been carried out by an ELISA.

Conclusion

- It was observed that IL-1β does increase the expression of adhesion molecules on endothelial cells, and that the action of IL-1β can be prevented with the use of IL-1β blocker antibody. However when tested on patient samples, this was not observed, and thus needs further investigation.

Acknowledgements

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3 - Andreasson A., Karamanou D., Gillespie C., Özalp F., Butt T., Hill P., Jiwa K., Walden H., Greem N., Borthwick L., *et al*. Profiling inflammation and tissue injury markers in perfusate and bronchoalveolar lavage fluid during human ex vivo lung perfusion. European Journal of Cardio-Thoracic Surgery. 2017;51:577-586.