Report for Pathological Society of Great Britain **October 2012**
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Supervisors Dr Gareth Turner / Professor Nicholas Day

Host Department: RT Johnson Division of Neuroimmunology
Johns Hopkins School of Medicine,
Baltimore, USA
Supervisor Prof Monique Stins, PhD.

Study Title **Studying the Effects of Hypoxia on *P.falciparum* infected Erythrocyte Adhesion to Cerebral Endothelial Cells**
Study Period: May – July 2012

Background:

This award allowed me to visit Prof Stins laboratory in Baltimore, to learn the techniques of primary human cerebral endothelial cell culture. Using both primary and immortalised brain endothelial cell lines it was then aimed to study the effects of hypoxia on malaria infected red cell (PRBC) adhesion to human brain endothelial cells (HBEC), specifically resultant effects on barrier function, intracellular signalling or gene transcription in HBEC.

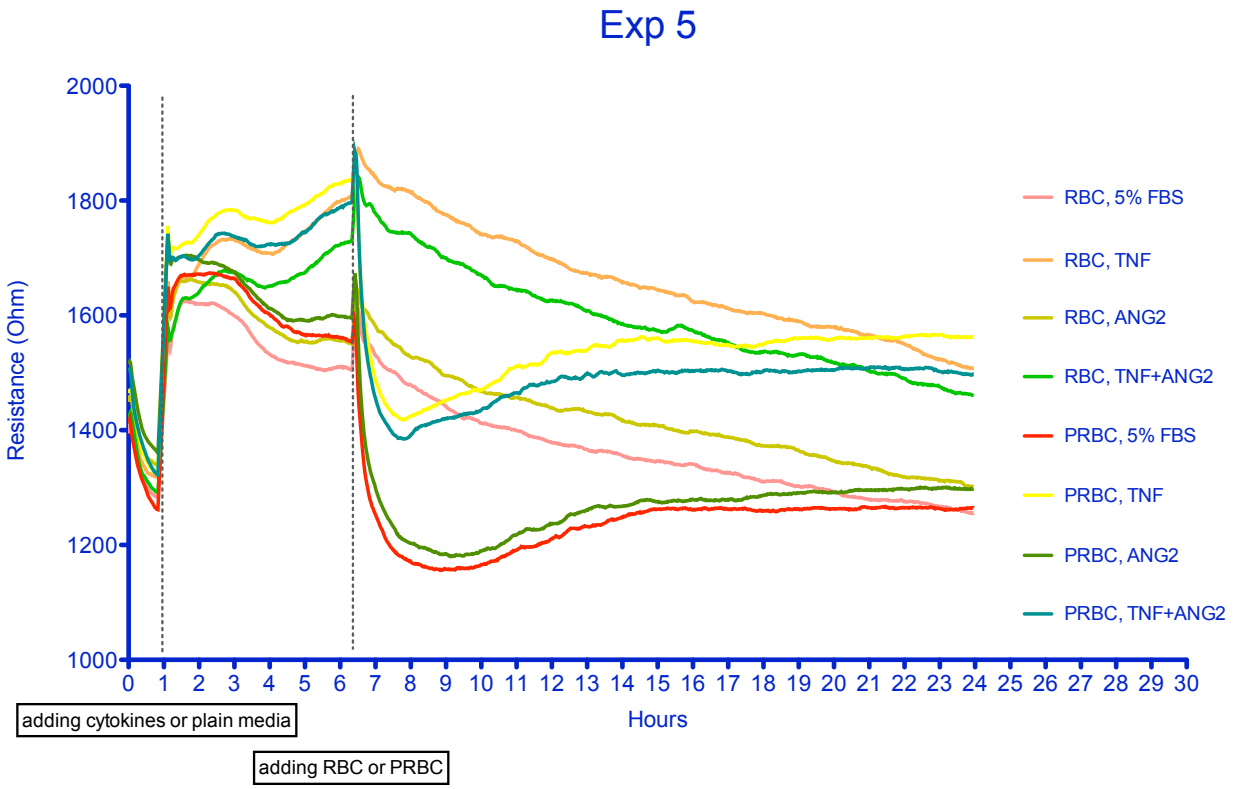
Report:

During my visit I learnt a number of techniques in order to isolate and characterise human brain endothelial cells from surgical specimens of brain. This included the protocol for separation and isolation of microvessles from brain tissue, selective tissue culture, FACS and immunohistochemistry for characterisation of resultant endothelial cells. I also worked with immortalised putative brain EC line cell lines (previously made in the laboratory) including HB56. In addition I learnt to use the ECIS system, which monitors electrical resistance across endothelial monolayers. I designed and made a hypoxic chamber to allow co-culture experiments, and used this to perform a series of experiments studying the adhesion of PRBC to HBEC.

Using PRBC from an ICAM-1 binding parasite strain I performed a series of experiments examining the effect of short (5 minutes) and longer term (12 hours) hypoxia on PRBC binding to HB56. Because one of the pathways of EC activation that I have been characterising as part of my DPhil was the Angiopoeitin-Tie 2 receptor pathway, I also examined the effect of exogenous cytokines including TNF and Ang2 on protection for responses to hypoxia. As well as examining the barrier function of the EC monolayer under these conditions using ECIS, I took samples of supernatant to examine release of soluble ICMA-1 and isolated total mRNA with the aim of performing qPCR for changes in gene expression for both control housekeeping genes tubulin, ICAM-1, HIF-1 alpha, Angiopoetin -1 and -2 and Tie-2.

The isolation of primary brain EC lines while I was visiting the lab was successful but the EC grew too slowly for me to use in the ECIS experiments. I switched to using HB56 and successfully performed a series of experiments using ECIS (figure attached). The addition of PRBC to the monolayer appeared to cause a decrease in blood-brain-barrier resistance which could be partially protected by addition of exogenous cytokines. Hypoxia decreased barrier resistance but this was a nonspecific effect which was not independently altered by PRBC binding. However because of the paradoxical effect of TNF on barrier function (it was expected to increase permeability) I repeated characterisation of the HB56 line using immunohistochemistry, which showed strong epithelial pan-cytokeratin staining but none for the endothelial markers CD31, Factor VIII. I confirmed on subsequent short tandem repeat analysis that the HB56 line was predominantly epithelial, presumably due to contamination of the original EC line. Therefore the results of my first set of experiments were not valid as they did not reflect PRBC binding to endothelial cells.

Having returned to Oxford I am finishing my DPhil studies, and we are aiming to repeat the experiments designed in Baltimore using commercial human brain EC lines. The experimental design and methods were validated using our approach and we aim to repeat our observation on barrier permeability, and extract both total and microRNA from EC to examine the synergy between PRBC binding, hypoxia and BBB function. This fellowship award allowed me to spend an extremely interesting and educational period in a high quality laboratory in Baltimore, and I learnt a lot of new techniques. I would like to thank the Society for giving me this opportunity.


Exp 5
Resistance (Ohm)
2000
1800
1600
1400
1200
1000
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Hours
adding cytokines or plain media
adding RBC or PRBC
RBC, 5% FBS
RBC, TNF
RBC, ANG2
RBC, TNF+ANG2
PRBC, 5% FBS
PRBC, TNF
PRBC, ANG2
PRBC, TNF+ANG2