5) Characterise the *in vivo* model with regards to cell movement and rearrangement within the culture using live imaging and confocal microscopy combined with fluorescent tagging of cells and cell-specific molecules.

After the establishment and characterisation of the culture system, changes in gene and protein expression as a result of contact with the other cell types, can be identified and monitored, using molecular biology techniques, FACS analysis sorting on known cell-specific markers, as well as ELISA and RIA techniques to measure the release of different cytokines and growth factors such as VEGF and Epo into the culture media. These methods will lead to identification of proteins expressed in the BBB under normal physiological conditions.

**Conclusions and relevance for 3R**

Importantly, this model system will provide a unique opportunity to study the BBB and reduce the need for difficult, invasive animal experiments. Given the large number of diseases characterised by BBB breakdown, such a model has wide applications in the medical and pharmaceutical industries, wherein drug and chemical testing could first be carried out exhaustively on the *in vitro* system. Establishment of this model will therefore promote the refinement of potential therapeutic tools and strategies, prior to animal testing and ultimately reduce animal experimentation, consumables and personnel costs. Furthermore, this model system will not only provide information on specific cellular interactions and signals, that promote induction of BBB formation during development, but will be readily manipulated and subjected to different insults such as hypoxia, glucose deprivation and chemical exposure to assist in understanding the breakdown of the BBB. Thus utilisation of this model means BBB research can be more focused and directed to the specific roles of individual cell types, as well as barrier function as a whole, with a minimal use of animal experimentation.

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**Adjuvanticity of Microbial-Derived Particles and Synthetic Analogs *In Vitro***

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**Keywords:** human, dendritic cells, T cell, cytokines, growth factors, vaccination, reduction, replacement, adjuvanticity

**Background and aim**

Vaccines represent the most successful immune-based medical treatments ever discovered and provide long lasting protection against acute infections. The task of limiting adverse reactions by using highly purified antigenic formulas has provided a safer, but nevertheless poor quality of immunogenic therapeutics. Thus, future immune intervention strategies, have a crucial dependence on the identification and characterisation of molecularly defined adjuvants, as well as, on the availability of adequate applicable methods for assessing adjuvanticity.

We would like to develop a system to identify human-compatible adjuvants based on lymphocyte cultures *in vitro*. We have two aims. Firstly, we will assess the capacity of microbial-derived particles and synthetic analogs of enhancing antigen-specific CD4+ T cell responses *in vitro*. Secondly, we will monitor the capacity of compounds endowed with adjuvant

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**Fig. 2:** Current status of 3-dimensional system for *in vitro* BBB model.

Endothelial cells (labelled with PECAM in red) produce tube-like structures in matrix that are contacted by astrocyte endfeet (GFAP staining in green), Ogunshola, O. (unpublished results).
activity to induce modulation of dendritic cells phenotype and the secretion of soluble factors, in order to identify profiles of activation that correlate to adjuvanticity.

Method and results
Our previous work has shown that di- and tri-palmitoylated bacterial lipopeptide analogs, act as adjuvants for antigen-specific CD8+ T cells reactivity. Based on these results we have developed two types of adjuvanticity tests for assessing the capacity of these compounds to enhance HLA class II-restricted T cell responses. The methods are described in Figure 1 and 2.

Conclusions and relevance for 3R
The assessment of adjuvanticity in animal models has a number of limitations. When applied to large-scale screening of chemical libraries, or to dose-dependent measurements, in vivo tests require the employment of high numbers of animals and become very costly procedures. Furthermore, chemicals may induce high toxic effects even if administered at low doses. In this case, animal death, often under painful conditions, hampers any experimental result. It should also be considered that T cell functions are ultimately measured in vitro, upon animal sacrifice. Finally, it is somewhat doubtful whether mice or rabbits have sensitivities to various drugs comparable to those of humans.

Our goal is to identify easily detectable immune bio-chemical parameters of adjuvanticity, in order to generate an applicable method for assessing potency and safety of human-suitable adjuvants in vitro.

References


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