

Generation of Thymus Cell Aggregates Using an *In Vitro* Co-Culture System

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INTRODUCTION: The thymus is a multicellular organ which acts as the central site for T cell lymphopoiesis from haematopoietic stem cell precursors, transformed via a series of interactions and gene rearrangements into thymocytes¹. These T cells have a large array of reactivity to foreign antigens and form the basis of immunity. Depletion of the circulating T cell population is the causative factor of Acquired Immunodeficiency Syndrome (AIDS) and is caused by the Human Immunodeficiency Virus (HIV) binding to and destroying T helper cells via the CD4 receptor². Formation of a controllable *in vitro* thymic system for the generation of T cells could allow for the immune reconstitution of carriers of this virus.

Therefore the aim of the current study is to generate aggregates of thymus cells using co-culture systems.

METHODS: Thymuses were isolated from 8 week old CD1 mice before being digested with trypsin/EDTA for 30 minutes at 37°C. The resulting cell suspension was filtered through a 70µm cell strainer (BD Biosciences) before incubation with Calcein AM / Ethidium Homodimer-1 (Molecular Probes). Thymus cells were then incubated with either mitomycin C incubated SNL fibroblasts or on tissue culture plastic with no other cells present using RPMI-1640 media supplemented with 10% FCS on orbital shakers (60rpm, 2 cm radius) at 37°C. Cells were maintained for 3 weeks and images were taken at 2 day timepoints

RESULTS: Figure 1A shows that at day 0 thymus cells in SNL fibroblast co-culture are in a single cell suspension, but figure 1B shows that at day 6 they formed large, approximately 400µm aggregates. Figure 1C shows thymus cells grown in monoculture at day 0 again the cells are in a single cell suspension but figure 1D shows that at day 6 the cells have only formed small aggregates of approximately 100µm.

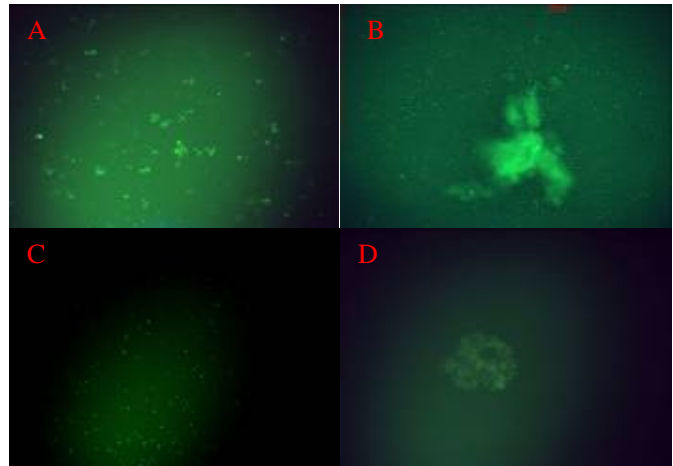


Fig. 1: Calcein AM stained thymus cell isolates cultured on SNL fibroblasts at 0 days (A) and 6 days (B; x10 magnification) and thymus cell isolates in monoculture at 0 days (C) and 6 days (D; x20 magnification).

DISCUSSION & CONCLUSIONS: From these observations it can be seen that thymus cells grown in a co-culture system with SNL fibroblasts more readily form large aggregates of cells. This is possibly due to signals received from the fibroblasts, mimicking the *in vivo* environment. Whether these aggregates are formed due to increased cell binding or proliferation is currently being investigated. Once the system for generating these aggregates is optimised they could then be used for further investigation.

REFERENCES: ¹ Anderson, G., Moore, N.C., Owen, J.J.T. and Jenkinson, E.J., (1996). *Annu. Rev. Immunol.* **14**: 73–99. ² Dalgleish, A.G., Beverley, P.C., Clapham, P.R., Crawford, D.H., Greaves, M.F. and Weiss, R.A., (1984). *Nature* **312**: 763-7.

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