Extrathymic T-cell differentiation *in vitro*

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The first 'Innovation' article in *Nature Reviews Immunology*[1] discussed the introduction of a technique for the extrathymic generation of T cells in culture[2], which was suggested as being a useful new tool for studying T-cell differentiation in a way that was not previously possible[1,3]. The originators of this technique showed that extrathymic differentiation from bone-marrow progenitors *in vitro* can be accomplished using the OP9 stromal cell line, ectopically expressing the Notch ligand delta-like 1 (REFS 1,2). It was stated that this allowed T-cell-differentiation processes to be studied in a simple cell-culture system for the first time. Although this might be true for mouse cells, it is not the case for human cells. Some years ago, we showed that $CD34^+$ lineage-negative human haematpoietic progenitors could acquire mature T-cell characteristics in a thymus-free culture system. This depended on the presence of peripheral-blood mononuclear cells (PBMCs) as feeder cells, cytokine cocktails and the use of serum-free media. Given the importance of delta-like 1, identified in the mouse system, and the fact that Notch ligands, including this one, are expressed by antigen-presenting cells[4], one could ask why previous attempts by others to establish extrathymic T-cell-differentiation systems using PBMCs were less successful[5–7]. We suggest that the reason for such failures was probably the choice of culture medium and, to a lesser extent, the choice of cytokine cocktail and feeder cells. We tested many different formulations over the years, at first containing human serum or fetal-calf serum, with little success. We also tested many serum-free formulations, with equal lack of success. However, the use of the then newly developed X-Vivo 10 medium from BioWhittaker allowed development, for the first time, of T cells in the absence of thymic components in a limiting-dilution cloning system. It was necessary to include stem-cell factor (SCF), fms-related tyrosine kinase 3 ligand (FLT3L), interleukin-2 (IL-2) and IL-3 in the cytokine cocktail. Cloning efficiencies were markedly increased by additional inclusion of oncostatin M or IL-7 (REF. 8). Feeder cells consisted of irradiated (at a dose such that no cells escaped proliferation blockade after irradiation) PBMCs pooled from 20 different donors. During the cloning procedure, these cells would have interacted with each other as a multi-way mixed-lymphocyte culture, resulting in cytokine release, and macrophage and dendritic-cell activation. The purity of the starting haematopoietic-cell population and the high cloning efficiency that could be achieved ruled out the possibility that the T cells isolated were derived from contaminants in the starting population. Derived T cells were not autoreactive, suggesting that negative selection had taken place in a system where the feeder cells did express MHC class II or class I molecules, unlike the OP9 mouse system recently discussed[1]. Derived clones were mostly $CD4^+$ αβ-T-cell receptor 2 $(TCR2)^+$, expressed a wide range of antigen-receptor clonotypes, responded to mitogenic stimulation by proliferation and cytokine release[8], and had the cell-surface markers and growth characteristics[9] typical of human $CD4^+$ T-cell clones. Currently, human T-cell clones derived in this manner are being examined[10] by European Union-supported consortia focused on ageing of the immune system, ImAginE and T-CIA (see further information for websites). Perhaps because of our focus on immunogerontology, and exclusively in humans, these previous findings might have been overlooked.

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FURTHER INFORMATION
ImAginE, Immunology and Ageing in Europe:
http://www.medizin.uni-tuebingen.de/imagine/
T-CIA, T Cell Immunity and Ageing:
http://www.medizin.uni-tuebingen.de/t-cia/
European Searchable Tumour Line Database:
http://www.medizin.uni-tuebingen.de/estdab/
Graham Pawelec's homepage: http://www.medizin.uni-tuebingen.de/tati/

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