Video Article

Erratum: Reaggregate Thymus Cultures

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Abstract

A correction was made to: Reaggregate Thymus Cultures. A revised abstract was republished due to a publisher error. The abstract was corrected to:

> Stromal cells within lymphoid tissues are organized into three-dimensional structures that provide a scaffold that is thought to control the migration and development of haemopoeitic cells. Importantly, the maintenance of this three-dimensional organization appears to be critical for normal stromal cell function, with two-dimensional monolayer cultures often being shown to be capable of supporting only individual fragments of lymphoid tissue function. In the thymus, complex networks of cortical and medullary epithelial cells act as a framework that controls the recruitment, proliferation, differentiation and survival of lymphoid progenitors as they undergo the multi-stage process of intrathymic T-cell development. Understanding the functional role of individual stromal compartments in the thymus is essential in determining how the thymus imposes self/non-self discrimination. Here we describe a technique in which we exploit the plasticity of fetal tissues to re-associate into intact three-dimensional structures *in vitro*, following their enzymatic disaggregation. The dissociation of fetal thymus lobes into heterogeneous cellular mixtures, followed by their separation into individual cellular components, is then combined with the *in vitro* re-association of these desired cell types into three-dimensional reaggregate structures at defined ratios, thereby providing an opportunity to investigate particular aspects of T-cell development under defined cellular conditions. (This article is based on work first reported Methods in Molecular Biology 2007, Vol. 380 pages 185-196).

from

> In the thymus, immature CD4+8+ thymocytes expressing randomly rearranged T-cell receptor α- and b-chain genes undergo positive and negative selection events based on their ability to recognize self-peptide/major histocompatibility complex (MHC) molecules expressed by thymic stromal cells. In vivo analysis of the role of thymic stromal cells during intrathymic selection is made difficult by the cellular complexity of the thymic microenvironment in the steady-state adult thymus, and by the lack of appropriate targeting strategies to manipulate gene expression in particular thymic stromal compartments. We have shown that the thymic microenvironment can be readily manipulated in vitro through the use of reaggregate thymus organ cultures, which allow the preparation of three-dimensional thymus lobes from defined stromal and lymphoid cells. Although other in vitro systems support some aspects of T-cell development, reaggregate thymus organ culture remains the only in vitro system able to support efficient MHC class I and II-mediated thymocyte selection events, and so can be used as an effective tool to study the cellular and molecular regulation of positive and negative selection in the thymus.

Protocol

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Disclosures

No conflicts of interest declared.