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Molecular definition of an *in vitro* niche for dendritic cell development

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Objective. Although dendritic cell (DC) precursors have been isolated from many lymphoid sites, the regulation and location of early DC development is still poorly understood. Here we describe a splenic microenvironment that supports DC hematopoiesis *in vitro* and identify gene expression specific for that niche.

Methods. The DC supportive function of the STX3 splenic stroma and the lymph node-derived 2RL22 stroma for overlaid bone marrow cells was assessed by coculture over 2 weeks. The DC supportive function of STX3 was identified in terms of specific gene expression in STX3 and not 2RL22 using Affymetrix microchips.

Results. STX3 supports DC differentiation from overlaid bone marrow precursors while 2RL22 does not. A dataset of 154 genes specifically expressed in STX3 and not 2RL22 was retrieved from Affymetrix results. Functional annotation has led to selection of 26 genes as candidate regulators of the microenvironment supporting DC hematopoiesis. Specific expression of 14 of these genes in STX3 and not 2RL22 was confirmed by reverse transcription-polymerase chain reaction.

Conclusion. Some genes specifically expressed in STX3 have been previously associated with hematopoietic stem cell niches. A high proportion of genes encode growth factors distinct from those commonly used for *in vitro* development of DC from precursors. Potential regulators of a DC microenvironment include genes involved in angiogenesis, hematopoiesis, and development, not previously linked to DC hematopoiesis. © 2004 International Society for Experimental Hematology. Published by Elsevier Inc.

Although dendritic cell (DC) precursors have been identified in various sites including bone marrow, blood and thymus, the process of DC hematopoiesis is still poorly understood. This is partly due to a lack of knowledge of the microenvironmental requirements for DC development. DC precursors have been shown to develop into functional DC after *in vitro* culture with cytokines like granulocyte-macrophage-colony-stimulating factor (GM-CSF), tumor necrosis factor- α (TNF- α), and Flt3 ligand (Flt3L). In fact, the earliest known bone marrow Lin⁻Flt3⁺Sca-1⁺c-kit⁺ DC progenitor population responds to these cytokines by development into DC [1]. Furthermore, common lymphoid progenitors and common myeloid progenitors from bone marrow, mouse blood monocytes, and CD4^{low} thymic precursors can develop into DC

after *in vitro* culture with appropriate growth factors [2–4]. Results from these *in vitro* protocols have identified the presence of DC precursor populations, which can respond to cytokines. However, these studies ignore the normal *in vivo* development of DC and the contribution of other components of the microenvironment such as stromal cells and extracellular matrix in DC hematopoiesis.

Previously, long-term cultures (LTC) have been used to define the contribution of the microenvironment in B-lymphopoiesis, erythropoiesis, megakaryocytopoiesis, and hematopoietic stem cells (HSC) self-renewal [5–8]. LTC from spleen have now been shown to produce DC [9]. This culture system maintains an adherent stromal cell layer as well as DC progenitors and fully differentiated DC [10]. By contrast, LTC of lymph node support stromal cell growth with only short-term production of hematopoietic cells and no long-term production of DC [9]. The STX3 splenic stroma has been derived from an established splenic LTC, which ceased production of DC after many culture passages, presumably

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