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# The Role of Stroma in Hematopoiesis and Dendritic Cell Development

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**Abstract:** Development of the immune system is depicted as a hierarchical process of differentiation from hematopoietic stem cells (HSC) to lineage-committed precursors, which further develop into mature immune cells. In the case of dendritic cell (DC) development, this linear precursor-progeny approach has led to a confused picture of relationships between various subsets of DC identifiable *in vivo*. A possible reconciliation of the diversity of DC precursors and DC subsets *in vivo* encompasses the role of the microenvironment in DC hematopoiesis. We propose here that various niches for DC hematopoiesis within lymphoid organs could account for the diversity of DC *in vivo*. A tridimensional space consisting of stromal cells which produce a range of membrane-bound and secreted molecules providing signals to DC progenitors would define these niches.

**Keywords:** Niche, endothelial cells, hematopoiesis, dendritic cells.

## INTRODUCTION

Stromal tissues within hematopoietic organs play a key role in the regulation of hematopoiesis. The stroma provides structural support for hematopoiesis by formation of a network of connective tissues. They also define a microenvironment suitable for maintenance of HSC as well as providing cues for development of all hematopoietic lineages [1].

The ontogeny of stroma with hematopoietic support capacity is not well understood. During embryonic development, the mesenchyme, a tissue of mesodermal origin, gives rise to stromal cells including fibroblasts, osteoblasts, chondrocytes, smooth muscle cells and endothelial cells [1]. Evidence has shown that mesenchymal stem cells persist in adulthood and can reconstitute all mesenchymal lineages [2,3]. These mesenchymal stem cells (MSC) are believed to follow a hierarchical development similar to hematopoiesis. At least *in vitro*, MSC can differentiate into osteoblasts, chondrocytes and adipocytes under specific culture conditions. It has been proposed that MSC differentiate into committed progenitors of each of the stromagenic, chondrogenic, adipogenic and osteogenic lineages by stochastic regulation of four different sets of genes [1]. Further development of committed progenitors into terminally differentiated cell types would then be regulated by specific growth factors, cytokines and hormones. Four main cell types within adult bone marrow are known to regulate hematopoiesis: macrophages, adipocytes, osteogenic cells and reticular cells [2].

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## DEFINITION OF A HEMATOPOIETIC STEM CELL NICHE

HSC show properties that distinguish them from any other cell, including the ability to undergo asymmetric cell divisions, extensive self-renewal capacity, existence in a mitotically quiescent form, and ability to clonally regenerate all of the cell types of its initial tissue [4]. Although HSC possess cell-intrinsic characteristics responsible for these functions, regulation of hematopoiesis often requires external signals provided by the microenvironment. Extrinsic regulation of HSC self-renewal and development has been proposed [4]. The plasticity of stem cells to adopt one fate instead of another has prompted a model for microenvironmental regulation of stem cell properties [5]. Indeed during embryonic development, HSC have been located in intimate contact with vascular endothelial cells both in the yolk sac and in the AGM (aorta-gonads-mesonephros) region of the embryo [6].

*In vitro* studies have been performed with the aim of delineating the microenvironment required to maintain stem cells properties of HSC. For instance, cell lines derived from fetal liver were examined for their ability to maintain HSC *in vitro*, and only a small number of cell lines were found to be supportive [7]. Investigation of expression of several cytokines and growth factors showed no correlation between cell line supportive function and pattern of cytokine transcription, which included SCF, LIF, IL-6, IL-7, IL-11, G-CSF, GM-CSF, IGF-1 and TGF [7]. Only a small number of cell lines was able to support HSC self-renewal and had ability to fully reconstitute the hematopoietic system after adoptive transfer into recipient mice [8]. Molecular delineation of the microenvironment using a panel of fetal liver cell lines has proven useful to identify new potential regulators of the HSC niche [9]. For example, this led to the identification of molecules with the Fringe motif, related to the Notch family, as well as cadherin molecules involved in extracellular matrix cell adhesion processes [9]. Osteopontin, a membrane protein involved in extracellular matrix formation expressed by osteoblasts, was identified as a key regulator of the HSC niche within bone marrow [10].

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Stromal components of the HSC niche within bone marrow were independently identified as osteoblasts and endothelial cells [11-13]. The *in vivo* HSC niche within bone marrow indicates a role for osteoblasts in regulating proliferation and niche size [11,12]. However, endothelial cells of sinusoidal vessels in bone marrow and spleen contain another pool of HSC available for blood mobilisation [13]. These rather opposite definitions of stromal niches for HSC could be explained by the needs of distinct pools of HSC, some quiescent and well-protected from any stress and shock, and others in a proliferative state readily available for mobilization [14].

Interactions between HSC and their microenvironment are critical for self-renewal of HSC [15]. Heterologous expression of  -catenin in HSC, or Wnt signals provided by the microenvironment, regulate HSC self-renewal. Sonic hedgehog (Shh) and Bone Morphogenetic Protein 4 (BMP-4) are also key regulators. Treatment of HSC with Shh led to expansion *in vivo*, while an inhibitor of BMP-4 inhibits Shh-induced HSC proliferation, indicating that Shh acts upstream of BMP-4 [16]. Together these results suggest that developmental pathways regulating hematopoiesis in the embryo could play an important role during hematopoiesis in the adult. Furthermore this evidence also supports a model of niche-directed hematopoiesis.

## VASCULAR NICHES FOR STEM CELLS AND HEMATOPOIETIC CELLS

Endothelial components of stem cell niches have been identified for HSC as well as for neural stem cells, and MSC have been located near vascular pericytes within bone marrow [3,13,17,18]. Endothelial cells of sinusoidal vessels in bone marrow and spleen also constitute another niche type for HSC, making them readily available for blood mobilisation [13]. Furthermore, a gradient of HSC potential has been observed within bone marrow, whereby HSC with full lineage reconstitutive capacity, line the bone endosteum, while more committed progenitors like maturing granulocytes increase in number toward the centre of the bone cavity [19].

Evidence for multiple distinct niches has also been shown in B lymphopoiesis. The migration of B lymphocyte precursors between bone marrow niches was revealed by close association between stromal cells expressing Cxcl12 and pre-pro-B cells, and between IL-7-expressing mesenchymal cells and pro-B lymphocytes [20]. A role for sinusoidal endothelial cells within bone marrow in supporting megakaryocytopoiesis has also been reported [21]. Recent evidence has now indicated that splenic endothelial cells play a specific role in development of a distinct subset of DC [22], as well as for *in vitro* development of immature myeloid DC from progenitors [23].

Overall, there are many examples of the critical role played by endothelial cells in various organs in the regulation of hematopoietic niches. Indeed, specialised sinusoidal endothelium was proposed to be important in regulating maintenance of HSC [13]. By their nature and capacity to form blood vessels, endothelial cells are constantly exposed to blood circulating cytokines, hormones and other soluble factors. Endothelial cells have a primary function to transduce messages from one organ to another *via* cell surface receptors and adhesion molecules. They are well placed to be involved in the regulation of hematopoietic niches within multiple organs.

## *IN VITRO* STUDIES OF HEMATOPOIESIS

Stromal cells obtained *in vitro* are derived from long term cultures (LTC) of dissociated lymphoid tissues. The initial development of an adherent stromal cell layer is critical to support production of mature hematopoietic cells from stem cells or progenitors [24]. Adherent stromal cell monolayers *in vitro* are quite heterogeneous and can include endothelial cells, fibroblasts, adipocytes and macrophages [24]. LTC have been described which can produce granulocyte/macrophage progenitors or B lymphocytes [25,26]. Bone marrow stroma can support HSC proliferation and myeloid cell development, myeloid/lymphoid development, B lymphopoiesis and megakaryocyte development [27-29]. Spleen stroma can support development of erythroid cells from fetal liver [30]. Fetal liver stromal cell lines can support maintenance of HSC [7]. Hence, various stroma define an *in vitro* microenvironment regulating proliferation and development of HSC into mature cells by providing specific signals through secreted and membrane-bound proteins. In many ways, stroma obtained from LTC mimic the various hematopoietic niches.

Most studies on stroma have focused on support function for hematopoiesis, with little definition of stromal cell types involved. Stromal cell lines supportive of different hematopoietic processes have been characterised in terms of cell surface marker expression according to the vascular smooth muscle cell (VSMC) pathway [31,32]. VSMC or pericytes are mural cells lining the external side of blood vessels [33]. During embryogenesis, angiogenesis and hematopoiesis occur simultaneously [34]. Formation of new vessels is a multistep process, involving first endothelial cells which later recruit mural cells [35]. Altogether, this supports the common view that stromal cells with hematopoietic support capacity could arise from a VSMC pathway related to angiogenesis.

## A NICHE MODEL FOR DENDRITIC CELL DEVELOPMENT

There has been extensive evidence for numerous *in vivo* subsets of functional DC. DC development studies based on precursor-progeny approaches imply the existence of a common DC progenitor. Recent evidence now confirms the existence of several lineages of DC including conventional DC of both myeloid and lymphoid origin, as well as the IFN-  -producing plasmacytoid DC and the Langerhans cells in skin [36]. However, even within these lineages many distinct DC subsets have been described representing cells in different states of maturation and function. While these cells maintain DC morphology and $CD11c^{+}$ phenotype, they express distinguishing markers and functions. The range of different subsets in spleen, lymph node, thymus and skin is shown in Table **1**.

One model to explain the existence of many DC subtypes *in vivo* could be that development of DC is

**Table 1. Tissue Location and Phenotype of Common DC Subsets**

| Site | Subtype | DC subset | Reference |
|---|---|---|---|
| Spleen | Conventional DC | CD11c$^{+}$CD11b$^{+}$CD8 $^{-}$MHC-II$^{+}$B220$^{-}$ | [37] |
| | | CD11c$^{+}$CD11b$^{-}$CD8 $^{+}$MHC-II$^{+}$B220$^{-}$ | [37] |
| | Plasmacytoid DC | CD11c$^{lo}$CD11b$^{-}$CD8 $^{-}$MHC-II$^{lo/+}$B220$^{+}$ | [38] |
| | Regulatory DC | CD11c$^{lo}$CD11b$^{+}$MHC-II$^{-}$CD45RB$^{+}$ | [39] |
| | | CD11c$^{lo}$CD11b$^{+}$MHC-II$^{lo}$CD45RB$^{+}$ | [40] |
| Thymus | Lymphoid-like DC | CD11c$^{+}$CD11b$^{-}$CD8 $^{+}$MHC-II$^{+}$B220$^{-}$ | [41] |
| | Plasmacytoid DC | CD11c$^{+}$CD11b$^{-}$CD8 $^{+}$MHC-II$^{lo}$B220$^{+}$ | [42] |
| Skin | Langerhans cells | CD11c$^{+}$CD8 $^{-}$MHC-II$^{+}$B220$^{-}$CD207$^{+}$ | [43,44] |
| Lymph node | Conventional DC | CD11c$^{+}$CD8 $^{-}$MHC-II$^{lo}$ | [45] |
| | | CD11c$^{+}$CD8 $^{+}$MHC-II$^{lo}$ | [45] |
| | Plasmacytoid DC | CD11c$^{+}$CD11b$^{-}$MHC-II$^{lo}$B220$^{+}$ | [46] |
| | Langerhans cells | CD11c$^{+}$CD8 $^{lo}$MHC-II$^{+}$CD205$^{+}$CD207$^{+}$ | [47] |

regulated by the microenvironment, rather than being a cell-intrinsic phenomenon. One hypothesis to reconcile evidence for multiple DC precursors is the existence of specific microenvironments within various organ sites. Various DC progenitors localising in specific tissue niches would therein develop into tissue-specific DC. Thus, multiple niches could contribute to the development of a diversity of DC subtypes [48]. Such a model offers strategic advantage for compartmentalisation of the immune system by production of site-specific DC with unique functional capacity as antigen presenting cells. A major component of this model is the stroma, which produces growth factors, extracellular proteins and cell-membrane molecules involved in adhesion of hematopoietic cells. Another main element of the niche model is a committed progenitor cell with capacity to self-renew and develop into DC. Together they would define a niche for development of tissue-specific DC subsets within various organs, such as the thymus, the skin and the spleen.

The thymus has been shown to contain lymphoid progenitors which can form both T lymphocytes and CD8 $^{+}$ lymphoid-like DC [41]. The thymus could contain stromal elements regulating the development of a specific subset of lymphoid-like DC, and human thymic stromal monolayers have been shown to support development of DC from progenitors present in cord blood [49].

The epidermis defines another microenvironment specific for the development of skin DC or Langerhans cells (LC). The secreted factors TGF- 1 and IL-4 are involved in LC development, and these have been shown to regulate the epidermal LC niche [50,51]. An *in vitro* system of coculture of CD34$^{+}$ cord blood cells with cutaneous fibroblast cell lines supports the development of dermal DC [52], indicating that specific stromal elements within dermis can regulate DC development. Self-renewing hematopoietic progenitors located in skin give rise to LC, and have been shown to be exclusively dependent on TGF- 1 for development and differentiation [53].

## STROMAL CELL FUNCTION IN SPLEEN DENDRITIC CELL DEVELOPMENT

Long-term stromal cultures (LTC) from murine spleen have been shown to support hematopoiesis of immature myeloid CD8 $^{-}$ DC [54]. More recent studies now confirm a specific role for stromal cell niches within spleen in the regulation of DC development. Murine spleen stromal cells which support hematopoiesis of plasmacytoid or regulatory DC were described as both fibroblastic cells expressing the ER-TR7 marker, and as resident CD68$^{+}$ macrophages [39]. Similarly, human CD34$^{+}$ cord blood cells cocultured with human spleen-derived myofibroblastic cell lines develop into DC *in vitro* [55]. This further supports the role of a fibroblast population within spleen in the regulation of DC development. However, other evidence indicates a role for endothelial cells within spleen in DC development. Primary cultures of murine splenic cells having endothelial-like properties have also been shown to support the development of a DC subset with distinct regulatory characteristics from hematopoietic progenitors [22,40]. Human endothelial umbilical vein cells (HUVEC) treated with TNF- , but not IL-1 or IL-4, support the development of DC from CD34$^{+}$ cells [56]. While contradictory in terms of stromal cell type responsible for DC development, these reports show that direct contact between stromal cells and hematopoietic progenitor cells is essential for DC development and function in spleen.

The molecular interactions which support DC development in splenic niches can be investigated *in vitro* using murine spleen LTC producing DC [57]. During establishment of spleen LTC, an adherent layer is formed which supports production of non-adherent hematopoietic cell populations. These comprise populations of small DC progenitors and large immature DC. Progenitors maintained within LTC proliferate, differentiate and are released into supernatant as immature myeloid DC. Small DC progenitors are heterogeneous for markers like Sca-1, c-kit, CD11c and

CD11b and have poor endocytic activity. Larger immature DC represent a homogeneous cell population expressing CD80, CD86, low levels of MHCII and showing good endocytic activity [57,58]. Over many cultures, these cells reproducibly resemble immature myeloid DC [54]. The STX3 splenic stroma was isolated from a LTC which no longer produced DC due to loss of progenitors [59]. It can, however, support DC development from overlaid bone marrow [59]. The development of myeloid DC from bone marrow progenitors which colonise STX3 stroma is shown in Fig. **1**. Small DC progenitors isolated from LTC by cell sorting specifically develop into large immature DC upon coculture above the competent STX3 splenic stroma [50]. Committed DC progenitors require stromal cell contact for growth and proliferation, and substitution of stroma with growth factors like IL-1 , IL-3, IL-6, IL-7, Flt3L, GM-CSF, SCF or TNF- does not lead to DC development from isolated LTC progenitors [60].

We have therefore utilised the LTC model producing DC to investigate the splenic microenvironment for *in vitro* DC development. Dissection of the splenic microenvironment has involved the STX3 splenic stroma as support for overlaid DC precursors in bone marrow. Genome-wide expression analysis of STX3 was performed using Affymetrix GeneChips with generation of a database of expression levels of all genes available on the Murine Genome U74Av2 GeneChip. In parallel, STX3 was cloned by single-cell sorting and a panel of 102 stromal cell lines were established. The Affymetrix results proved useful for delineating cell types and for identification of potential regulators of DC development. The panel of cloned lines revealed heterogeneity amongst stromal cells in terms of morphology and support function for hematopoietic cell development. This combined study has identified an immature endothelial cell type in splenic stroma as an essential component of a splenic niche for DC development.

## INVESTIGATION OF THE NICHE ENVIRONMENT FOR DENDRITIC CELL DEVELOPMENT IN SPLEEN

Stromal cells derived from spleen provide a model microenvironment for DC development. The mixed splenic stromal cell population STX3 defines an *in vitro* microenvironment supportive of DC development from overlaid bone marrow or spleen. Gene profiling using Affymetrix GeneChips has produced a dataset of 154 genes specifically expressed in STX3 and not in the control non-supporter lymph node stroma 2RL22 [61]. Functional annotation has led to the selection of 26 genes as candidate regulators of the STX3 microenvironment supporting DC hematopoiesis. The specific expression of 14 of these genes in STX3 and not 2RL22 was confirmed by reverse transcription-polymerase chain reaction. The expression of genes encoding endothelial-cell markers, candidate growth factors and known regulators of DC development was also investigated in the Affymetrix dataset. This data-mining approach revealed common gene expression between STX3 and primary cultures of endothelial-like splenic stromal cells shown to be supportive of DC development [22]. Several endothelium-related genes were also found to be expressed by STX3. This study has clearly defined the DC supportive stromal cells in STX3 as immature endothelial cells. Overall, genes specifically expressed in STX3 are known to be associated with functions of angiogenesis, growth factor and cytokine activity, and development. These are summarised in Table **2**.

Dissection of cellular components of the STX3 stroma by single-cell cloning and analysis of DC support function of individual clones has also provided information on the niche environment for DC development in spleen. A panel of 102 splenic stromal cell lines was established, and a range of clone morphologies was identified as reflective of

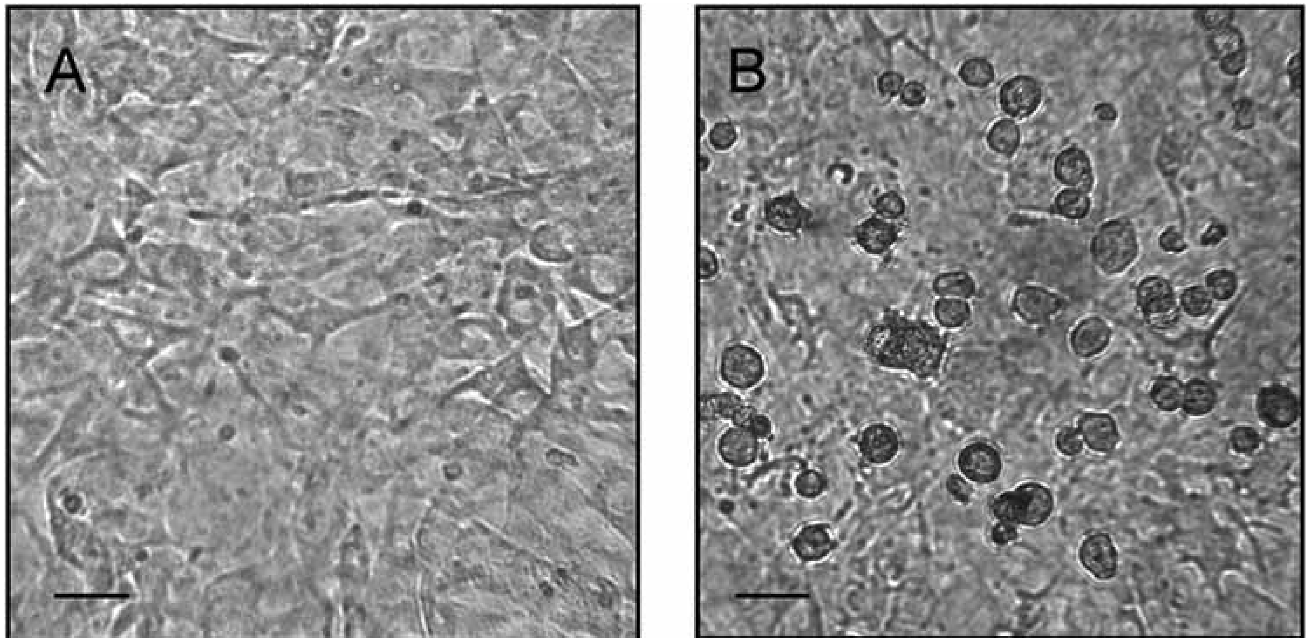


**Fig. (1). Development of DC from bone marrow overlaid on STX3 stroma.** Phase contrast microscopy of stromal cultures. A. STX3 stroma alone. B. Cells developing at 14 days after overlay of unfractionated bone marrow above STX3. Bar represents 30µm.

**Table 2. Potential Regulators Specifically Expressed by the STX3 Microenvironment Supportive of Dendritic Cell Development**

| Biological process | Molecular function | Gene |
|---|---|---|
| Angiogenesis | Cell surface molecules | Cd34, Mcam, Thy1 |
| | Receptors | Acvrl1, EphB2 |
| | Extracellular matrix proteins | Fbln1, Col18a1 |
| | Matrix remodelling | Mmp8, Mmp12, Mmp10, Mmp16, Plau |
| Secreted factors | Growth factors | Igf2, Gdf10, Hgf |
| | Cytokines | G1p2, Gdf10, Ccl8, Ccl11, Ccl5, Cxcl10, Ctf1 |
| Development | Thymopoiesis | Slfn2 |
| | Embryo segmentation | Odz3 |
| | Wnt pathway | Dkk3, Col18a1, Lef1 |
| | TGF pathway | Cktsf1b1, Gdf10, Acvrl1, Grfa2 |

heterogeneity in STX3 [48]. These included stromal cells which grew as cuboidal cells and others which grew as elongated spindle-shaped cells, However, similar expression levels for the endothelial genes *Acvrl1/Alk1*, *Col18a1* and *Mcam* in 13 splenic stromal cell lines with either cuboidal or spindle-shaped morphology suggested that cells with both morphologies were of endothelial origin. The hematopoietic support function of stromal clones was tested in coculture assays using a bone marrow cell overlay. Splenic stromal clones with different morphologies were found to be both supporters and non-supporters of hematopoiesis, evidenced as either foci formation or release of DC in suspension. Four representative splenic stromal cell lines which support hematopoiesis from bone marrow were further characterised in terms of endothelial cell function [48]. All four stromal lines express the endothelial genes *Acvrl1*, *Cd34*, *Col18a1*, *Eng*, *Flt1*, *Mcam* and *Vcam1* but not *Cd31* or *Vwf*, which are expressed by mature endothelial cells. Three lines with cuboidal morphology form tube-like structures when cultured on Matrigel. Capacity for formation of tube-like structures correlates with ability to support myeloid DC development from overlaid bone marrow. A fourth cell line, with spindle-shape morphology and unable to form tube-like structures in Matrigel, produced large granulocytic cells expressing CD11b and CD86, but not the CD11c and CD80 markers reflective of DC. These supporter and non-supporter lines will be used in studies to identify differentially expressed markers for identification of endothelial cells which specifically support DC differentiation. Conditioned medium from splenic stromal cell lines also showed support for DC production from precursors in bone marrow, confirming that soluble factors and cytokines produced by stromal lines can drive DC development. However, long term production of DC from progenitors in bone marrow requires the presence of stroma.

In conclusion, a niche-directed model for DC development is presented, which supports hematopoiesis of progenitors and differentiation of precursors into immature myeloid DC. We have specifically studied the splenic microenvironment for DC development, and identified immature endothelial cell lines derived from spleen which are supportive of DC development. These results predict the existence of an immature endothelial cell type *in vivo* which regulates DC development within spleen. Our model under test is that this niche environment supports hematopoiesis of an endogenous DC progenitor resident in spleen. Given evidence for heterogeneity amongst endothelial cells within the sinusoidal microvasculature of spleen [62], future studies will be directed at location of niches and characterization of endothelial cells which support DC hematopoiesis.

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## REFERENCES

[1] Dennis JE, Charbord P. Origin and differentiation of human and murine stroma. Stem Cells 2002; 20: 205-14.

[2] Deans RJ, Moseley AB. Mesenchymal stem cells: biology and potential uses. Exp Hematol 2000; 28: 875-84.

[3] Short B, Brouard N, Occhiodoro-Scott T, Ramakrishnan A, Simmons PJ. Mesenchymal stem cells. Arch Med Res 2003; 34: 565-71.

[4] Morrison SJ, Shah NM, Anderson DJ. Regulatory mechanisms in stem cell biology. Cell 1997; 88: 287-98.

[5] Fuchs E, Tumbar T, Guasch G. Socializing with the neighbors: stem cells and their niches. Cell 2004; 116: 769-78.

[6] Bertrand JY, Giroux S, Golub R, *et al.* Characterisation of purified intraembryonic hematopoietic stem cells as a tool to define their site of origin. Proc natl Acad Sci USA 2005; 102: 134-9.

[7] Wineman J, Moore K, Lemischka I, Müller-Sieburg C. Functional heterogeneity of the hematopoietic microenvironment: rare stromal elements maintain long-term repopulating stem cells. Blood 1996; 87: 4082-90.

[8] Moore KA, Ema H, Lemischka IR. *In vitro* maintenance of highly purified, transplantable hematopoietic stem cells. Blood 1997; 89: 4337-47.

[9] Hackney JA, Charbord P, Brunk BP, Stoeckert CJ, Lemischka IR, Moore KA. A molecular profile of a hematopoietic stem cell niche. Proc Natl Acad Sci USA 2002; 99: 13061-6.

[10] Nilsson SK, Johnston HM, Whitty GA, *et al*. Osteopontin, a key component of the hematopoietic stem cell niche and regulator of primitive hematopoietic progenitor cells. Blood 2005; 106: 1232-9.

[11] Calvi LM, Adams GB, Weibrecht KW, *et al*. Osteoblastic cells regulate the haematopoietic stem cell niche. Nature 2003; 425: 841-6.

[12] Zhang J, Niu C, Ye L, *et al*. Identification of the haematopoietic stem cell niche and control of the niche size. Nature 2003; 425: 836-41.

[13] Kiel MJ, Yilmaz ÖH, Iwashita T, Yilmaz OH, Terhorst, Morrison SJ. SLAM family receptors distinguish hematopoietic stem and progenitor cells and reveal endothelial niches for stem cells. Cell 2005; 121: 1109-21.

[14] Suda T, Arai F, Hirao A. Hematopoietic stem cells and their niches. Trends Immunol 2005; 26: 426-33.

[15] Reya T, Duncan AW, Ailles L, *et al*. A role for Wnt signalling in self-renewal of haematopoietic stem cells. Nature 2003; 423: 409-14.

[16] Bhardwaj G, Murdoch B, Wu D, *et al*. Sonic hedgehog induces the proliferation of primitive human hematopoietic cells *via* BMP regulation. Nat Immunol 2001; 2: 172-80.

[17] Palmer TD, Willhoite AR, Gage FH. Vascular niche for adult hippocampal neurogenesis. J Comp Neurol 2000; 425: 479-94.

[18] Shen Q, Goderie SK, Jin L, *et al*. Endothelial cells stimulate self-renewal and expand neurogenesis of neural stem cells. Science 2004; 304: 1338-40.

[19] Taichman RS. Blood and bone: two tissues whose fate are intertwined to create the hematopoietic stem-cell niche. Blood 2005; 105: 2631-9.

[20] Tokoyoda K, Egawa T, Sugiyama T, Choi BI, Nagasawa T. Cellular niches controlling B lymphocyte behaviour within bone marrow during development. Immunity 2004; 20: 707-18.

[21] Avecilla ST, Hattori K, Heissig B, *et al.* Chemokine-mediated interaction of hematopoietic progenitors with the bone marrow vascular niche is required for thrombopoiesis. Nat Med. 2004; 10: 64-71.

[22] Zhang M, Tang H, Guo Z, *et al*. Splenic stroma drives mature dendritic cells to differentiate into regulatory dendritic cells. Nat Immunol 2004; 5: 1124-33.

[23] Despars G, O'Neill HC. Splenic endothelial cell lines support development of dendritic cells from bone marrow. Stem Cells in press.

[24] Dexter TM, Testa NG. *In vitro* methods in haemopoiesis and lymphopoiesis. J Immunol Methods 1980; 38: 177-90.

[25] Whitlock CA, Robertson D, Witte O. Murine B cell lymphopoiesis in long term culture. J Immunol Methods 1984; 67: 353-68.

[26] Okubo T, Yanai N, Obinata M. Self-renewal and differentiation of a novel bipotent myeloid progenitor clone in the stroma-dependent culture. Exp Hematol 2000; 28: 651-9.

[27] Friedrich C, Zausch E, Sugrue SP, Gutierrez-Ramos JC. Hematopoietic supportive functions of mouse bone marrow and fetal liver microenvironment: dissection of granulocyte, B-lymphocyte, and hematopoietic progenitor support at the stroma cell clone level. Blood 1996; 87: 4596-606.

[28] Koguma M, Matsuda KI, Okuyama R, Yanai N, Obinata M. Selective proliferation of lymphoid cells from lineage$^-$ c-kit$^+$ Sca-1$^+$ cells by a clonal bone marrow cell line. Exp Hematol 1998; 26: 280-7.

[29] Zweegman S, Veenhof MA, Huijgens PC, Schuurhuis G-J, Dräger AM. Regulation of megakaryocytopoiesis in an *in vitro* stroma model: preferential adhesion of megakaryocytic progenitors and subsequent inhibition of maturation. Exp Hematol 2000; 28: 401-10.

[30] Yanai N, Matsuya Y, Obinata M. Spleen stromal cell lines selectively support erythroid colony formation. Blood 1989; 74: 2391-7.

[31] Remy-Martin JP, Marandin A, Challier B, *et al*. Vascular smooth muscle differentiation of murine stroma: a sequential model. Exp Hematol 1999; 27: 1782-95.

[32] Charbord P, Oostendorp R, Pang W, *et al*. Comparative study of stromal cell lines derived from embryonic, fetal, and postnatal mouse blood-forming tissues. Exp Hematol 2002; 30:1202-10.

[33] Jain RK. Molecular regulation of vessel maturation. Nat Med 2003; 9: 685-93.

[34] Baron MH. Embryonic origins of mammalian hematopoiesis. Exp Hematol 2003; 31: 1160-9.

[35] Cleaver O, Melton DA. Endothelial signaling during development. Nat Med 2003; 9: 661-8.

[36] O'Garra A, Trinchieri G. Are dendritic cells afraid of commitment? Nat immunol 2004; 5: 1206-8.

[37] Vremec D, Pooley J, Hochrein H, Wu L, Shortman K. CD4 and CD8 expression by dendritic cell subtypes in mouse thymus and spleen. J Immunol 2001; 164: 2978-86.

[38] Asselin-Paturel C, Boonstra A, Dalod M, *et al.* Mouse type I IFN-producing cells are immature APCs with plasmacytoid morphology. Nat Immunol 2001; 2: 1144-50.

[39] Svensson M, Maroof A, Ato M, Kaye PM. Stromal cells direct local differentiation of regulatory dendritic cells. Immunity 2004; 21: 805-16.

[40] Tang H, Guo Z, Zhang M, Wang J, Chen G, Cao X. Endothelial stroma programs hematopoietic stem cells to differentiate into regulatory dendritic cells through IL-10. Blood 2006; DOI10.11282/blood-2006-01-007187.

[41] Wu L, Shortman K. Hetergeneity of thymic dendritic cells. Semin Immunol 2005; 17:304-12.

[42] Okada T, Lian ZX, Naiki M, Ansari AA, Ikehara S, Gershwin ME. Murine thymic plasmacytoid dendritic cells. Eur J Immunol 2003; 33: 1012-1019.

[43] Anjuere F, del Hoyo GM, Martin P, Ardavin C. Langerhans cells develop from a lymphoid-committed precursor. Blood 2000; 96: 1633-37.

[44] Valladeau J, Ravel O, Dezutter-Dambuyant C, *et al.* Langerin, a novel C-type lectin specific to Langerhans cells, is an endocytic receptor that induces the formation of Birbeck granules. Immunity 2000; 12: 71-81.

[45] Dakic A, Shao QX, D'Amico A, *et al.* Development of the dendritic cell system during mouse ontogeny. J Immunol 2004; 172: 1018-27.

[46] Nakano H, Yanagita M, Gunn MD. CD11c(+)B220(+)Gr-1(+) cells in mouse lymph nodes and spleen display characteristics of plasmacytoid dendritic cells. J Exp Med 2001; 194: 1171-8.

[47] Henri S, Vremec D, Kamath A, *et al.* The dendritic cell populations of mouse lymph nodes. J Immunol 2001; 167: 741-8.

[48] Despars G, O'Neill HC. A role for niches in the development of a multiplicity of dendritic cell subsets. Exp Hematol 2004; 32: 235-43.

[49] Miralles GD, Smith CA, Whichard LP, Morse MA, Haynes BF, Patel DD. CD34+CD38-lin- cord blood cells develop into dendritic cells in human thymic stromal monolayers and thymic nodules. J Immunol 1998; 160: 3290-8.

[50] Borkowski TA, Letterio JJ, Farr AG, Udey MC. A role for endogenous transforming growth factor beta 1 in Langerhans cell biology: the skin of transforming growth factor beta 1 null mice is devoid of epidermal Langerhans cells. J Exp Med 1996; 184: 2417-22.

[51] Elbe-Bürger A, Egyed A, Olt S, *et al*. Overexpression of IL-4 alters the homeostasis in the skin. J Invest Dermatol 2002; 118: 767-78.

[52] Mollah ZU, Aiba S, Manome H, Yoshino Y, Tagami H. Cord blood CD34$^+$ cells differentiate into dermal dendritic cells in co-culture with cutaneous fibroblasts or stromal cells. J Invest Dermatol 2002; 118: 450-60.

[53] Merad M, Manz MG, Karsunky H, *et al.* Langerhans cells renew in the skin throughout life under steady-state conditions. Nat Immunol 2002; 3: 1135-41.

[54] O'Neill HC, Wilson HL, Quah B, Abbey JL, Despars G, Ni K. Dendritic cell development in long-term spleen stromal cultures. Stem Cells 2004; 22: 475-86.

[55] Briard D, Azzarone B, Brouty-Boyé D. Importance of stromal determinants in the generation of dendritic and natural killer cells in the human spleen. Clin Exp Immunol 2005; 140: 265-73.

[56] Moldenhauer A, Nociari M, Lam G, Salama A, Rafii S, Moore MA. Tumor necrosis factor alpha-stimulated endothelium: an inducer of dendritic cell development from hematopoietic progenitors and myeloid leukemic cells. Stem Cells 2004; 22: 144-57.

[57] Ni K, O'Neill HC. Long-term stromal cultures produce dendritic-like cells. Brit J Haematol 1997; 97: 710-25.

[58] Wilson HL, Ni K, O'Neill HC. Identification of progenitor cells in long-term spleen stromal cultures that produce immature dendritic cells. Proc Natl Acad Sci USA 2000a; 97: 4784-9.

[59] Ni K, O'Neill HC. Hemopoiesis in long-term stroma-dependent cultures from lymphoid tissues: production of cells with

myeloid/dendritic characteristics. *In vitro* Cell Dev Biol Anim 1998; 34: 298-307.

[60] Wilson HL, Ni K, O'Neill HC. Proliferation of dendritic cell progenitors in long term culture is not dependent on granulocyte macrophage-colony stimulating factor. Exp Hematol 2000b: 28: 193-202.

[61] Despars G, Ni K, Bouchard A, O'Neill TJ, O'Neill HC. Molecular definition of an *in vitro* niche for dendritic cell development. Exp Hematol 2004; 32: 1182-93.

[62] Blazseks M, Harvath G, Grama L, Balogh P. Phenotypic identification and development of distinct microvascular compartments in the postnatal mouse spleen. Cell Immunol 2001; 212: 126-37.

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