Stromal cells of the mouse spleen

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INTRODUCTION

The structure and organization of the spleen differ in many aspects from other secondary lymphoid organs such as lymph nodes, related to the complex function of the spleen as a filter of the blood as well as a lymphoid organ. The spleen is composed of compartmentalized lymphoid tissue, the white pulp, which resembles the organization of lymph nodes (Mebius and Kraal, 2005). The venous part of the spleen, the red pulp, is composed of intricate blood endothelial sinuses lined with macrophages, essential for particle clearance of the blood and removal of effete red blood cells. In addition to the immune and filter function of the spleen, the organ is a large reservoir of monocytes and can play a role in hematopoiesis during ontogeny and under pathological conditions. This variety of functions will be reflected in the local composition and function of stromal cells in the spleen, such as fibroblast reticular cells (FRC) and endothelial cells. Here, we will describe what is known about the different stromal cell types in the compartments of the spleen and their contribution to the function of the organ.

ONTGENY

The distinct position of the spleen is reflected in its ontogeny. The molecular and cellular requirements that are essential for the development of lymph nodes and mucosal-associated lymphoid organs have been described in large detail. Studies in mice deficient in various genes have made it clear that the interaction of lymphoid tissue inducer (LTi) cells and stromal lymphoid tissue organizer (Lto) cells is crucial for the development of lymph nodes (Mebius, 2005). The hematopoietic LTi cells, expressing lymphotoxin-β (LTβ), seed the lymph node anlage and interact with the mesenchymal Lto cells that express the lymphotoxin-β receptor (LTβR). The interaction between the two cell types and the resulting upregulation of adhesion molecules, cytokines and chemokines production is instrumental for further local development of lymph nodes (Vondenhoff et al., 2009a). Interestingly, deficiency of either the lymphotoxin receptor or ligand leads to a complete absence of lymph node development. Similarly, deficiencies described for genes that are crucial for the differentiation or the homing and clustering of LTi cells prevent the formation of lymph nodes (Yoshida et al., 2002; Vondenhoff et al., 2009a). Yet, under all these circumstances the spleen will still be formed.

Keywords: spleen, white pulp, red pulp, fibroblast reticular cells, marginal zone, marginal reticular cell

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The stromal cells play an important role in the support and guidance of lymphocytes and dendritic cells. That B cells play an important role in this process can be the result of altered induction of chemokines that are necessary for the homing and retentions of lymphocytes and dendritic cells (DCs). In its absence, T and B cell compartments do form but B cell follicles lack functional follicular dendritic cells (FDCs) and there is a conspicuous absence of macrophages in the marginal zone. This impaired development can be the result of altered induction of chemokines that are necessary for the homing and retentions of lymphocytes and dendritic cells (DCs). That B cells play an important role in this process was demonstrated in mice that lack B cells (Ngo et al., 2001; Nolte et al., 2004). A profound effect was seen on the organization of the splenic T cell zone (periarteriolar lymphoid sheath, PALS) and on the number of DCs in the white pulp. This was dependent on the production by stromal cells of CCL21, a T cell zone chemokine, and the induction of gp38 (podoplanin), expression, a stromal cell marker. This induction required the interaction of B cell and stromal cells and depended on LTβR expression by B cells (Ngo et al., 2001).
In other studies, it was demonstrated that the chemokines CCL21, and to a lesser extent CCL19, are involved in the localization of the marginal zone macrophages in the marginal zone (Ato et al., 2004).

STROMAL CELLS IN THE SPLEEN: THE WHITE PULP

Even though it is obvious that stromal cells are important for the organization of the white pulp and marginal zone, the question is whether there are differences between stromal cells from spleen and other lymphoid organs. The organization of the white pulp of the spleen in discrete T and B cell areas closely resembles that of the lymph nodes, but there are two major differences, the absence of high endothelial venules (HEVs) and the absence of a subcapsular sinus.

High endothelial venules are crucial for the entrance of naïve, recirculating lymphocytes from the blood into the lymph nodes. In the absence of the homeodomain transcription factor Nkx2.3 HEV do develop in the spleen and can mediate l-selectin-dependent homing of lymphocytes (Csonpany et al., 2011). In a normal spleen, both lymphocytes and antigens will enter the lymphoid white pulp from the surrounding marginal zone (Bajenoff et al., 2008). Here, the blood vessels partially end and blood-borne antigens will be picked up by macrophages and DCs, whereas lymphoid cells can actively migrate into the white pulp, depending on the expression of adhesion molecules and chemokine receptors. From homing studies using blocking antibodies the involvement of the adhesion molecules LFA-1 and α4β1 on migrating cells and the ligands ICAM-1 and VCAM-1 on stromal cells has been inferred (Nolette et al., 2002; Lo et al., 2003).

Data from intravital microscopy have confirmed earlier observations that lymphocytes predominantly enter the white pulp from the marginal zone via the bridging channels and from there into the T cell-dependent area surrounding the central arteriole (Bajenoff et al., 2008). These bridging channels are also involved in the exit of effector cells that have been activated in the white pulp and leave for the red pulp or further dissemination into the blood (Figure 1). The migration of cells into the white pulp is an active process by which a layer of stromal cells, the marginal reticular cells (MRC) has to be passed. These cells form the boundary between marginal zone and the T and B cell areas of the white pulp and are characterized by the expression of MARCAM-1 and the production of CXCL13 (Katakai et al., 2008). Furthermore, the production of chemokines by these cells may lead to the formation of local niches for cells in the marginal zone, such as the marginal zone B cells and the marginal metallophilic macrophages, and possibly DCs.

The position of the MRC at the border of marginal zone and white pulp emphasizes the overall structural resemblance of the white pulp with a lymph node. In a lymph node, the MRC form the bottom of the subcapsular sinus and play a similar role as in the marginal zone of the spleen by regulating cell entrance into the T cell zone (Katakai et al., 2008; Koning and Mebius, 2012). In contrast to most connective tissues, in which the extracellular components that are produced by the fibroblasts surround the cells, in lymphoid organs a special adaptation leads to a situation where the fibroblasts surround the extracellular matrix they produce. In addition, the FRC are connected to each other forming a three-dimensional reticulum, and lymphocytes fill up the spaces of this network (Geiss et al., 1997). The extracellular matrix is highly organized in a collagen fiber network, consisting of 20–200 parallel bundles, up to 1 micron in diameter, of macrophages have important functions in both the innate and the adaptive immune system by producing type I IFN and viral antigens after infection, by transferring antigens to B cells and DCs, and by directly stimulating NKT cells (Phan et al., 2009; Vondenhoff et al., 2009b; Backer et al., 2010). Once lymphoid and myeloid cells have entered the white pulp they are able to migrate further along a network of stromal cells, the FRC. The network extends throughout the T cell zone of the white pulp and is connected to the marginal zone (Mueller and Ahmed, 2008). Although the structure of the FRC network in the spleen has not been studied in as much detail as has been done for the comparable network in lymph nodes, based on functional studies it is very likely that the overall composition is similar to the lymph node situation.

In contrast to most connective tissues, in which the extracellular components that are produced by the fibroblasts surround the cells, in lymphoid organs a special adaptation leads to a situation where the fibroblasts surround the extracellular matrix they produce. In addition, the FRC are connected to each other forming a three-dimensional reticulum, and lymphocytes fill up the spaces of this network (Geiss et al., 1997).
is not complete and a conduit system still exists in adult B cell follicles where it provides an efficient mechanism for delivery of small antigens and chemokines such as CCL19 to B cells that are in direct contact with the conduits (Roosenraad et al., 2009). Larger antigens and complexes are transported into the follicles by macrophages. In addition, it has been shown that marginal zone B cells can shuttle between the marginal zone and follicles using a combination of the chemokine receptor CXCR5 and the sialoglycoprotein 1-phosphate receptors 1P1 and 1P3 (Cainmon et al., 2008). This is regarded as an additional mechanism for systemic antigen capture and delivery to FDCs and may not involve migration via the bridging channels. FDCs trap and present antigen to B cells via the binding of immune complexes to complement receptors 1 and 2 and FcγRIIB (El Shikh et al., 2010). In addition, they provide survival and proliferation signals to both naïve B cells and germinal center B cells and thereby are necessary for the integrity of both primary B cell follicles and germinal centers (Wang et al., 2011). The factors secreted by FDCs include RAFF (Goerlik et al., 2003), IL-6, and the chemokine CXCL13 to attract B cells (Ansel et al., 2000). FDC generation is dependent on IL-4,8,2 and TNFα expression by B cells and there are no differences reported between lymph node and splenic FDCs. 

**OTHER STROMAL CELLS IN THE SPLEEN**

With minor local differences the general picture of the stromal network of the lymphoid compartment as an important scaffold in the T and B cell zones is quite comparable between spleen and lymph nodes. In addition, the spleen contains the red pulp. This compartment has unique venous sinuses in which the lining endothelial cells are positioned in parallel way. The endothelial cells contain stress fibers that run along the long axis of the endothelial cells and which are attached to annular fibers that run around the sinuses (Mebius and Kraal, 2005). Contraction of the stress fibers leads to the formation of small slits between the endothelial through which red blood cells can leave the sinuses for the venous system of the spleen. Membrane stiffening as a result of aging will eventually prevent erythrocytes from leaving the sinuses and will result in elimination by macrophages present in these sinuses. For the human spleen it has been suggested that endothelium-derived litoral cells are important for this process of filtration and elimination (Ogembo et al., 2012).

In addition to the presence of these red pulp macrophages with their important function in erythrocyte turnover and iron metabolism, the spleen is also a reservoir of macrophage precursors, monocytes (Swirski et al., 2009), which are thought to reside in the red pulp. This monocyte depot can play an important role under circumstances of acute inflammation, where rapid recruitment of monocytes leads to improved clinical outcome, as has been demonstrated in a model of acute myocardial infarction (Leuschner et al., 2012). Interestingly, in a mouse tumor model it has recently been demonstrated that the spleen also harbors neutrophil precursor cells and that both the monocyte and neutrophil reservoir can lead to tumor progression by infiltrating the tumor (Cortes-Retamozo et al., 2012). The recruitment of the cells from the spleen was mediated by the chemokine receptor CCR2. Based on histology, it was deduced that the myeloid progenitor
CXCL12 were able to produce IL-6, an important factor for anti-
body production, and expressed the adhesion molecule CD54, which is a ligand for CD11a, which is found on plasma cells (Ellyard et al., 2005).

**CONCLUDING REMARKS**

Both in the white pulp and in the red pulp of the spleen different stromal cells make up the basic framework of the respective compartments. Through differential production of chemokines and growth factors stromal cells can form local niches that are essential for the maintenance and function of a variety of cell types. In the white pulp, these niches are essential for the generation of immune responses. In the red pulp, stromal cells are involved in the localization of effector cells such as plasma cells, but they have also been suggested to be central to the support of myeloid progenitor cells. Together, the data emphasize the important role of various stromal cell types in creating environments with highly specific functions in lymphoid organs. Future work is needed to further phenotype the different stromal cell subsets that mediate these specific functions, further allowing the identification of the different molecules involved in these processes.

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