



Review

The extracellular matrix of the spleen as a potential organizer of immune cell compartments

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Abstract

Until recently little information was available on the molecular details of the extracellular matrix (ECM) of secondary lymphoid tissues. There is now growing evidence that these ECMs are unique structures, combining characteristics of basement membranes and interstitial or fibrillar matrices, resulting in scaffolds that are strong and highly flexible and, in certain secondary lymphoid compartments, also forming conduit networks for rapid fluid transport. This review will address the structural characteristics of the ECM of the murine spleen and its potential role as an organizer of immune cell compartments, with reference to the lymph node where relevant.

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1. Introduction

Cellular compartmentalisation within secondary lymphoid organs is essential for normal immune function. Over the past years, the complex relationship between cell–cell adhesion molecules, cytokines and chemotactic factors involved in the maintenance of immune cell compartments within lymph nodes and the spleen has started to be elucidated [1–3]. Only recently, however, has attention turned to stromal cells, the cellular part of the non-hematopoietic scaffold, and their impact on establishing the milieu in which immune reactions take place. Even though the acellular component of this scaffold, the reticular fibre network, has long been recognised to form the backbone of secondary lymphoid organs, relatively little is known of this acellular compartment and whether it does more than support the fibroblastic reticular cells of secondary lymphoid organs.

Historically, electron microscopic studies have been employed to decipher the nature of the extracellular matrix (ECM) of secondary lymphoid tissues, revealing the reticular fibre network or “reticulum” [4], but details on molecular composition remained sparse not only due to the strong focus on immunology and cellular composition of secondary lymphoid tissues, but also due to the absence of specific tools for the detection of defined ECM molecules. Several isolated studies on the spleen demonstrated the presence of both interstitial matrix molecules (collagen types I, II and III, fibronectin, tenascin-C) and basement membrane components (laminins, collagen type IV, heparan sulfate proteoglycans, nidogen) (summarised in Table 1). However, the generic nature of these studies together with the species differences observed in spleen architecture [5,6] has made it difficult to draw conclusions apart from the presence or absence of defined molecules in certain sites. It is only recently, as a consequence of systematic analyses of the localisation of defined basement membrane versus interstitial matrix molecules with respect to immune cell populations, that the existence of distinct matrices associated with different immune cell compartments has become apparent [7]. This review gives an overview on how the differential expression of ECM molecules defines lymphoid compartments, with focus on the spleen, and will provide a framework for future studies

Abbreviations: BM, basement membrane; ECM, extracellular matrix; HEVs, high endothelial venule; MZ, marginal zone; PALS, periarterial lymphoid sheath; WP, white pulp; RP, red pulp.

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Table 1
Summary of studies performed on extracellular matrix molecules expressed in the spleen

ECM type	ECM molecule	Mouse	Rat	Human	Guinea fowl
Interstitial matrix	Collagen I	[38]		[33]	[77]
	Collagen II			[78]	
	Collagen III			[33,79]	[77]
	Fibronectin	[50]		[33]	[77]
	Tenascin	[46,51]		[79]	[77]
	Vitronectin			[33]	
Basement membrane	Laminins	[42,48,80]	[80]	[7,33,79]	[77]
	Collagen IV			[7,33,60,79]	
	Perlecan			[81]	
	Heparan sulfates	[49]	[49]		
	Hyaluronan			[82]	

elucidating the influence of the non-immunological scaffold on immune cell function.

2. Extracellular matrices

Two structurally and functionally distinct extracellular matrices can be distinguished in most tissues, the loose connective tissue of the interstitial matrix and the thin sheet-like structure of the basement membrane. The interstitial matrix represents a network that loosely connects mesenchymal cells or fibroblasts and is typically composed of the fibrillar-forming collagens (such as collagen types I, II, III, V and XI), which convey great flexibility and tensile strength to these matrices, plus non-collagenous glycoproteins (such as tenascin, fibronectin, vitronectin, chondroitin-, dermatan-, keratan-sulfate proteoglycans), which are highly charged molecules with a high capacity for intermolecular interactions not only with other ECM molecules but also with growth factors and cytokines [8–11]. Specialised interstitial matrix molecules associated with highly elastic tissues include the microfibrillar proteins, such as fibrillin 1 and 2, and elastin. In contrast, basement membranes are highly interconnected glycoprotein networks that act principally to separate tissue compartments. They consist of a scaffold of collagen type IV that is interconnected to a laminin network via molecules such as the heparan sulfate proteoglycan, perlecan, and the nidogens. Apart from these four main components, molecules such as agrin, fibulin-1 and -2, BM-40/osteonectin/SPARC, collagen types VII, VIII, XV and XVIII are minor components of some basement membranes, which nevertheless have distinct functional roles [12].

Laminins, heterotrimeric glycoproteins composed of α , β , and γ chains, are considered to represent the biologically active component of basement membranes, with the α chains carrying distinct cell binding sites that signal specific information to different cell types that controls their growth, migration and differentiation. To date 5α , 4β and 3γ laminin chains have been identified that may combine to form at least 16 different isoforms [13] that are named according to their $\alpha\beta\gamma$ chain composition. For example, laminin 111 is composed of laminin $\alpha 1$, $\beta 1$ and $\gamma 1$ chains [14]. The major receptors for the laminins include $\beta 1$ and $\beta 3$ integrins, and α -dystroglycan of the dystrophin-glycoprotein complex (as reviewed by [15]), all of which interact only with

laminin α chains. Apart from direct signalling to cells via such receptors, laminins and other basement membrane components, in particular the highly charged heparan sulfate proteoglycans, perlecan and agrin, can also function as storage sites for growth factors and cytokines as described for the interstitial matrix glycoproteins above.

Reticular fibres have long been known as argyrophilic structures that are highlighted by silver staining and commonly found in parenchymal tissues such as the liver, bone marrow, lung and kidney, typically underlying epithelia or covering the surface of muscle, adipose and Schwann cells [4,16,17]. Ultrastructurally, these fibres consist of both basement membrane and interstitial matrix components [4,18,19]. Particularly prominent reticular fibres occur in secondary lymphoid organs where, because of their fluid draining function (described below), they have also been termed “conduits”. Conduits in this context are, therefore, reticular fibres with an unusually large diameter (1–2 μm), characterised by an outer basement membrane layer, and a central fibrillar collagen core as a defined substructure. In the following paragraph, we describe the ECM of the spleen, which is particular due to the heterogeneity of molecularly distinct reticular fibres that define different lymphoid compartments.

3. Types of ECM in the spleen

The main splenic structures that contain ECM components are the capsule, trabecules, vascular walls and reticular fibres. A dense connective tissue layer of fibrillar collagen, a typical interstitial matrix, and elastic fibres surround the spleen and encapsulate trabecules and incoming arteries. The functional compartments of the spleen are the white pulp and red pulp which are connected by the marginal zone. Endothelial cell basement membranes are found in the vascular walls of the central artery and branching capillaries of the white pulp, as well as in the venous sinuses of the red pulp. In addition, the endothelial cell basement membrane of the marginal sinus, underlying the marginal zone, shows unique characteristics that are discussed below. Apart from their identification by the immune cells residing in these niches, each of the three splenic compartments – white pulp, red pulp and marginal zone – can also be distinguished by the organization and composition of the basement membrane of the reticular fibre network. This becomes appar-

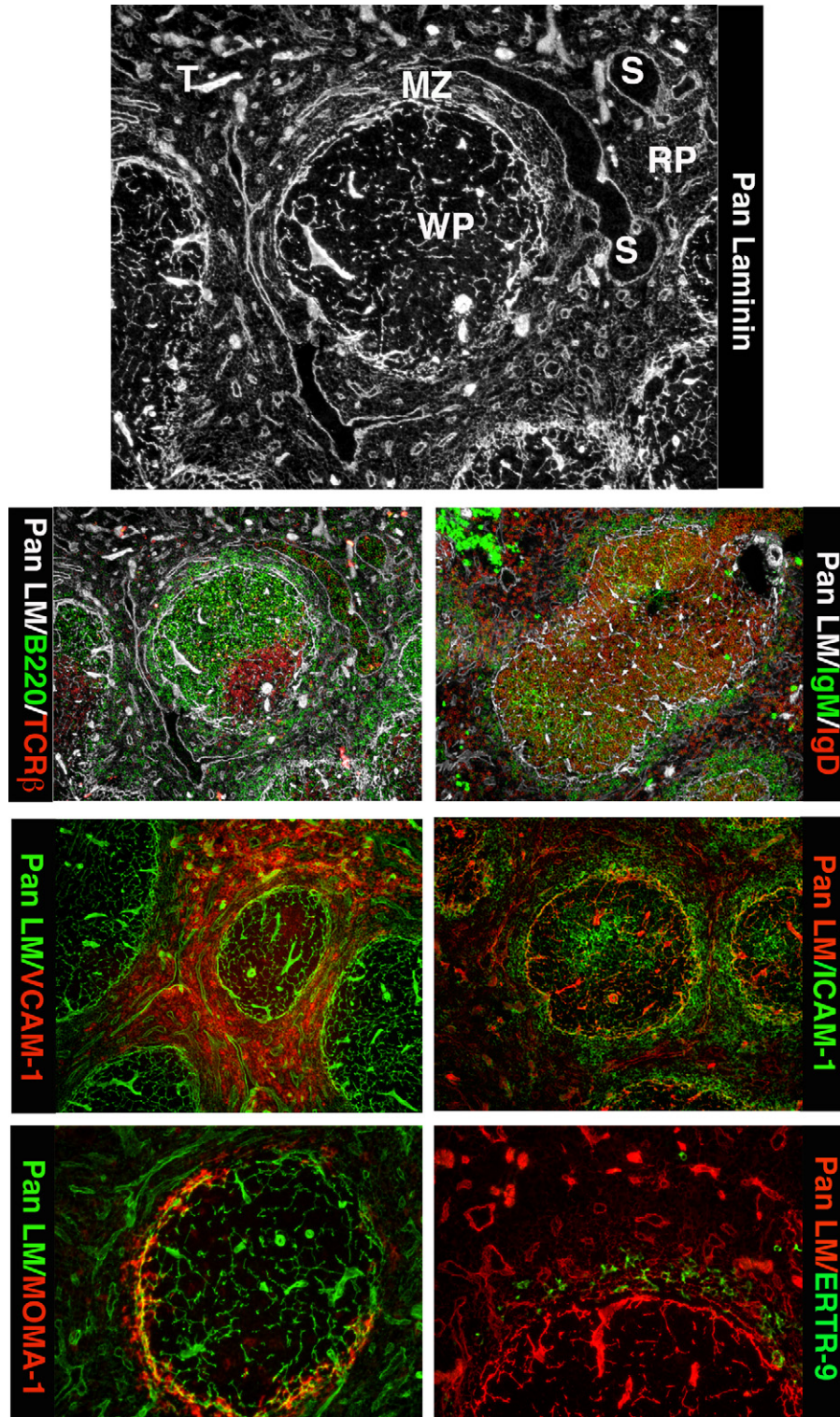


Fig. 1. Pan-laminin immunofluorescence (Pan LM) staining of adult murine spleen in combination with markers of specific immune cell compartments, including B220 for B cells, T cell receptor β (TCR β) for T cells, IgM/IgD to distinguish MZ B cells (IgM^{high}/IgD^{low}) from follicular B cells (IgM^{low}/IgD^{high}), VCAM-1 and ICAM-1 as markers of stromal cell compartments, MOMA-1 as a marker of the metallophilic sinus-lining macrophages, and ERTR-9 as a marker of marginal zone macrophages. The sinus-lining basement membrane appears as quasi-continuous pan-laminin staining surrounding the white pulp. MZ is marginal zone; WP is white pulp; RP is red pulp; S is venous sinus, T is trabeculae.



	Fibre diameter	Collagen core	Microfibril layer	Basement membrane	Fibroblastic reticular cell
LN	1-2 μ m	Coll I/III	ERTR-7	LM 511/411/332	gp38, α SM, Des
Spleen					
WP/PALS	1-2 μ m	Coll I/III	ERTR-7	LM 511/411/332	gp38, α SM, Des
WP/B cell	1-2 μ m	Coll I	-	LM 511/411/211	α SM, Des
MZ	<1 μ m	-	ERTR-7	LM 521/ Agrin	Des
RP	30-50nm	-	ERTR-7	LM ?	gp38, Des

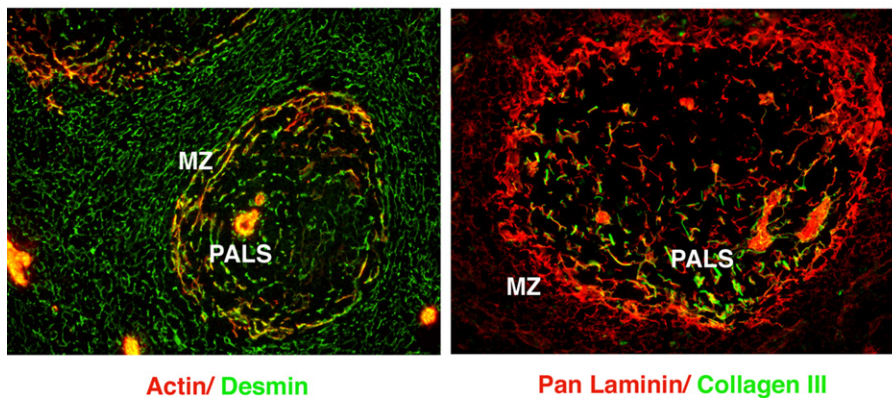


Fig. 2. Schematic representation of the typical structure of a reticular fibre found in the lymph node. Table lists significant differences between reticular fibre networks found in lymph nodes and at different sites in the spleen. In addition to the molecules listed, in all tissues the microfibrillar layer also contains collagen type VI and fibrillins 1 and/or 2, while the basement membrane layer contains collagen type IV, perlecan and nidogens 1 and/or 2. Immunofluorescence images illustrate examples of molecular differences in reticular fibre networks in the spleen. PALS is periarterial lymphoid sheath; MZ is marginal zone.

ent by staining of adult murine spleen sections with antibodies specific for one of the major basement membrane components, such as pan-laminin antibody. Even at low magnifications, the splenic compartmentalisation is evident: The white pulp, delineated by the quasi-continuous marginal sinus-lining basement membrane, contains a network of larger diameter reticular fibres that show wider spacing compared to the filigran, dense reticular fibre network of the marginal zone and red pulp (Fig. 1). Co-staining for basement membranes and markers of B- and T-lymphocytes, and/or macrophage populations specific for the marginal zone and marginal sinus demonstrates a correlation between localisation of immune cells and distinct ECM compartments (Fig. 1), which are explained in detail below.

3.1. White pulp reticular networks (conduits)

The reticular fibres of the T cell area of the splenic white pulp, the periarteriolar lymphoid sheath (PALS), closely resemble the well-described reticular fibres of the T cell cortex of lymph node (Fig. 2). The basement membrane layer of these reticular fibres

is characterised by the presence of laminin isoforms 511, 411 and 332, the heparan sulfate proteoglycan, perlecan, collagen type IV and nidogen 1, while the fibrillar core typically contains collagen types I [20–22] and III [23,24]. These two distinct ECM layers are interconnected by a microfibrillar layer [19,25] characterised by fibrillin 1 and/or fibrillin 2, collagen type VI and, the yet to be defined, ER-TR7 antigen [20,23] (Fig. 2). The entire ECM core is largely ensheathed by reticular fibroblasts that may interdigitate with other cells types, such as dendritic cells as has been shown in the lymph node [20], but also remains naked in certain regions [26].

Although the overall multilayered structure is similar, the reticular fibres of the lymphoid follicle (B cell area) of the white pulp have several unique characteristics that distinguish them from reticular fibres of lymph nodes and PALS. Most notably they show little or no ERTR-7 antigen expression and they lack collagen type III in their core; apart from the characteristic laminins 511 and 411 in the basement membrane layer they also express laminin 211 and not laminin 332 found in the PALS and lymph nodes (Fig. 2) (manuscript in preparation).

The differential expression of collagen types I and III in the reticular fibre core is likely to control fibre diameter, and strength and flexibility of the network. While collagen type I fibrils can form long-range, highly stable structures, the inclusion of collagen type III into these fibrils confers increased elasticity and is associated with reduced fibril diameter [16,17,27]. Collagen type VI, found in the microfibrillar mantle, is a short-chained collagen that forms filamentous networks commonly found in elastic tissues [28,29]. Collagen type VI and the fibrillins have been postulated to interact with both basement membrane and interstitial matrix components and thereby probably form an elastic link between the collagen core and the outer basement membrane of the reticular fibre [30].

The outer basement membrane is most likely to act as an adhesive substrate for the ensheathing fibroblastic reticular cells. Why different laminin isoforms occur in this basement membrane in different reticular fibres is not clear, but several possibilities exist. Laminins 511, 211, 411 and 332 are not only biochemically distinct molecules but are also structurally distinct, as shown by rotary shadowing [15]. This results in large variations in their abilities to form interactions both within a laminin network and with other basement membrane components [15,31], which may influence the tightness of the barrier formed by the basement membrane and thereby influence the conduit function of reticular fibre networks. In addition, these different laminin isoforms impart different cellular signals via several receptors, many of which have been identified on fibroblastic reticular cells [32,33], that control cell growth, differentiation and migration [13,31]. The laminin composition of the basement membrane underlying the fibroblastic reticular cells may, therefore, influence their differentiation state and/or the expression of defined marker molecules, contributing to the observed heterogeneity of this cell population (reviewed in Allen and Cyster, this volume; [34–36]).

Basement membranes normally restrict the movement of large (>70 kDa) and positively charged molecules. This selective barrier to soluble molecules both into and out of the reticular fibre contributes to the fluid-conduction or so-called conduit function that has been demonstrated for reticular fibres of lymph nodes [20–22,37]. Tracer injection experiments in mice [38] have revealed that low molecular weight tracers accumulate in the core of reticular fibres of the B cell area of the splenic white pulp in a similar manner as has been described for the lymph node [20,37], suggesting that it may also provide a fluid transport mechanism. In the lymph node this conduit system has been shown to be connected to the afferent lymphatics and to transport both chemokines (CCL21, CCL19) and small molecular weight antigens [21,22,37] from peripheral sites of inflammation to the high endothelial cell venules (HEVs). Resident dendritic cells which interdigitate with the reticular fibroblasts within T cell areas of lymph nodes are capable of taking up antigen from the core of the conduit, presumably for the presentation to local T cells although this has not yet been conclusively demonstrated [20]. Unlike the lymph node, the splenic conduit system is not connected to an afferent lymphatic system. Rather, there is most likely a tight connection between the vascular system and the conduit system. The central collagen core of the white pulp retic-

ular fibre emerges from the interstitial matrix of the trabecular arteries (unpublished observation). Time course tracer experiments have shown that small molecular weight tracers (<70 kDa) arriving in the bloodstream are deposited in the marginal zone and move rapidly to the red pulp. A small proportion of the incoming tracer, however, moves from the marginal zone in the direction of the center of the follicle within the reticular fibre, where it colocalises with chemokines such as CXCL13 (in the B cell area) and CCL21 (in the PALS) in the conduit core [38]. The white pulp reticular fibre network may, therefore, provide a means of transport of blood borne factors from the blood stream which may influence immune interactions in the lymphoid follicles.

Apart from the conduit function, the reticular fibre network of the lymph node has recently been shown to provide a scaffold which supports the migration of naive T cells and B cells between the afferent lymphatics and HEVs and border of the B cell areas [39]. It is unlikely that the ECM of the reticular fibre network plays a role in this migration process, rather that lymphocytes migrate on the fibroblastic reticular cell layer, which in turn is anchored to the ECM scaffold. The reticular fibroblasts that enfold the splenic conduit fibres have been suggested to bind and present chemokines in the PALS (CCL21) and in the lymphoid follicles (CXCL13) [38], consistent with a role in migration of lymphocytes. As the white pulp reticular fibre network interconnects the marginal sinus with the white pulp and PALS, and the outer sheath of the central artery [18,38] it is possible that, as in lymph node, it provides a route for lymphocytes moving either into or out of the white pulp.

3.2. *Red pulp reticular networks*

Within the spleen, the reticular fibre network of the red pulp differs from that of the white pulp in several ways: reticular fibres of the red pulp have a considerably smaller diameter (30–50 nm) than those of the white pulp (1–2 μ m) [4,40] and they lack the characteristic pronounced fibrillar collagen core (Fig. 2). Rather, the red pulp reticular fibre network consists of a typical basement membrane component (containing collagen type IV, perlecan, nidogen 1 and an as yet undefined laminin isoform), plus an extensive microfibrillar layer containing ERTR-7 antigen, collagen type VI and fibrillin 2 (Fig. 2) (manuscript in preparation). The spatial organization of these two ECM layers to each other and to the stromal cells of red pulp is not clear, due to the absence of appropriate electron microscopic studies. Apart from its function in the retention of aging erythrocytes, the dense red pulp fibre network may provide the stable backbone for red pulp macrophages and other red pulp stromal cells. Its impact on proliferation and differentiation of these cells as well as its direct influence on recirculating lymphocytes remains to be elucidated.

3.3. *Marginal sinus and marginal zone reticular networks*

The marginal zone is described as a layer surrounding the B cell follicles, with the marginal sinus facing to the WP

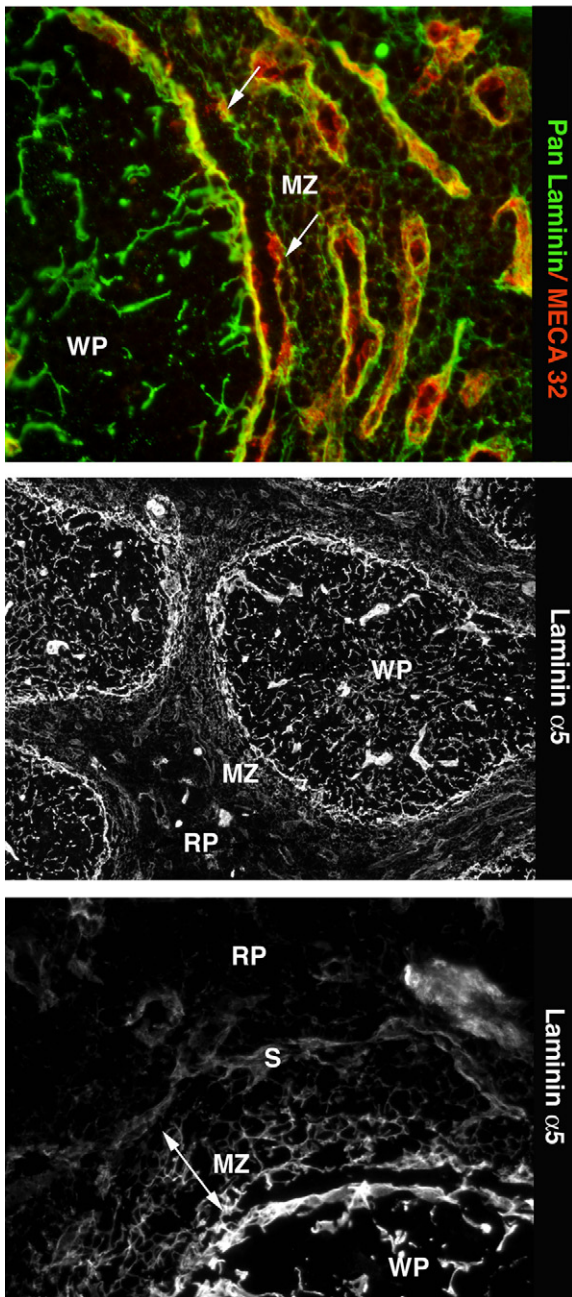


Fig. 3. Immunofluorescence staining for pan-laminin and the endothelial cell marker, MECA-32, showing the sinus-lining basement membrane delineating the border to the white pulp and an outer basement membrane underlying the MECA-32+ endothelial cells of the marginal sinus (marked with arrows). Black and white images illustrate laminin $\alpha 5$ immunofluorescence staining which occurs predominantly in the reticular fibre network of the white pulp and in a diffuse manner in the surrounding marginal zone. The higher magnification image shows the staining of laminin $\alpha 5$ in the marginal zone but not red stroma. Basement membranes of the venous sinus in the red pulp also contain laminin $\alpha 5$. The gap between sinus-lining basement membrane and marginal zone is the outer basement membrane of the marginal sinus vessels as also seen in the uppermost panel.

defining its innermost border and the RP its outermost border and is the only B cell-dependent area in the body where B lymphocytes are not organized in a follicular structure (Figs. 1 and 3).

3.4. Marginal sinus

In the spleen all incoming cells arrive via the central artery. The capillaries of splenic follicles are branches of the central artery in the white pulp that anastomose to form a vascular net covering the white pulp and terminate in the marginal sinus. The contents of the marginal sinus pass into the marginal zone, while the flat sinus-lining reticular fibroblasts characterised by expression of the mucosal addressin cell adhesion molecule (MAdCAM) [41] and the Ig superfamily adhesion molecule member, L1 [42], are located at the inner border of the marginal sinus and separate the white pulp from the marginal zone. A sinus-lining basement membrane occurs at this site containing all components that are typically present in basement membranes, including: laminins (isoforms laminin 511, 521 and some laminin 411 and 421), collagen type IV, perlecan and nidogens 1 and 2 (Fig. 3).

The basement membrane at this site in rats and humans has been described to have a double layered structure, with one basement membrane underlying the endothelial cells of the incoming blood vessels, separating the marginal sinus from the marginal zone, while the other forms the inner border between marginal sinus and white pulp and underlies the MAdCAM positive sinus-lining reticular cells [43–46]. Electron microscopy has identified discontinuities in the two basement membranes: Pores sufficiently large to allow the passage of cells occur in the basement membrane layer bordering the marginal zone which correspond to gaps between adjacent endothelial cells, thereby, permitting the free passage of blood borne leukocytes. In contrast, smaller discontinuities, which would not permit the easy passage of cells, occur both in the basement membrane layer and the sinus-lining reticular cells on the inner border to the white pulp [43–46]. The marginal sinus is discontinuous and varies in width. In the regions where there are discontinuities in the marginal sinus, the white pulp is separated from the marginal zone by the sinus-lining basement membrane alone. It has been speculated that these sites of direct connection also represent sites of lymphocyte migration from the marginal zone into the lymphoid follicle. Pan-basement membrane staining (Fig. 3) suggests a close association between the sinus-lining basement membrane and subjacent white pulp reticular fibre network [43,44], potentially forming a route for lymphocyte migration from the marginal zone into the follicle as described above.

3.5. Marginal zone reticular networks

The marginal zone is described as a region surrounding the B cell follicles, with the marginal sinus defining its innermost border and the red pulp its outermost border and is the only B cell-dependent area in the body where B lymphocytes are not organized in a follicular structure (Figs. 1 and 3). The inner border can be clearly defined by MAdCAM expressing sinus-lining reticular cells and the underlying basement membrane, while the outer border is defined by the distribution of IgM^{high}/IgD^{low} B cells. The reticular fibre network of the marginal zone is characteristically much denser than that in the red pulp or the

white pulp [47] (Fig. 3) and has a unique expression of laminin 521 [7] and heparan sulfate proteoglycan, agrin (manuscript in preparation), plus a dense expression of other basement membrane molecules including collagen type IV, nidogen 1 [48] and perlecan [49]. Some interstitial matrix molecules occur here, including fibronectin [50,51] and the ERTR-7 antigen, but not fibrillar collagens or the typical microfibrillar molecules (fibrillin 1 and 2), indicating an important structural difference to the matrices of the white pulp and red pulp [7,46,52,53]. Unpublished confocal microscopy data suggests that the ECM of the marginal zone does not share the conduit-like structure of white pulp reticular fibre network but rather more closely resembles a classical basement membrane structure, which encases individual cells or groups of cells, resulting in a basket-like structure (Fig. 3). Electron microscopic studies have suggested that there is frequent contact between the reticular fibres of the marginal zone and lymphocytes [46], further substantiating the concept that the reticular fibre network is distinct at this site.

The function of the specialised ECM in the marginal zone is unclear, as is the precise spatial relationship between the ECM and the cells resident at this site, including reticular cells and the VCAM-1+/ICAM-1+ stromal cells [54], marginal zone B cells (MZ B cells), and the ERTR-9+ [55] and MOMA-1+/SER-4+ [56,57] macrophages. The ECM may either provide an adhesive niche for one or more of these cell populations, or it may indirectly influence these cell populations by trapping specific chemokines or cytokines at this site.

It is known that resident stromal cells in the marginal zone constitutively express the cell adhesion molecules, ICAM-1 and VCAM-1. Interactions with these adhesion molecules and $\alpha 4\beta 1$ and LFA-1 expressed on MZ and follicular B lymphocytes play important roles in the long-term retention of MZ B cells at this site [54], and for B- and T-lymphocyte migration into the white pulp [50]. In addition to these adhesive events, a balance between sphingosine-1-phosphate (S1P) and CXCL13 is required for maintaining MZ B cells in the marginal zone. Normally, S1P interactions with S1P-1 receptor are associated with egression of lymphocytes from lymph nodes. However, in the case of MZ B cells, which have high levels of S1P-1 receptor, engagement of the receptor is required for their retention in the marginal zone [58]. This effect is partially due to failure to respond to the chemoattractant, CXCL13, produced in the lymphoid follicle, and upregulation of ICAM-1/VCAM-1-mediated adhesion. However, molecules other than S1P are involved in retention of MZ B cells in the marginal zone, since in the absence of S1P and CXCR5 (the receptor for CXCL13) MZ B cells are still maintained at this site [58]. Whether the specialised ECM of the marginal zone contributes either directly or indirectly to any of the events described above remains to be investigated.

4. Potential function of ECM in the spleen

The close correlation between immune cell localisation and structurally and biochemically distinct ECM compartments in the spleen leads to the question whether the reticular fibre net-

works of the spleen have an impact on immune cell reactions. A major direct effect on the trafficking of the highly dynamic recirculating lymphocytes is unlikely, as most of the reticular fibres are covered by the cellular processes of reticular fibroblasts and/or endothelial cells [18,45,59–61]. However, the few sites where the ECM is exposed [26] it may provide anchorage for sessile leukocyte populations such as MZ B cells, macrophages in marginal zone and red pulp, or resident dendritic cells in the marginal zone and white pulp. In addition, it is likely that the ECM exerts indirect effects on immune cells of the spleen by 1) storage or presentation of cytokines, growth factors or chemokines (as discussed above), and/or 2) as a major element for survival, proliferation and differentiation of mesenchymal and endothelial stromal cells which have been shown to influence trafficking [62–64], differentiation [65,66] and commitment [67] of immune cells in the spleen.

4.1. Cellular sources of ECM in the spleen

There is growing evidence for the heterogeneity of splenic stromal cells with the identification of new markers (reviewed in Allen and Cyster, this volume; [34,35]) Fibroblastic reticular cell populations can be distinguished by antibodies against gp38 (white pulp PALS), smooth muscle actin (white pulp), desmin (white pulp and red pulp) (see Fig. 2), BP-1 (white pulp follicle), ICAM-1 (marginal zone and white pulp), VCAM-1 (marginal zone and red pulp) and antibodies IBL-10 (white pulp) and IBL-11 (white pulp PALS) against unknown epitopes [36]. In the B cell follicle, follicular dendritic cells (FDC) represent another mesenchymal FRC which is not tightly associated with the ECM [68,69]. Also endothelial cells show heterogeneity: Apart from the ubiquitous endothelial expression of MECA-32Ag, Endomucin and PECAM-1 (not sinus-lining endothelium) (unpublished observations), antibodies have been generated that define distinct endothelial cell compartments in the spleen [70,71]. Investigation of correlations between stromal cell and ECM markers will contribute to the understanding of which cells produce the matrix of the reticular fibres in the red pulp, marginal zone and white pulp.

In vitro data from fibroblastic reticular cell lines derived from lymph nodes has shown that these cells are capable of synthesising ERTR-7 antigen, laminins and fibronectin, and that their extracellular deposition is strongly promoted by either contact with lymphocytes or exogenously added $\text{TNF}\alpha/\text{LT}\alpha$ [63], indicating an interplay between immune cells and stromal cells. Macrophages are an additional immune cell located within the stroma that have been postulated to secrete ECM molecules and are certainly sources of ECM modifying proteases and cytokines that can alter ECM secretion by other cells [72,73].

Whether additional stromal cells exist in either the white pulp or red pulp of the spleen which may contribute to ECM structures is not clear. Indeed, studies identifying the precise cells responsible for synthesis of individual matrix components and regulating the ECM deposition and remodelling during secondary lymphoid organ development and homeostasis remain to be performed.

4.2. Animal models for splenic ECM investigation

As discussed above, spleen compartments can now be characterised not only by their immune cell composition, but also by their ECM structure and molecular composition. However, the relationship between ECM and immune cell function remains entirely open to investigation. Due to the complexity of different ECM types and the difficulty in reconstituting extracellular matrices *in vitro* that faithfully reflect the *in vivo* situation, such analyses will require the use of transgenic mice deficient in defined ECM molecules, but also investigation of ECM molecules in mice known to lack specific splenic immune cell or stromal cell populations. Not only will structural analyses be important in these mice, but also studies of chemokine and/or cytokine localisation.

Several transgenic mouse strains exist, which lack ECM molecules normally expressed in different splenic compartments that are viable but have not been examined from point of view of spleen structure or function. These include mice lacking the microfibrillar molecules collagen type VI and fibrillin 1, the small leucine-rich proteoglycans, fibromodulin, biglycan and decorin, expressed in the collagen core of reticular fibres [20], or specific isoforms of basement membrane components such as nidogens 1 and 2, and laminin $\alpha 2$ which occurs exclusively in the reticular fibre network of the B cell area of the white pulp (as described above) (ECM mouse mutants are reviewed in Refs. [74,75]). Although complete elimination of the major basement membrane or interstitial matrix components, such as the laminins, collagen type IV, perlecan and fibronectin (see Refs. [74,75]) results in mouse embryos that do not survive sufficiently long for investigation of the spleen, several tissue-specific knockout mice are currently being generated that will not only provide models for investigation of the significance of splenic ECM but also identification of their cellular source.

Non-ECM transgenic mice which will be particularly important to investigate with respect to ECM expression include mice deficient in the TNF superfamily members, which show anatomical abnormalities in the formation of distinct B and T cell areas as well as the marginal zone [76]. In particular, mice lacking lymphotoxin (LT) α in which T and B cells have no separate areas and are completely intermingled, and LT β deficient mice which show B cells localized in a ring around a central T cell area [76] are likely to provide information on whether ECM is directly or indirectly involved in immune cell compartmentalisation.

5. Conclusion

The spleen is traditionally characterised by its complex immune cell compartmentalisation, but can also be characterised by its unique ECM. Substantial differences have been identified in ECM structure and molecular composition in the red pulp, white pulp and the marginal zone. Most significant are the variations in molecular composition of the reticular fibre networks in red pulp versus white pulp compartments, and the unique expression of laminin 521 isoform and the heparan sulfate proteoglycan, agrin, characteristic of the marginal zone. Differences in localisation of the various ECM molecules in

the spleen suggests that the ECM plays a role in the compartmentalisation of the immune cells to their respective niches, an area which remains to be investigated. It is clear that future analyses of ECM animal models require consideration of possible immunology defects, an aspect rarely considered to date, while chemokine/immunological mouse models cannot overlook the need for analysis of stromal cells and the ECM.

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