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# The Influence of the Extracellular Matrix in Inflammation: Findings from the SPARC-null mouse

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## Abstract

Matricellular proteins are secreted proteins that, among other functions, can contribute to extracellular matrix (ECM) assembly including modulation of cell:ECM interactions. Recent discoveries have indicated a fundamental role for the ECM in the regulation of inflammatory responses including cell extravasation and recruitment, immune cell differentiation, polarization, activation, and retention in tissues. Secreted Protein Acidic and Rich in Cysteine (SPARC) is a matricellular collagen binding protein implicated in fibrillar collagen assembly in the ECM of connective tissue as well as in basal lamina organization. Functions of SPARC in modulating cell adhesion events are also reported. Studies of phenotypic responses observed in SPARC-null mice to a variety of injury models have yielded interesting insight into the functional importance of SPARC production and aberrations in ECM structure that occur in the absence of SPARC that influence immune cell behavior and inflammatory pathways. In this review, we will discuss several examples from different tissues in which SPARC-null mice exhibited an inflammatory response distinct from those of SPARC expressing mice and provide insight into novel ECM-dependent mechanisms that influence these responses.

### Keywords

Matricellular; collagen; immunity; Discoidin domain receptor 2; glycocalyx

## Introduction

Matricellular proteins are defined as extracellular matrix (ECM) associated proteins that function to modulate cell:ECM interactions rather than play central roles in ECM structural complexes. Matricellular proteins include the Secreted Protein Acidic and Rich in Cysteine (SPARC) family, osteopontin, thrombospondins, tenascins, periostin, fibulins, and the CCN family among others (Murphy-Ullrich and Sage, 2014). Characteristically, matricellular

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proteins tend to be highly expressed in developing tissues and exhibit reduced levels of expression in healthy adult tissues. However, expression of matricellular proteins is often elevated in response to injury and/or stress particularly at sites of ECM remodeling. Given the increase in expression of matricellular proteins in injury and disease, the concept that matricellular proteins influence immune cell recruitment and activity is increasingly appreciated. In fact, a number of studies have supported critical and distinct functions for different matricellular proteins in inflammation (Kirk and Cingolani, 2016; Midwood et al., 2016; Rienks and Papageorgiou, 2016; Frangogiannis, 2017; Kim et al., 2018).

As matricellular proteins are secreted into the extracellular space to influence cell:ECM interaction and ECM deposition, the cellular source of these proteins is likely be an important determinant of activity. For example, fibrosis associated with inflammation frequently arises from focal points that progress to larger lesions. Localized expression of structural ECM and matricellular proteins have the potential to drive initiating deposition of disorganized ECM that leads to fibrotic ECM accumulation. Mounting evidence that the structure and composition of the ECM is a critical factor influencing inflammatory cell activation in tissues implies that expression of matricellular proteins by infiltrating and/or resident cells will modulate inflammatory progression and ECM remodeling (Gordts and Esko, 2018; Stephenson and Yong, 2018; Yamauchi et al., 2018). SPARC (BM40/osteonectin) is a secreted, collagen-binding protein shown to be an important modulator of procollagen processing and collagen fibril assembly in connective tissues. In addition, expression of SPARC is closely associated with fibrotic events. Notably, SPARC-null mice are unable to mount a fibrotic response in heart, lung, liver, kidney and skin (Trombetta-Esilva and Bradshaw, 2012). As fibrosis is almost always accompanied by inflammatory cell expansion, a provocative concept is that inflammatory cells provide an important cellular source of SPARC. Recently, we reported that macrophages recruited to the myocardium following pressure-overload induced hypertrophy, a model of cardiac fibrosis, express SPARC (McDonald et al., 2018).

The diversities of activities attributed to SPARC and differences observed in tissue-specific outcomes in SPARC-null mice complicate the task of neatly packaging the function of SPARC into a simple biological sound bite. For example, in many fibrosis injury models SPARC-null tissues including peritoneum, skin, lung, and liver, exhibit reduced collagen levels in association with a reduction in tissue macrophages (Trombetta-Esilva and Bradshaw, 2012). Hence, SPARC is implicated in facilitating monocyte recruitment and/or macrophage differentiation and tissue retention. In contrast, in studies using different tumor models, SPARC-null mice are reported to display increases in tumor-associated macrophages versus WT mice perhaps reflecting differences in tumor-associated vasculature as well in tumor stromal ECM that affect monocyte behavior (Arnold et al., 2010) (Sangaletti et al., 2003). Finally, increased numbers of macrophages were also observed in SPARC-null mice afflicted with myocarditis (Rienks et al., 2018).

In this review, we will discuss the influence of SPARC on macrophage polarization in culture and focus on the functional significance of increased SPARC expression at sites of injury with an eye to the consequences of the lack of SPARC expression in terms of ECM assembly, cell:cell and cell:ECM interaction. The review is not meant to serve as

an inclusive list of inflammatory outcomes shown to be influenced by SPARC expression, but to provide examples representing the importance of SPARC-dependent processes in extracellular events that ultimately affect immune responses in different tissues.

## Macrophage Polarization

In a recent study by Toba et al., the effects of SPARC expression on age-related cardiac inflammation in a murine model were examined (Toba et al., 2015). Global deletion of SPARC suppressed age-related increases in collagen content, wall thickness, and age-related myocyte hypertrophy. Interestingly, SPARC-null mice also displayed a shift in the production of proinflammatory cytokines in aged hearts that was associated with a decrease in the cardiac macrophage population. To further examine the relationship between SPARC and macrophage protein expression, peritoneal macrophages isolated from young mice were cultured with and without exogenous addition of SPARC. Cells exposed to SPARC were found to have increased levels of cytokines indicative of pro-inflammatory M1-type macrophages, whereas a reduction in the expression of markers attributed to the M2-type, anti-inflammatory, macrophage, was found (Toba et al., 2015). Assessment of proteins with known expression by macrophages in aged myocardium demonstrated an increase in M1-type transcripts in SPARC-null versus wild-type (WT) hearts. Several markers of M1-type macrophages increased in an age-dependent manner in WT mice, however significant differences in these markers were not detected in SPARC-null mice with age. Likewise, markers of M2 macrophages were reduced in aged WT mice, whereas no significant age-dependent reductions in M2 markers were found in SPARC-null hearts.

Hence, the expression of SPARC was suggested to contribute to macrophage polarization toward an M1-type phenotype, and because SPARC levels increased with age, older animals were predicted to have an increase in pro-inflammatory type M1 macrophages in the myocardium. The authors proposed a model in which the absence of SPARC altered cytokine expression and resulted in decreased macrophage numbers with age. Both the absence of SPARC and the reduction in the macrophage population were thought to reduce overall levels of collagen and ECM deposition in the aged myocardium. The mechanism by which SPARC influences macrophage polarization is currently unknown, however, further studies into SPARC activity on macrophage phenotype will likely lead to insight into the regulation of age-related inflammation and fibrosis.

## Extracellular Matrix: Connective Tissue Fibrillar Collagen

In addition to direct effects of SPARC predicted on macrophages, SPARC also influences ECM composition and structure. Hence, the function of SPARC in ECM assembly affords an opportunity to uncover unique functions of the ECM in the regulation of inflammation when viewed through the point of view of the inflammatory response in SPARC-null mice. Accordingly, phenotypic differences in SPARC-null ECM structures have lead to interesting insight into prospective roles of collagen networks in immune cell behavior and activation.

With the exception of a curly tail, SPARC-null mice are indistinguishable from WT littermates. However, closer inspection of some tissues, particularly those rich in connective

tissue, reveal significant differences in ECM structure. In the skin, for example, collagen fibrils are smaller and more uniform in diameter versus those of WT mice. The dermal collagen content is also reduced (Bradshaw et al., 2003). Experiments in cultured fibroblasts have revealed a role of SPARC in procollagen processing and deposition *in vitro* (Rentz et al., 2007). How might reductions in fibrillar collagen content and size influence immune cell behavior?

Sangaletti et al. reported that dendritic cell migration in the skin of SPARC-null mice is accelerated in comparison to WT mice (Sangaletti et al., 2005). Thus, T-cell priming in response to stimulation, occurs more rapidly in SPARC-null mice by virtue of the fact that dendritic cells are able to migrate more quickly through a dermis with fewer and thinner collagen fibers. Using bone marrow transplant, the authors were able to show that increased dendritic cell migration in the absence of SPARC was not attributed to a lack of SPARC expression by dendritic cells but instead to the intrinsic differences in the ECM assembled in the absence of SPARC (Sangaletti et al., 2005). The function of SPARC as a regulator of collagen deposition, and possibly cross-linking, suggests that Langerhans cell migration in SPARC-null skin is enhanced due to a less dense collagenous ECM, allowing the cells to reach the lymphatic vessels more efficiently while also requiring less ECM degradation for movement. This, in turn, accelerates T cell priming by hastening Langerhans dendritic cell migration in a cell nonautonomous manner.

Because the collagen content and fibril morphology is significantly affected by the absence of SPARC, the level of collagen-binding moieties in the extracellular space are likely to be altered as well in SPARC-null tissues. For example, proteoglycans such as the small leucine-rich proteoglycans (SLRP) family that includes decorin, biglycan, lumican and fibromodulin bind to collagen fibrils and fibers in the ECM. Hence, reductions in the size and number of collagen fibrils and fibers will influence amounts of SLRPs accommodated in the ECM. As SLRPs are known to play important roles in inflammatory processes, the changes in the profile of SLRPs in SPARC-null connective tissue is likely to also influence inflammatory cell behavior (Stephenson and Yong, 2018). For example, Arnold et al. reported a reduction in levels of decorin expression in the ECM surrounding pancreatic tumors grown in SPARC-null mice (Arnold et al., 2010). As SLRPs such as decorin can serve as ECM-derived DAMPs that activate pro-inflammatory pathways, one might predict that reductions in decorin in SPARC-null ECM would lead to decreased inflammation at site of ECM turnover. These authors reported however, that in tumors grown in SPARC-null mice, levels of infiltrating alternatively activated macrophages were actually increased in comparison to tumors grown in WT mice despite the observation that decreases in levels of decorin were noted in tumors grown in SPARC-null versus WT mice (Arnold et al., 2010). In this study, differences in macrophage infiltration were suggested to arise from increased vascular permeability of the SPARC-null tumor vasculature, a phenotype likely to arise from differences in basal lamina collagen IV organization and/or changes in the endothelial glycocalyx.

## Basal Lamina and the Endothelial Glycocalyx

The function of SPARC in ECM deposition is also significant to basal lamina assembly through the capacity of SPARC to bind to collagen IV. In *Drosophila melanogaster*, for example, the assembly of the basal lamina surrounding fat body cells is dependent upon SPARC expression. When SPARC production is perturbed, basal lamina ECM components, including collagen IV, accumulated in the pericellular space and resulted in the rounding of the adipocytes in the fat body (Shahab et al., 2015). Interestingly, in experiments performed in *C. Elegans*, Morissey *et al*. reported that both over and under expression of SPARC influenced collagen IV deposition in basal lamina (Morrissey et al., 2016). Over-expression of SPARC in worms was linked to a lack of collagen IV in the basal lamina and resulted in enhanced cell invasion while a lack of SPARC led to an interruption in delivery of collagen IV to cells distant from the origin of expression, similar to *Drosophila*. One scenario is that SPARC binds to a specific site on collagen IV necessary for network assembly so that collagen IV bound by SPARC remains soluble for transport to basal lamina distant from cells of origin. A lack of SPARC would result in inefficient transport of collagen IV to sites of assembly. Conversely, over-expression of SPARC might result in decreased collagen IV incorporation to insoluble networks due to a saturation of binding sites necessary for collagen IV assembly and/or stability.

In mammals, evidence for a function of SPARC in basal lamina assembly is represented by early onset cataractogenesis in SPARC-null mice that was associated with aberrant basal lamina morphology assembled by lens epithelial cells particularly with respect to collagen IV distribution (Yan et al., 2002). Disruptions in basal lamina integrity in tumor associated vasculature were also apparent in SPARC-null versus WT mice. Sangaletti *et al*. reported that the absence of SPARC expression by stromal cells, but not bone-marrow derived cells, in a mammary tumor model led to significant increases in leukocyte infiltration surrounding the tumor (Sangaletti et al., 2003). The increase in leukocyte invasion was attributed to aberrant collagen IV assembly in tumor-associated stroma that allowed a more permissive environment for inflammatory cell recruitment. Hence, mechanistically, aberrant basal lamina assembly might give rise to a more permissive environment for extravasation of inflammatory cells.

Another extracellular network produced by endothelial cells implicated in immune cell recruitment is the glycocalyx. The glycocalyx resides on the apical surface of endothelial cells and is composed of proteoglycans and glycosaminoglycans. This outer coating provides an important point of contact in the capture and recruitment of circulating leukocytes. Rienks *et al*. used a murine model of cardiac myocarditis to demonstrate an increase in leukocyte recruitment to the myocardium associated with a reduction in the endothelial glycocalyx in SPARC-null hearts (Rienks et al., 2018). These authors hypothesized that SPARC is critical for proper assembly and/or production of the glycocalyx and that decreases in glycocalyx integrity, brought about by the absence of SPARC, resulted in enhanced adhesion of circulating immune cell and subsequent increases in leukocyte extravasation to the myocardium.

Studies of both basal lamina and glycocalyx suggest that SPARC-null endothelium might provide a more permissive environment for the recruitment of immune cells during inflammatory events. However, as pointed out above, in several scenarios, SPARC-null mice exhibit a reduction in macrophage populations in inflamed tissues with the exception of myocarditis-induced inflammation in the heart and in several tumor-bearing models. Hence, likely distinct mechanisms of inflammatory cell extravasation are in place in different settings and/or tissue-specific differences in basal lamina/glycocalyx composition influence immune cell invasion and response in a site-specific manner.

## ECM Compartmentalization

Another key point of immune cell regulation that centers on ECM assembly is illustrated by phenotypic changes in secondary lymphoid organs (SLO) that were apparent in SPARC-null mice. Rempel *et al.* first reported that spleens from SPARC-null mice demonstrated alterations in structure including an increase in white pulp and a reduction in marginal zones (Rempel et al., 2007). In WT mice, SLO compartments that gave rise to lymphoid and myeloid lineages were neatly defined and separated by collagenous ECM components. Sangaletti *et al.* showed that in SPARC-null mice, aberrant collagen assembly led to poor compartment separation and differentiation of immune cells (Sangaletti et al., 2014). For example, activated neutrophils that came in contact with CD5+ B cells in the poorly organized SLOs in SPARC-null mice gave rise to malignant transformation of B cells due to increased contact with neutrophil-derived factors versus WT conditions. On neutrophils, leukocyte associated immunoglobulin like receptor I (LAIR-1) is an immune inhibitory receptor. LAIR-1 binds to collagen IV and sends a “self signal” to limit apoptosis in these cells. In a Fas mutant autoimmune SPARC-null model, the reduction in ECM led to a lack of inhibitory signaling down-stream of LAIR-1 binding to collagen IV which primed neutrophils to undergo NETosis, a process in innate immune infection in which decondensed chromatin and granule containing proteins are released to the extracellular space (Sangaletti et al., 2014). Thus, another example of the importance of proper ECM assembly in immune cell function is represented in SPARC-null mice.

## SPARC and Cell Surface Receptors

Evidence for increased levels of fibrillar collagen I and basal lamina collagen IV preferentially associated with SPARC-null cell surfaces in comparison to those of WT cells suggested that SPARC might interfere with binding to cell surface collagen receptors. An intriguing candidate in this regard are the Discoidin Domain Receptors (DDR) 1 and 2. DDRs are collagen binding tyrosine kinase receptors that undergo slow activation when bound by collagen (Borza and Pozzi, 2014). DDR1 is expressed primarily by cells of epithelial origin and with a greater affinity for collagen IV whereas DDR2 is more highly expressed in mesenchymal cells and binds preferentially to collagen I. Interestingly, DDR2 and SPARC bind collagen at the same GVMGFO motif (Giudici et al., 2008; Hohenester et al., 2008). Because DDR2 and SPARC bind at the same location on collagen, these proteins are predicted to compete for binding to collagen. In SPARC-null cells, an increase in collagen engagement by DDR2 might increase cell-associated collagen. A similar scenario is also possible for SPARC and collagen IV in basal lamina. Tethering of collagen to the

cell surfaces by virtue of increased DDR engagement might impede collagen deposition to insoluble ECM and thus lead to decreased collagen content and ECM disorganization characteristic of SPARC-null tissues. Interestingly, DDR2 has been characterized as a critical receptor for mediating cell migration in cell types such as fibroblasts, dendritic cells, and neutrophils, particularly in 3D cultures (Poudel et al., 2012; Afonso et al., 2013; Majkowska et al., 2017). Engagement of collagen by DDR2 led to down-stream increases in matrix metalloproteinase (MMP) expression that was hypothesized to facilitate movement of cells in 3D. The absence of SPARC would then be predicted to increase DDR2 engagement of collagen and thus induction of increased MMPs. An increase in MMPs would be expected to increase collagenolytic activity and perhaps contribute to reduced amounts of collagen in the ECM.

Historically, SPARC is known to induce endothelial and epithelial cell rounding when added to cells in culture (Sage et al., 1989). The cellular mechanism by which SPARC influences cell rounding *in vitro* is not completely defined but may include interference with integrin engagement to the ECM (Barker et al., 2005). In lens epithelial cells, for example, SPARC was shown to interact with β1 integrins and integrin linked kinase (ILK) to enhance cell survival (Weaver et al., 2008). On endothelial cells, VCAM-1 is a cell adhesion receptor expressed that forms either a homomeric attachment or binds to integrin α4β1 on adjacent cells to facilitate endothelial barrier function. SPARC has been reported to interact with the extracellular domain of VCAM-1, thereby releasing the homomeric association between VCAM-1 molecules and thus creating a break in the endothelial barrier (Kelly et al., 2007). In fact, binding of SPARC to VCAM-1 induced alterations in the actin cytoskeleton of endothelial cells that was followed by the formation of an intercellular gap. SPARC binding to VCAM-1 is anticipated to also alter binding to integrin α4β1 and thus might influence integrin signaling on endothelial cells as well. SPARC expression by leukocytes was thus one mechanism proposed to ease extravasation of recruited cells through the endothelial layer to the underlying tissue. SPARC-null mice were used to validate that irregular leukocyte migration was observed in the peritoneum of SPARC-null mice in an inflammatory model. As pointed out above, in the majority of injury models carried out in SPARC-null mice, a reduction in the level of macrophages in inflamed tissues has been reported which would suggest that SPARC expression might facilitate cell extravasation. On the other hand, bone-marrow transplant studies performed in SPARC-null mice do not support an absolute requirement of SPARC for leukocyte recruitment in some tissues.

## Conclusion

The majority of phenotypic characteristics described to date in SPARC-null mice reside in defects in ECM assembly, composition and/or deposition. Studies that have evaluated the profile of circulating immune cells in SPARC-null mice have not indicated significant differences in the profile of circulating immune cells versus that of WT. Successful bone-marrow transfer experiments using SPARC-null donors also support that bone marrow produced in the absence of SPARC is sufficient to repopulate and engraft irradiated WT mice. Recently, Tanaka et al. reported that T-cells derived from SPARC-null mice were not able to support a robust inflammatory response in RAG1 mice challenged in a colonitis model of inflammation (Tanaka et al., 2018). Whether expression of SPARC by

T-cells was required for the inflammatory response and/or that T-cells differentiated in the absence of SPARC were intrinsically less capable of eliciting an inflammatory response, was not directly assessed in this study. Hence, possibly SPARC expression in some immune cell populations is also critical to modulate activity. Notably, SPARC production by macrophages might influence polarized cytokine expression as well.

The capacity of SPARC to bind both fibrillar collagens, such as type I and III, as well as the primary basal lamina collagen, collagen IV, clearly leads to alterations in connective tissue as well as basal lamina in SPARC-null mice. Interestingly, the alterations that arise from ECM assembled in the absence of SPARC affect inflammatory response illustrating the critical role of the ECM in modulating immune responses. SPARC is also reported to bind to VCAM, thereby promoting endothelial cell de-adhesion to facilitate extravasation of recruited leukocytes. Future experiments in which cell-type specific knock-down of SPARC expression is assessed in response to inflammatory stimuli will undoubtedly shed more light into the differing functions of SPARC in inflammatory processes and in specific immune populations. To date, the variety of elegant experiments that have evaluated inflammation and immune system activity in SPARC-null mice has provided valuable clues as to the ECM-dependent mechanisms, that influence inflammatory response and tissue recovery.

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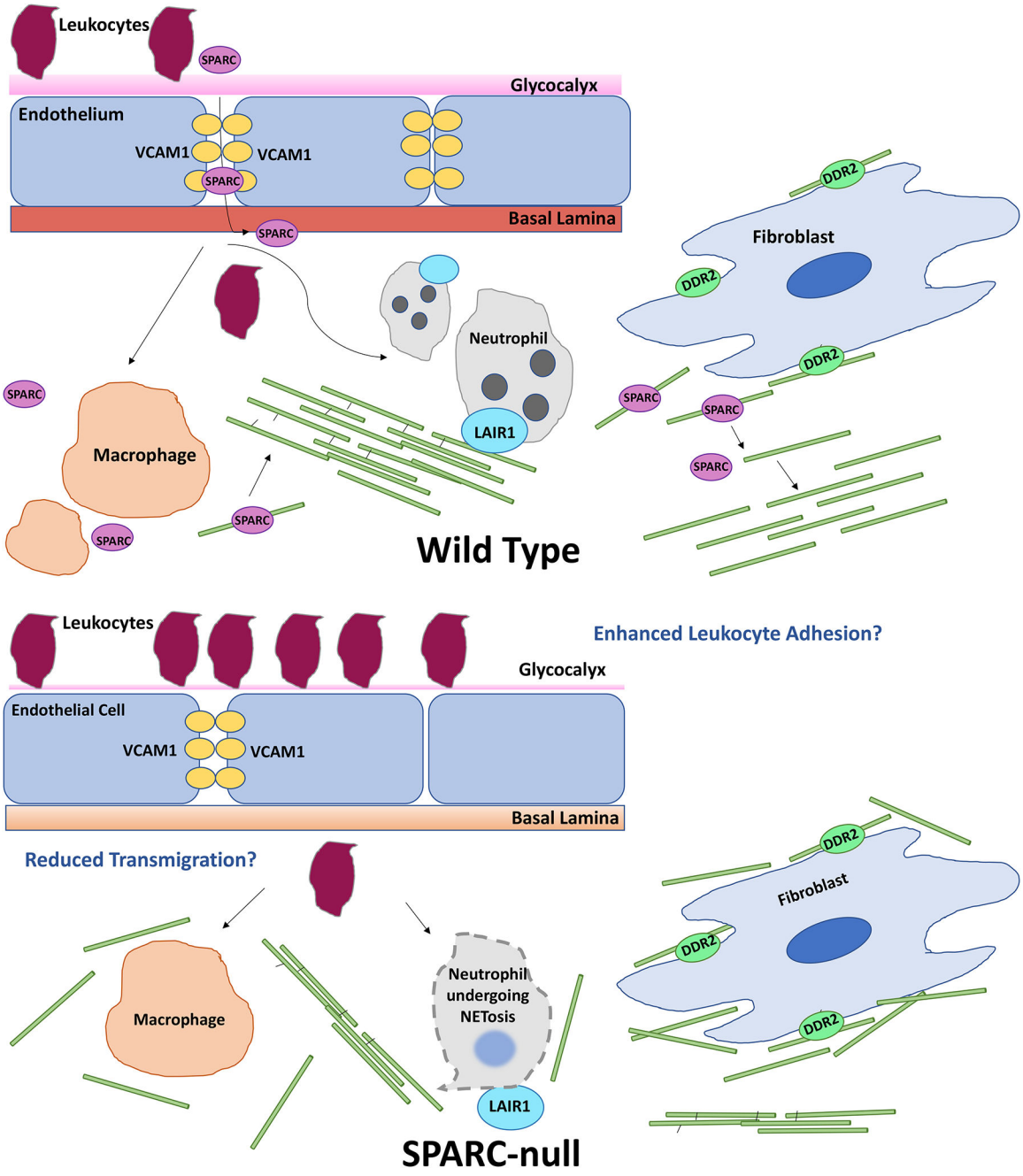


**Figure 1.**

Proposed roles of SPARC in cell:cell and cell:extracelluar matrix (ECM) interactions that influence inflammation.

A). In wild-type (WT) conditions, SPARC interacts with the extracellular domain of VCAM1 to facilitate leukocyte transmigration. SPARC competes with DDR2 for collagen binding on fibroblasts. Collagen bound by SPARC transports away from the cell surface and is incorporated into insoluble cross-linked ECM. Structural ECM integrity preserves tissue compartmentalization in secondary lymphoid organs. LAIR1 binds to collagen fibers in the ECM and prevents neutrophil cell death via NETosis. Proper assembly of the basal

lamina and the endothelial glycocalyx occur in the presence of SPARC. (B) In the absence of SPARC (SPARC-null), leukocyte extravasation via disengagement of VCAM1-mediated endothelial cell adhesion is compromised. Engagement of collagen fibers on fibroblast surfaces by increased DDR2 engagement limits deposition of insoluble collagen. Decreased collagen content and ECM disorganization in SPARC-null tissues leads to decreases in LAIR1 binding thus enhancing neutrophil NETosis and impairing the CD5+ B cell population. Aberrant basal lamina and glycocalyx assembly lead to vascular leakiness and increased leukocyte association with an activated endothelium.