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Review

Decidual stromal cells: fibroblasts specialized in immunoregulation during pregnancy

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Decidual stromal cells (DSCs) are involved in immunoregulatory mechanisms that prevent fetal rejection by the mammalian maternal immune system. Recent studies using single-cell RNA sequencing demonstrated the existence of different types of human and mouse DSCs, highlighting corresponding differentiation (decidualization) pathways, and suggesting their involvement in the immune response during normal and pathological pregnancy. DSCs may be considered tissue-specialized fibroblasts because both DSCs and fibroblasts share phenotypic and functional similarities in immunologically challenged tissues, especially in terms of their immune functions. Indeed, fibroblasts can initiate, support, or suppress immune responses and these functions are also performed by DSCs. Moreover, fibroblasts and DSCs can induce ectopic foci as tertiary lymphoid structures (TLSs), but also contribute to endometriosis. Thus, understanding DSC immunoregulatory functions is of timely relevance.

Highlights

Mammalian decidual stromal cells (DSC) control trophoblast invasion into the decidua during pregnancy and play a role in maternal–fetal immune tolerance.

Recent single-cell RNA sequencing (ScRNAseq) studies demonstrated the existence of different DSC populations with different functions: angiogenesis, immunoregulation, and involvement in the onset of labor.

ScRNAseq analysis also implicated DSCs and endometrial stromal cells (EnSC) in immune-mediated obstetric and gynecological pathologies, such as recurrent pregnancy loss (RPL), preterm birth, pre-eclampsia, and endometriosis.

Secondary lymphoid organ (SLO) fibroblasts and non-SLO fibroblasts set up, support, and suppress immune responses.

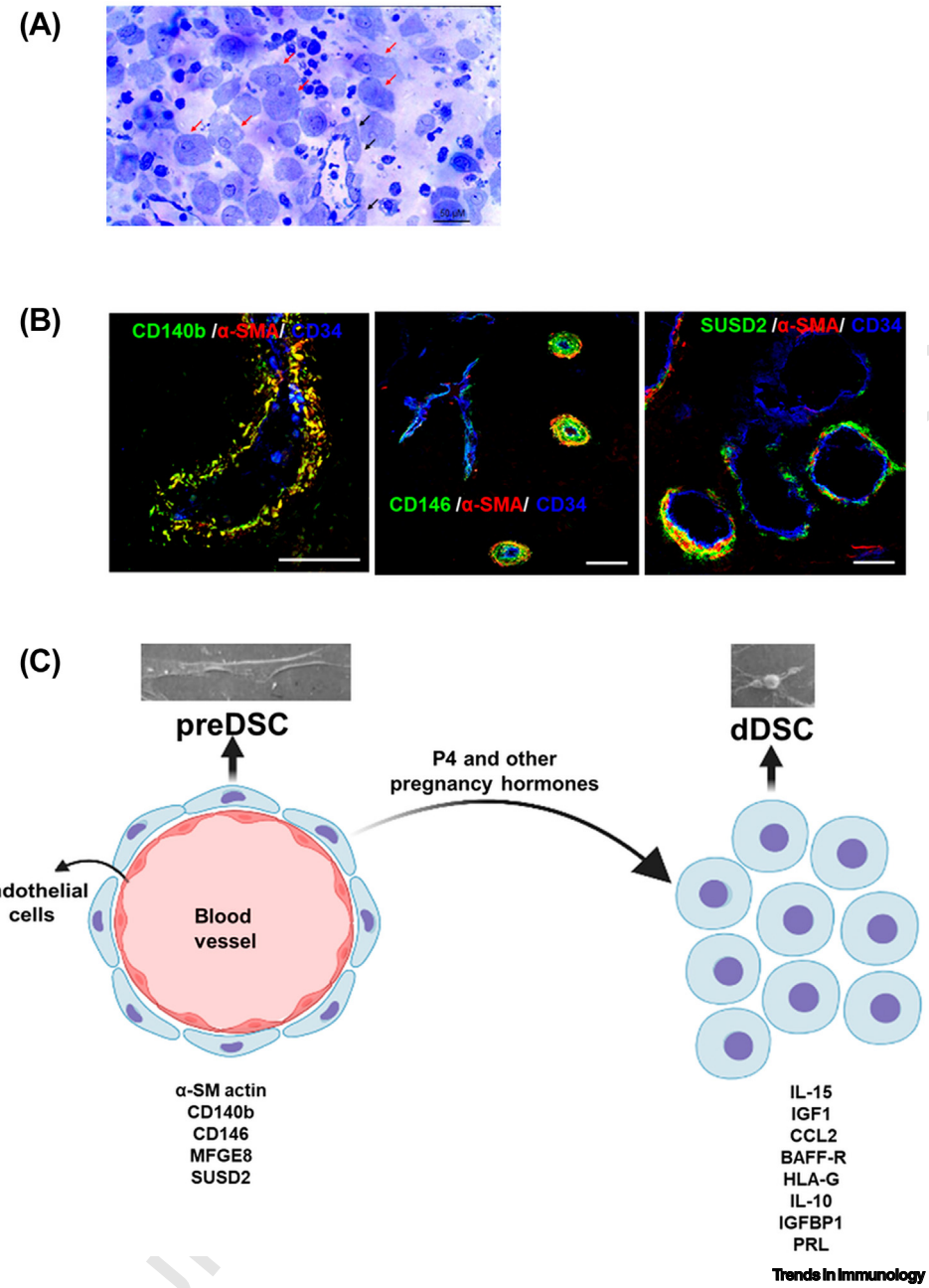
Functional and transcriptomic studies and antigenic phenotyping provide evidence of a close relationship between DSCs and fibroblasts of immunologically active tissues.

Significance

Human decidual stromal cells (DSCs) can be considered tissue-specialized fibroblasts, because they share phenotypic and functional similarities with fibroblasts of immunologically challenged tissues, particularly in terms of immune functions. The identification of common functions and molecules responsible for these activities may help to further understand the physiology of these stromal cells interacting with immune cells during pregnancy and non-pregnancy. This may help uncover potential targets for treating diseases associated with DSCs.

DSCs and pregnancy homeostasis

It is well-established that DSCs are the most abundant cell type in first-trimester human decidua (≈50%) (Figure 1A), the maternal part of the **placenta** (see Glossary), which is in close contact with the fetal **trophoblast**. The decidua is derived from the non-pregnant **endometrium**, which differentiates into the decidua through the effects of progesterone (P4) and other pregnancy hormones when pregnancy occurs. This process, called decidualization, involves all endometrial/decidual cell types, and prepares the endometrium for **implantation** of the **blastocyst**. Pregnancy can be considered a semi-allogeneic graft in which the maternal immune system establishes various local and systemic mechanisms to prevent fetal rejection [1] (Box 1). In mice and humans, DSCs are involved in the control of trophoblastic invasion into the decidua and participate in local immunoregulatory activities by interacting with different decidual immune cells (Box 2) [2–5]. Single-cell RNA sequencing (ScRNAseq) technology has made it possible to distinguish different types of DSCs and **endometrial stromal cells (EnSCs)** – the endometrial counterpart of DSCs – and to infer their possible functionality and interactions with decidual or endometrial immune cells [6–8]. ScRNAseq has also provided insights into the involvement of DSCs in labor onset [9–12] and in various obstetric and gynecological pathologies [13–15]. DSCs are derived from perivascular precursors (preDSCs) with fibroblastic morphology which, under the effect of P4 and other pregnancy hormones, differentiate (decidualize) into rounded or polygonal cells that leave the vessels and occupy extravascular spaces [decidualized DSCs (dDSCs)] (Figure 1) [16]. dDSCs secrete prolactin (PRL) and insulin-like growth factor-binding protein1 (IGFBP-1), both of which are considered to be markers of decidualization [17]. The establishment of human DSC lines from first-trimester human decidua has made it possible to study the antigenic phenotype and some of the functions of these cells [18–20] (Table 1). In the absence of decidualizing factors (P4 + cAMP) in the culture medium, DSC lines consist of preDSCs, cells with fibroblastic morphology that do not secrete PRL. However, when P4 and cAMP are added to the culture medium *in vitro*, preDSCs decidualize into



Q2 Figure 1. Decidual stromal cells (DSCs) in first-trimester human decidua. (A) The representative micrograph shows a semithin cryostatic section of early human decidua stained with toluidine blue, indicating the DSC precursors (preDSCs, black arrows) and decidualized DSCs (dDSCs, red arrows). Scale bar: 50 μm (B) The representative micrographs show perivascular α-SM actin-positive cells (yellow) coexpressing CD140b, CD146, and SUSD2. Endothelial cells are stained with anti-CD34-antibody (blue). Scale bars: 100 μm. (C) The cartoon depicts decidualization from preDSCs to dDSCs. The expression of molecules is characteristic of each stage of differentiation. Top shows scanning electron micrographs of a preDSC and a dDSC from a cultured DSC line. Abbreviations: α-SM actin, alpha-smooth muscle actin; BAFF-R, B cell activating factor receptor; IGF1, insulin-like growth factor 1; IGFBP-1, insulin-like growth factor-binding protein1; MFGE8, milk fat globule-epidermal growth factor 8; P4, progesterone; PRL, prolactin; SUSD2, sushi domain-containing 2. Figure 1B created with BioRender.com. Figure 1A from [18], with permission. Figure 1B from [16], with permission.
Q1 Figure 1C, scanning electron micrographs from [119], with permission.

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Box 1. The decidua and maternal immune response during mammalian pregnancy

Many of the mechanisms of maternal–fetal immune tolerance, although occurring in the decidua, extend their effects to the systemic maternal immune system [98]. One of these mechanisms has been thought to be based on the T helper 1–T helper 2 (Th1–Th2) cell balance. A role of Th1 cells has been associated with spontaneous abortion, because these cells activate cytotoxic lymphocytes – similar to the immune response in organ transplantation – which attack fetal tissues [99]. In contrast, Th2 cells have been associated with normal pregnancy because they inhibit Th1 differentiation [100]. However, the Th1–Th2 cell paradigm has been viewed as being too simplistic with the discovery of new subsets of Th cells [1]. Moreover, a report suggested that a certain degree of inflammation along with Th1 cells, rather than being detrimental, can play a physiological role in certain stages of normal pregnancy, such as implantation and parturition [39]. These findings support the concept that inflammation plays a role in physiological processes [101]. Nevertheless, recent views maintain that, with the exception of the beginning (implantation) and end of pregnancy (parturition), when inflammation and Th1 cells play a role, Th2 cells – as part of broader type 2 immune response – are key elements in maternal–fetal tolerance during the long intermediate period of pregnancy. Th2 cells can block the abortigenic activity of Th1 cells during this stage, irrespective of the contribution of other Th subsets [39,98]. The importance of a Th1–Th2 balance is exemplified by the fact that normal pregnancy ameliorates certain Th1-mediated diseases, while increasing susceptibility to infection by intracellular pathogens (where immune defense depends on Th1 cells) and the worsening of certain Th2-associated diseases. These observations also suggest that a Th1–Th2 balance is not limited to the decidua, but may have a systemic effect on the immune system of pregnant women [39,98].

dDSC, and *in vivo*, become rounder, secreting PRL and IGFBP-1 (Figure 1) [18]. The antigenic phenotype and morphology of preDSC and dDSC lines correlate with those of perivascular cells and extravascular DSCs of the first-trimester decidua, respectively, as observed via confocal microscopy (Table 1, Figure 1B) [16,21] and ScRNAseq [6]. Furthermore, DSC lines have a functional profile that seems to be equivalent to that of primary DSCs, as defined by ScRNAseq in mice and humans, including for myofibroblast-related cells [22,23]; this has also been noted during angiogenesis [6,8,18], immune cell recruitment, and immunosuppression to achieve pregnancy homeostasis [2,8], or to induce apoptosis [8,24] or tumor cell cytolysis [8,16].

PreDSCs constitute the perivascular niche of the decidua, which is also observed in the endometrium. From this niche, the cells secrete cytokines and growth factors and interact with endothelial cells that they surround, promoting angiogenesis. PreDSCs also interact with decidual immune cells and the extracellular matrix, attracting peripheral blood immune cells to the decidua [25,26]. dDSCs, located in the extravascular space, also form a niche where they crosstalk with decidual immune cells, epithelial cells, and trophoblasts [27–29]. Through these homeostatic mechanisms (i.e., angiogenesis, immunoregulation, and interactions with epithelial cells and trophoblasts) DSCs can support embryo implantation [30–32].

Decidualization induces changes in cell morphology, tissue location, as well as DSC antigenic phenotypes and functions

Decidualization is essential for pregnancy to proceed. It is a multi-step process of differentiation that affects all cells of the endometrium and decidua, but is particularly evident in DSCs as they differentiate from preDSCs to dDSCs. This process involves a change in cell morphology from a fibroblastic appearance to a polygonal or rounded shape, along with a change in tissue localization, where preDSCs leave the perivascular niche to become extravascular dDSCs (Figure 1) [21]. Additionally, decidualization also changes the antigenic phenotype of preDSCs, as dDSCs downmodulate the expression of α-smooth muscle (SM)-actin, CD140b, CD146, and sushi domain-containing 2 (SUSD2) (all four **pericyte** markers) (Figure 1). Upon decidualization, dDSCs in their extravascular location downregulate the expression of these pericyte molecules involved in interactions with endothelial cells [6,16,18]. Obviously, changes that the DSCs undergo during decidualization are accompanied by corresponding changes in function, particularly in DSC immune activities: among the molecules produced by DSCs (and involved in interactions with decidual immune cells), IL-15, insulin-like growth factor 1 (IGF1), CCL2, B-cell activating factor (BAFF)-R, and CXCL12 increase with decidualization [16,33–36], while CXCL9, CXCL10, CXCL11, and CCL5 decrease [2,4]. Other molecules expressed or secreted by DSCs during

Glossary

Blastocyst: a structure that begins to form 5 days after oocyte fertilization in humans. Approximately 7 days later, the blastocyst undergoes implantation and becomes embedded in the endometrium. The embryo and trophoblast are derived from the blastocyst.
Cellular niche: a specialized microenvironment of cell–cell and cell–extracellular matrix component interactions to support the growth and differentiation of specific cell types.
Cellular senescence: a state of cessation of cell division and secretion of proinflammatory molecules.
Endometrial stromal cells (EnSC): cells of the non-pregnant endometrium that are equivalent to DSCs. During the menstrual cycle, EnSCs are also decidualized by the effects of ovarian hormones; however, if pregnancy occurs, the EnSCs continue as DSCs (Box 5). 'Uterine stromal cells' is a collective term for both DSCs and EnSCs.
Endometriosis: presence of endometrial tissue outside the uterus, most commonly in the peritoneum and on the ovaries. This recurrent chronic inflammatory disease causes pelvic pain and infertility.
Endometrium: the inner layer of the mammalian uterus. It differentiates under the effects of P4 and estrogen and becomes receptive to implantation of the blastocyst. If pregnancy occurs, the endometrium continues its differentiation (decidualization) to become the decidua. If pregnancy does not occur, this tissue is eliminated with menstruation.
Graft-versus-host disease: in bone marrow transplantation, rejection of the recipient's tissue by the donor's immune cells.
High endothelial venules (HEVs): specialized venules for lymphocyte migration with plump endothelial cells, which are present in secondary lymphoid organs (with the exception of the spleen).
Implantation: adhesion and invasion of the endometrium by the blastocyst 7 days after fertilization in humans.
Pericytes: cells that surround microvascular endothelial cells and regulate vascular structure and homeostasis. Pericytes produce angiogenic factors and exhibit contractile, chemotactic, phagocytic, and immunoregulatory activities.

Box 2. Immune cell composition of the human decidua

The human decidua is composed of epithelial and endothelial cells, leukocytes, and DSCs (Figure 1A). Under physiological conditions, the leukocyte content of first-trimester decidua is unusually high for a noninflammatory tissue (30–40% of all decidual cells) [102]. The most abundant immune cells are distinctive $CD56^{brigth}$ $CD16^{-}$ NK cells: decidual NK (dNK) cells (~70% of all decidual leukocytes in early human decidua) [103], of which three subsets have been identified [6], with their main function seeming to be the control of trophoblastic invasion and vessel development in the decidua [104,105]. Macrophages are the next most abundant leukocyte in first-trimester decidua (~20% of all decidual leukocytes) [106]. M1-like (proinflammatory) and M2-like (anti-inflammatory) macrophages have been identified by ScRNAseq and mass cytometry [6,106]. T cells (~10%) [106] include $CD4^{+}$ and $CD8^{+}$ T cells, along with Tregs [6]. Small populations of DC1 and DC2 dendritic cells, and group 3 innate lymphocyte cells (ILC3s), have also been detected [6,106]. Two subsets of ILC3s have also been identified: natural cytotoxicity receptors $(NCR)^{+}$ and NCR^{-} ILC3 [77]. The proportions of first-trimester leukocytes evolve throughout pregnancy. Although there are discrepancies across different publications, the general consensus is that dNK cells reach a maximum number in the first trimester and progressively decrease until term, while T cells steadily increase until term [106]. Despite the abundance of lymphocytes, the existence of stromal cells (SCs) with immune activities, and the presence of high endothelial venules (HEVs) [107] (Figure I) – all of which are characteristic of SLOs – the human decidua cannot be considered an SLO. It lacks the T and B cell compartmentalization that is typical of these organs and, in addition, is a site of antigenic capture rather than antigen presentation [108].

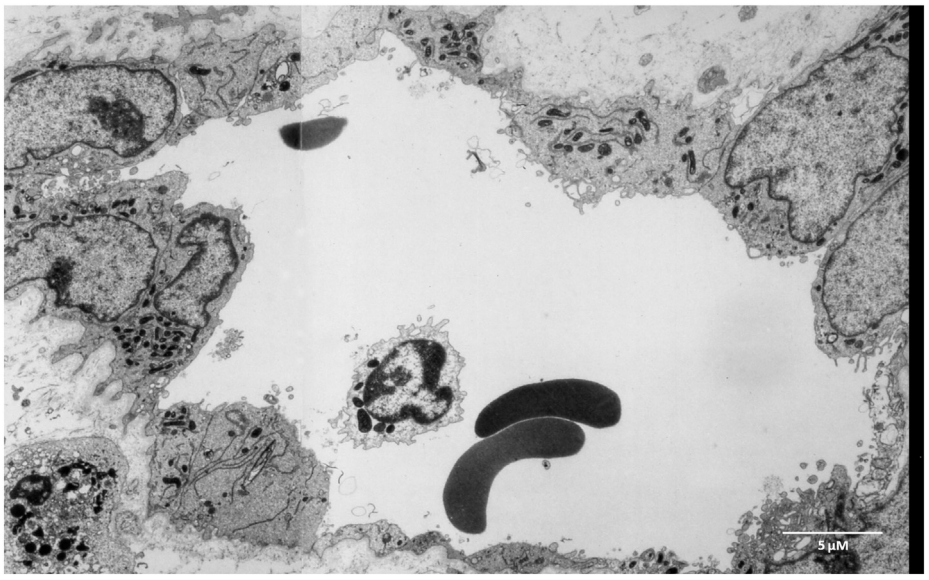

Figure I. Representative electron micrograph of a high endothelial venule (HEV) from a first-trimester human decidua. Scale bar: 5 µm.

Placenta: organ that connects the mother to the fetus. It provides oxygen and nutrients to the fetus and removes waste products. The placenta consists of two interconnected parts: the decidua, of maternal origin, and the trophoblast, of fetal origin. During physiological pregnancy in humans, the trophoblast invades the vessels of the decidua – a process controlled by decidual immune cells.

Recurrent pregnancy loss (RPL): the loss of two or more consecutive pregnancies before 24 weeks of gestation.

Trophoblast: fetal part of the placenta derived from the outer cell layer of the blastocyst. Trophoblast cells are involved in embryo nourishment and implantation. They also invade the decidua and replace blood vessel endothelial cells. This process ensures adequate blood supply during pregnancy.

Type 2 immune response: adaptive immune response centered on differentiated Th2 cells and the secretion of a distinct repertoire of cytokines, including IL-4, IL-5, and IL-13. The type 2 immune response promotes antihelminthic immunity, suppresses type 1-driven immune responses, and regulates wound repair and tissue regeneration.

decidualization that contribute to maternal–fetal immune tolerance processes include HLA-G, which may block dNK cell cytotoxicity, and IL-10, which promotes a **type 2 immune response** [37]. In general, decidualization induces an immunoregulatory profile in dDSCs [2,37]; a biphasic process, it involves the transition of DSCs from a proinflammatory phase that is associated with preDSCs, to an anti-inflammatory phase that is associated with dDSCs [2,38]. While the inflammatory activity of preDSCs promotes the implantation process [39], dDSCs – which expand during the post-implantation period – contribute to the development of a type 2 immune response that favors maternal–fetal immune tolerance [2,5,40,41].

Decidualization and subpopulations of uterine stromal cells (SCs)

Although studies with DSC lines have provided some valuable insights into antigenic phenotypes and functions, to our knowledge, this approach has not adequately identified the various cellular

Table 1. Antigen expression by human bone marrow MSC, preDSC, and preFDC lines obtained and maintained in equivalent culture conditions, as determined by flow cytometry [16,18–20,35,76,112][a]

Antigen[b]	BM-MSCs	preDSCs	preFDCs
CD10[c]	+	+	+
CD13	+	+	+
CD15	–	–	–
CD19[d]	–	–	ND
CD29	+	+	+
CD31	–	–	–
CD34[d]	–	–	–
CD44	+	+	+
CD45[d]	–	–	–
CD62P	–	–	ND
CD73[d]	+	+	+
CD90[d]	+	+	+
CD105[d]	+	+	+
CD140b[e,f,g]	ND	+	+
CD146[e,f]	+	+	–
α-SM actin[f]	+	+	+
BAFF[h,i]	–	+	+
CXCL12	ND	+	+
CXCL13[h]	–	+	+
Cytokeratin	ND	–	ND
HLA-DR[d]	–	–	–
HLA-G	–	+[g]	ND
ICAM-1[g]	+	+	+
MFGE8[h]	ND	+	+
Nestin	+	+	+
Podoplanin	+	+	+
Prolactin[h]	+	+	+
STRO-1[f]	+	+	+
SUSD2[e,f]	+	+	ND
VCAM-1[g]	+	+	+

[a]From [21], with permission.
[b]BM-MSCs, bone marrow mesenchymal stem cells from bone marrow aspirates; ND, not determined; preFDCs, precursors of follicular dendritic cells from tonsillectomies.
[c]Endometrial stomal cell marker [113].
[d]Antigens meeting the minimal criteria for identification as mesenchymal stem cells [50].
[e]Endometrial mesenchymal stem cell marker [51].
[f]Pericyte marker [114].
[g]Immunofibroblast marker [69,70].
[h]Follicular dendritic cell marker [115].
[i]Under decidualization conditions.

steps in the decidualization process [16]. In contrast, ScRNAseq technology has facilitated a more precise analysis of cellular pathway(s) in the differentiation process, as well as of cell–cell communications [25]. This technology has been used to study decidualization in human and mouse endometrium and decidua, where different numbers of subpopulations or clusters of

EnSCs or DSCs (uterine SCs) have been identified [7,25–29]. Stemming from the gene expression profiles of these cells, the functionality of each cluster can be inferred, and the interactions of uterine SCs with other cells (i.e., immune, endothelial, epithelial, and trophoblast cells) can be defined in their respective **cellular niches** [7,25–29]. Among the many DSC interactions and functions identified thus far, here we focus specifically on immune responses. Although some differences in the results obtained have been noted across ScRNAseq studies, several trends are consistently observed: (i) a gradual increase in the expression of the decidualization markers PRL and IGFBP1 in the DSC pathway [6,8,10,42], (ii) the presence of clusters involved in immune responses [8,22,43], and (iii) a decrease in these clusters as decidualization progresses [6]; these results are consistent with those reported in DSC lines [2]. The presence of human DSC clusters that are enriched for inflammation-associated genes, termed inflammatory DSCs (iDSCs) [22], and of $CD24^+$ DSCs [43], has been observed. Similarly, in mice, a cluster of DSCs that express genes related to immune responses was identified and named immune-featured DSCs (also iDSCs) [8]. ScRNAseq technology has also implicated human and mouse DSCs during parturition [9–12]. In pathologies involving altered decidualization, such as **endometriosis**, antiphospholipid syndrome, **recurrent pregnancy loss (RPL)**, and pre-eclampsia [44–47], an immunoactivating response seems to predominate and can negatively affect pregnancy [2,38] (Box 3).

Decidualization, senescent uterine SCs, and dNK cell embryonic biosensing

Decidualization of human EnSCs progresses along a continuous trajectory towards **cellular senescence**, resulting in the formation of a subpopulation of senescent EnSCs (snEnSCs). These are P4-resistant cells that express abundant extracellular matrix proteins, secrete proinflammatory cytokines and chemokines, and also induce secondary senescence in neighboring EnSCs, impairing interactions with trophoblast cells and hindering subsequent embryo implantation [48]. Experimental evidence suggests that under normal conditions, endometrial NK cells

Box 3. Uterine stromal cell (SC) pathology

ScRNAseq has indicated the involvement of uterine SC subpopulations in recurrent pregnancy loss (RPL). In general, an increase in SC clusters with enriched expression of genes important in cell apoptosis and senescence [13,28], as well as in immune responses [22,43,47], has been observed. In addition, interactions of DSCs with inflammatory decidual macrophages, as well as with dNK cells, have been demonstrated [22]. Other work shows that DSCs from RPL fail to induce the differentiation of naïve $CD4^+$ T cells into regulatory T cells (Tregs) [43]. A relevant finding in RPL was that there was defective DSC decidualization [28]. Moreover, a pre-pregnancy study assessing the endometrium of patients with RPL showed an increase in snEnSCs, along with a deficiency in endometrial NK cells, relative to non-RPL controls; this supported the notion that the failure of endometrial NK cells to eliminate snEnSCs can lead to miscarriage [13]. In a mouse model of immune-based RPL, a DSC cluster enriched with chemokine genes was detected, whereas vascularization of a second DSC cluster and cytolytic functions of a third DSC cluster were inhibited. This led to abnormal immune cell enrichment and inflammation, along with impaired vascularization and cytolysis, ultimately resulting in pregnancy loss [8]. Similar findings have been observed in other obstetrical pathologies such as preterm labor, defined in humans as labor occurring between 20 and 37 weeks of gestation. In a mouse model of preterm labor, DSCs were enriched for genes related to leukocyte migration and chemotaxis [109]. In human preterm labor, differences in gene expression compared to normal parturition have also been detected in DSCs and certain immune cells [110].

Ectopic foci in endometriosis consist of endometrial tissue and leukocytes, which trigger a local inflammatory response [92]. In ectopic areas, EnSCs may attract and interact with leukocytes (mainly macrophages), thereby contributing to the development of endometriotic lesions. Interactions between EnSCs and macrophages seem to be essential for the maintenance of these foci [26,94]. Accordingly, ScRNAseq technology showed that two clusters of decidualized EnSCs along with endometrial M1-like and M2-like macrophage populations were enriched for the expression of genes associated with endometriosis risk variants. These results suggested dysregulated EnSC–macrophage homeostasis, potentially contributing to the characteristic inflammation seen in endometriosis [26,94]. Other single-cell studies have also reported EnSC and immune cell dysregulation in endometriosis (e.g., EnSC clusters with enriched expression of genes important for immune responses and senescence), as well as a reduction in endometrial NK cell numbers compared to controls [45,46,111]. The association of increased EnSC senescence with decreased endometrial NK cell populations may indicate an impaired ability of these latter cells to clear senescent EnSCs (SNEnSCs), which in turn might contribute to the inflammation associated with endometriosis [13,45,46].

eliminate these snEnSCs by granule exocytosis to limit the potentially deleterious effects of excessive inflammation induced by snEnSCs in a future pregnancy [13,49]. Moreover, this process seems to be part of a broader mechanism of embryo biosensing by dNK cells. *In vitro* studies have demonstrated that low-quality embryos secrete substances (such as high-molecular-weight hyaluronic acid) that inhibit the cytotoxic activity of endometrial NK cells on snEnSCs. This inhibition may increase the number of snEnSCs, promoting an excessively inflammatory environment that may impede pregnancy [31]. Senescent DSCs have also been found in human pregnancy, where DSC subpopulations exhibit high expression of genes related to cell apoptosis and senescence. This suggests that a similar mechanism for the elimination of senescent DSCs by dNK cells might also operate during normal pregnancy [28,30] (Box 3).

DSCs are related to mesenchymal stromal/stem cells (MSCs)

Human preDSC lines meet the minimal criteria established by the International Society for Cellular Therapy to define human MSCs [50], namely, expression of CD73, CD90, and CD105, and lack of expression of CD19, CD34, CD45, and HLA-DR (Table 1), adherence to plastic culture dishes, and the capacity to differentiate into adipocytes, osteoblasts, and chondrocytes [19]. In addition, preDSCs express CD140b, CD146, and SUSD2, which are markers of endometrial MSCs (eMSCs) that can be detected in the non-pregnant endometrium (Table 1) [21]. PreDSCs and eMSCs share several characteristics, including perivascular localization, an antigenic phenotype, a clonogenic and self-renewal potential, as well as the capacity to differentiate into mesenchymal lineages *in vitro* [19,21,51]. Like MSCs, DSCs also show immunoregulatory activities both *in vitro* and *in vivo*; furthermore, they have shown certain therapeutic effects for some immune-related diseases [19,52,53] (Box 4). The similarity between preDSCs and MSCs suggests that DSCs or their endometrial counterparts, EnSCs, might potentially originate from bone marrow MSCs. These latter cells may colonize the endometrium and decidua, where the molecular microenvironment might enable them to fully acquire the characteristics of uterine SCs [20,35]. Several lines of evidence support this possibility. Bone marrow-derived MSCs can differentiate *in vitro* via the DSC pathway [54]. Donor-derived EnSCs have also been identified in women having undergone bone marrow transplantation [55] – a finding that was also confirmed in mouse models [56,57] (Box 5). Additionally, in both mice and humans, endometrial inflammation and pregnancy can mobilize bone marrow MSCs into circulation, and bone marrow-derived cells, likely stemming from MSCs, have been shown to colonize the uterus, proliferate, and differentiate into SCs, thus contributing to the regeneration of the endometrium [58–60]. This migration is mediated by CXCL12 [61]. Furthermore, transcriptomic analysis of human EnSCs, demonstrated similarities between these cells and MSCs [62]. Experimental evidence suggested that MSCs and pericytes likely constitute the same population, although this remains to be demonstrated [63]. Accordingly, like

Box 4. Decidual stromal cells (SCs) and endometrial SCs may be the same cells under different contexts and with different decidualization capacities

Although DSC and EnSC can be considered the same cell type under different physiological situations (pregnant endometrium or decidua, and non-pregnant endometrium, respectively), there is considerable confusion in the literature regarding their nomenclature. Some authors refer to EnSCs as non-decidualized cells and to DSCs as decidualized cells. These terms do not accurately reflect the location and differentiation status of these cells, given that the process of decidualization takes place in both the endometrium and the decidua, and therefore there are precursor and differentiated cells in both the endometrium (preEnSCs and dEnSCs) and the decidua (preDSCs and dDSCs) [26]. Furthermore, DSCs have a greater capacity for decidualization than EnSCs, as evidenced by the characteristics and molecules associated with the process of decidualization: secretion of PRL and IL15, round morphology, and apoptosis [6,7,35]. These differences are probably due to the different environments (non-pregnant endometrium vs. pregnant endometrium) in which EnSCs and DSCs are found, respectively, making it necessary to clearly differentiate one cell type from the other [21,35]. The fact that it is the decidua and not the endometrium that faces the allogenic challenge of the trophoblast suggests that immunological and cellular mechanisms may occur in the decidua that determine the functional differences between SCs in these two tissues.

Box 5. Decidual stromal cell (SC) therapy

Although the therapeutic effects of bone marrow MSCs are promising because of their immunoregulatory properties, the results of clinical trials have been inconsistent. This may be due to the lack of a standardized method for sample collection, and the possibility that cell preparations contain mixtures of different subpopulations with different activities. In addition, *in vitro* expansion is required for clinical use, and culture procedures may induce cell differentiation, leading to the loss of stem properties [119]. Furthermore, bone marrow aspiration is a painful procedure, prompting interest in alternative tissues such as the placenta or menstrual blood, where SCs are more easily accessible. DSCs have been proposed as an alternative to MSCs, not only because their immunoregulatory activities suggest a therapeutic potential – especially in Th1 cell-mediated diseases [2] – but also because they can be obtained painlessly from term placentas [89]. The therapeutic effect of DSCs [3] has been demonstrated in several murine models and human clinical trials. In a mouse model of Th1-mediated recurrent miscarriage (RPL) using female CBA/J mice mated with male DBA/2 mice – characterized by a high rate of embryonic resorption – inoculation of human DSCs into pregnant CBA/J mice significantly reduced the abortion rate [19,53]. Other reports also documented the beneficial effects of human DSCs on steroid-refractory graft-versus-host disease (a Th1-mediated process) in humans (i.e., a greater therapeutic efficacy than bone marrow MSCs) [52,120]. This finding suggests that DSCs can be a potentially important component of cell-based therapies to treat certain immune-mediated diseases. In fact, DSCs have also shown promising results for the treatment of coronavirus disease 2019 (COVID-19)-induced acute respiratory distress syndrome [121]. EnSCs – DSC-equivalent cells in the non-pregnant endometrium – have also demonstrated numerous therapeutic effects. In this regard, EnSCs derived from menstrual blood are a potential noninvasive, easily obtainable source of these cells, which might be used in autologous therapies [122].

pericytes, preDSCs express pericyte markers, are detected in perivascular locations, and exhibit cell contractility [18]. In summary, we posit that DSCs can be considered cells of mesenchymal origin (i.e., uterine fibroblasts) that perform immune functions to support pregnancy.

DSCs as specialized fibroblasts

Although there seem to be no fibroblast-specific markers, antigens such as CD140b, podoplanin (PDPN), CD90, and α-SMA are commonly associated with fibroblasts. These cells are also characterized by the absence of molecules associated with other lineages, such as endothelial (CD31), epithelial (EPCAM), or hematopoietic (CD45) cells [64]. Of note, human preDSCs exhibit this antigenic profile (Table 1) [21]. Fibroblasts constitute a heterogeneous population that is difficult to classify yet which is widely distributed throughout the mammalian organism. These cells are not only involved in tissue repair and organization, but also perform immunoregulatory functions [65]. One of the best studied types of fibroblasts are those found in secondary lymphoid organs (SLOs), known as fibroblastic reticular cells (FRCs), which display distinctive characteristics. These cells interact with immune cells, facilitate their differentiation and survival, regulate some of their responses, and stimulate the activation of an adaptive immune response to prevent the dissemination of pathogens in the body [66]. Classically, three major FRC subpopulations have been well defined: follicular dendritic cells (FDCs), located in the B-zone of lymphoid follicles; T-zone reticular cells (TRCs; in the parafollicular region); and marginal reticular cells (in the marginal zone). Recently, additional stromal subpopulations were identified via ScRNAseq [67]. Fibroblasts distributed throughout the rest of the body are referred to as non-SLO fibroblasts or tissue fibroblasts. There is considerable evidence that these fibroblasts are also actively involved in the immune system [65]. In fact, in local immune responses such as infection, chronic inflammation, transplantation, or cancer, proinflammatory cytokines can activate tissue fibroblasts, causing them to acquire immune characteristics, and have been termed immunofibroblasts. These cells are also responsible for the formation of TLSs at sites of chronic inflammation (see later) [64,65,68,69].

Since the decidua is not an SLO (Box 2), DSCs should be classified in the group of non-SLO fibroblasts. Within this group, preDSCs are comparable to immunofibroblasts. Murine and human immunofibroblasts express PDPN, CD140b, ICAM-1, VCAM-1, and RANK-L, as well as CXCL10, CXCL13, CCL5, CCL19, and BAFF [69,70], all of which are also expressed by preDSCs (Table 1) [2,21] (T. Llorca, doctoral thesis, University of Granada, 2024). Like tissue

fibroblasts that undergo differentiation into immunofibroblasts in response to local immune signals, MSC-related preDSC progenitors, when exposed to the decidua-associated immune response (Box 1), may also differentiate into a form of immunofibroblast. Indeed, tissue fibroblasts in mice differentiate into immunofibroblasts under the effect of IL-13 and IL-22 [69]. In human decidua, IL-13 is produced by dNK cells [41], and IL-22 is produced by DSCs, dNK cells [71], and $CD4^+$ T cells [72].

Reports suggest that immunofibroblasts are phenotypically and functionally similar to FRCs [69,70]. Thus, DSCs also seem to be related to FRCs, particularly to FDCs. Several lines of evidence support these relationships. Regarding the origin of FRCs, during human and mouse embryonic development, FRCs are derived from lymphoid tissue organizer (LTo) cells, which are located in perivascular sites and are related to MSCs. LTo cells interact with lymphoid tissue inducer (LTi) cells, which are a distinctive type of innate lymphocyte cell [73]. This LTo–LTi interaction contributes to attracting B and T lymphocytes, as well as DCs, into the SLO primordium, and to further differentiate into LTo cells, which then express adhesion molecules ICAM-1 and VCAM-1, thus contributing to the organization of lymphoid tissues [74]. In adults, FRC precursors are also related to perivascular cells or pericytes [75,76]. Like the embryonic LTi–LTo interaction in the SLO primordium for lymph node formation [73], decidual group 3 innate lymphoid cells (ILC3) interact with DSCs. This interaction increases the expression of adhesion molecules ICAM-1 and VCAM-1 on DSCs, suggesting that these cells are involved in leukocyte attraction and, presumably, tissue remodeling [77]. In addition, there is evidence of phenotypic and functional similarities between DSCs and FDCs, as summarized in Tables 1 and 2 [18–20,75,78]. Lastly, the posited relationship between DSCs and FDCs is supported by transcriptomic analysis of human FDCs, EnSCs, and MSCs, confirming that FDCs and EnSCs are closely related and that both cell types are also related to MSCs [62]. Despite their similarities, DSCs are not the same as FDCs; these two types of cells are comparable but exhibit characteristic differences [21,115].

Table 2. Comparison of phenotypic and functional characteristics of human preDSCs and preFDCs[a]

Criteria considered	preDSCs	preFDCs	Refs
Antigenic phenotype (see Table 1)			[16,18–20,35,76,112]
-Endometrial stromal cell marker	+	+	
-MSC/pericyte markers	+	+	
-eMSC markers	+	+	
-FDC markers	+	+	
Perivascular location of $CD140b^+$ $MFGE8^+$ precursors	+ (Decidua)	+ (Secondary lymphoid organs)	[18,75,76]
Mesenchymal differentiation into adipocytes, osteoblasts, and chondrocytes	+	+	[19,50,76,116]
Decidual differentiation into prolactin-secreting cells	+	+	[20]
Inhibition of B cell apoptosis	+	+	[18,20,76]
Cell contractility	+	+	[18,112,117][b]
Chemotactic activity	+	+	[2,16,18,76]
In vitro immunoregulatory activity	+	+	[20,76,112,116]
Hematopoietic cell supportive activity	NK cells	B cells	[20,76,112,118]
Presence in ectopic sites	+[c]	+[d]	[69,92]

[a]From [21] with permission.
[b]TRCs are also contractile cells.
[c]Induced by endometrial stromal cells.
[d]FDC-like cells from immunofibroblasts.

DSCs set up, support, and suppress decidual immune responses

According to one report, fibroblasts can help initiate, govern, and moderate certain immune responses [64]. Similarly, the general functions of FRCs and non-SLO fibroblasts have been reported to set up, support, and suppress immune responses [65]. For setup, fibroblasts secrete chemokines that attract immune cells, thereby helping to organize SLOs (in the case of FRCs), or TLSs (in the case of non-SLO fibroblasts in chronic inflammation) [65]. For support, fibroblasts secrete antiapoptotic factors and induce immune cell differentiation [65]. For suppression, fibroblasts can regulate immune responses to mediate immune tolerance [65]. FRCs display their functional activities mainly on T and B lymphocytes, whereas non-SLO fibroblasts have a more evident regulatory effect on innate immune cells [65].

Like FRCs and non-SLO fibroblasts, DSCs can perform the general functions of fibroblasts, setting up, supporting, and suppressing an immune response (Figure 2). For setup, DSCs participate in the recruitment of peripheral blood NK (pbNK) cells by secreting chemokines CX3CL1, CXCL10, and CXCL12 [79]. PreDSCs secrete CXCL9, CXCL10, and CXCL11, which attract Th1 and Tc lymphocytes from peripheral blood [2]. Although these T cells are potentially abortigenic, they may be important in the defense against infection and during the inflammatory phase that is associated with implantation [39]. Nevertheless, Th1 and Tc chemotaxis can be controlled by dDSCs during the anti-inflammatory phase of decidualization [2] (see later). For support, DSCs secrete antiapoptotic factors affecting dNK, pbNK, and peripheral blood T cells [16]. DSCs also induce differentiation from pbNK to dNK cells through the effect of transforming growth factor β1 (TGF-β1), IL-15, IL-18, and IL-24 [80–82]. Additionally, DSCs can interact with decidual $CD34^{+}CD45^{+}$ hematopoietic progenitor cells and induce their differentiation into dNK cells [80,83]. These two possible origins of dNK cells may not be mutually exclusive, because different subsets of dNKs have been identified [6]. Upon secreting macrophage colony-stimulating factor, DSCs also favor the differentiation of monocytes into M2-like (anti-inflammatory) macrophages, which are predominant in human decidua [84]. For suppression, DSCs drive dNK to a non-cytotoxic state by producing prostaglandin E_2 (PGE_2), indoleamine 2 3-dioxygenase (IDO) [85], TGF-β [81], IL-24 [82], IL-33 [41], and IGF1 [36]. Decidual dendritic cells (dDCs) are maintained in an immature state by the effects of PGE_2, IDO, and macrophage inhibitory cytokine-1 (MIC-1) that are secreted by DSCs [85,86]. Immature DCs exhibit tolerogenic activity; indeed, first-trimester dDCs produce little IL-12, promote differentiation toward Th2 cells, and prevent the activation of abortive Th1 and Tc cells [87]. dDCs also induce regulatory T cell (Treg) by secreting TGF-β1 [88]. Mouse DSCs undergo epigenetic silencing of T cell attracting inflammatory chemokine genes (*CXCL9*, *CXCL10*, *CXCL11*, and *CCL5*), which prevent the arrival of harmful Th1 and Tc cells to the decidua, and this effect seems to be induced by decidualization [4]. A similar effect has been observed in human dDSCs [2] (T. Llorca, doctoral thesis, University of Granada, 2024). Furthermore, dDSCs inhibit the expression of *IFNG* and *TNFA* by activated T lymphocytes and secrete low-molecular-weight thermostable factor(s) that inhibits Th1 and Tc chemotaxis [T cell chemotaxis-inhibiting factor (TCIF)] [2]. Additionally, DSCs induce differentiation into Tregs from peripheral blood T cells through the action of IDO [89] and TGF-β [84], and from decidual T cells through the action of IL-33 [5]. By secreting CCL2 and IL-33, DSCs enhance Th2 cytokine production and inhibit Th1 cytokine secretion [40,41]. In general, DSCs interact with different immune cells of the decidua, establishing the predominance of a type 2 immune response that favors immune tolerance to fetal tissues [2,5,40,41].

Ectopic sites: TLSs and endometriosis

In addition to the three criteria for the general immune functions of fibroblasts, a fourth criterion can be posited: the presence of these cells in ectopic sites. Under chronic inflammation (e.g., autoimmunity, infection, and cancer), tissue fibroblasts differentiate into immunofibroblasts that

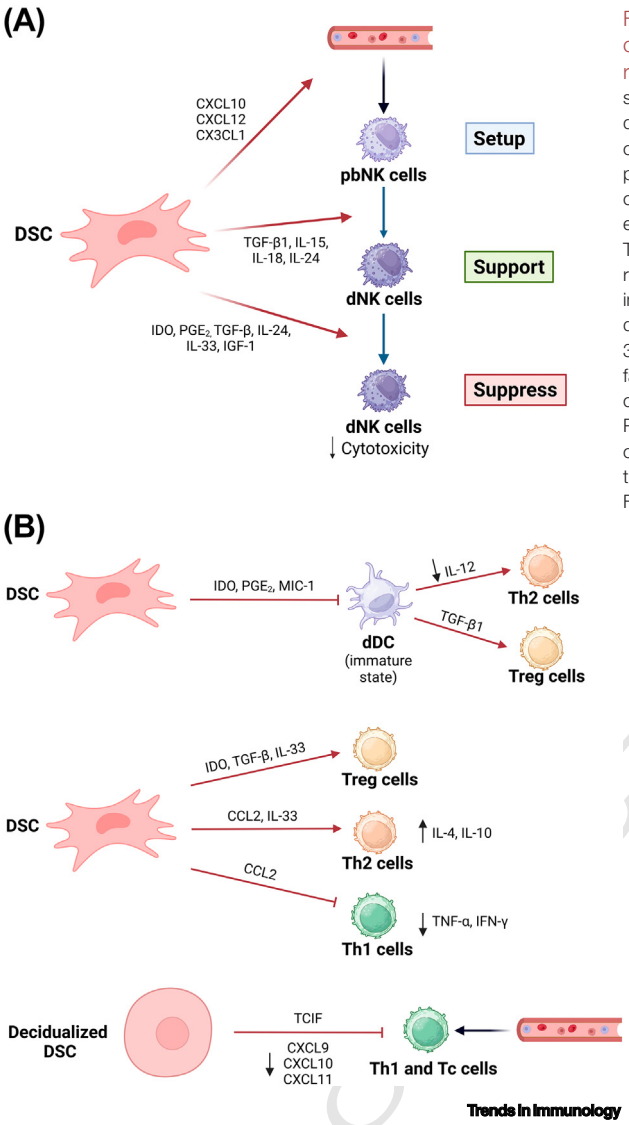

Figure 2. Effects of decidual stromal cells (DSCs) on mammalian immune responses. (A) DSCs are involved in the setup, support, and suppression of decidual NK (dNK) cells (decreased cytotoxicity). The relevant molecules and pathways implicated are shown. (B) The cartoon depicts the immunoregulatory effects of DSCs on T cell subsets [Th1, Th2, and regulatory T cells (Tregs)]. The relevant molecules and pathways implicated are shown. Abbreviations: dDC, decidual dendritic cells; IDO, indoleamine 2 3-dioxygenase; IGF1, insulin-like growth factor 1; MIC-1, macrophage inhibitory cytokine-1; pbNK, peripheral blood NK; PGE_2, prostaglandin E2; TCIF, T cell chemotaxis-inhibiting factor; TGF-β1, transforming growth factor β1. Figure created with BioRender.com.

phenotypically and functionally resemble FRCs and are capable of organizing TLSs [64,65,68,69]. The cellular organization of these TLSs is similar to that of SLOs, with the presence of TRC- and FDC-like cells, along with **high endothelial venules (HEVs)** [90]. Although DSCs can be found in ectopic locations (deciduosis), it is much more common to find their endometrial counterparts, EnSCs, in ectopic sites during endometriosis [91]. Endometriotic foci are comparable to TLSs, because both types of structure constitute ectopic foci that are induced by SCs with immunofibroblasts characteristics [21,69,92] (T. Llorca, doctoral thesis, University of Granada, 2024) and both harbor HEVs [15,90,93]. Despite these similarities, their corresponding cellular organizations differ. TLSs contain more or less well-defined B and T cell areas [90], whereas such areas are not commonly detected during endometriosis, and a high proportion of macrophages and neutrophils are found instead [26,94,95]. These differences are likely due to the distinct types of associated SCs and chemokines produced in each case. TLS-associated FRC-like cells are

mainly involved in lymphocyte attraction and organization, whereas EnSCs mainly attract macrophages and neutrophils [26,94,95]. A certain study showed that injection of FRCs induced TLSs in mice [96]; other work showed that injection of human EnSCs in mice generated endometriosis-like nodules. The presence of human EnSCs along with murine macrophages and neutrophils in these nodules demonstrated the chemotactic action of human EnSCs on these leukocytes during nodule formation [97]. However, although endometriosis originates from a dysregulated interaction between EnSCs and macrophages [26,94], the chemokines produced as a result of this interaction may additionally attract lymphocytes [15]. In related findings, TLSs have been observed in some endometriosis lesions, which may suggest lymphoid formation in areas of chronic inflammation. Therefore, the formation of TLSs in endometriotic lesions might not be a driver for these lesions, but rather, a consequence of a persistent inflammatory response [15,93].

Concluding remarks

DSCs and EnSCs (uterine SCs) seem to be the same cells under different physiological contexts: non-pregnancy and pregnancy, respectively. The former are immunologically challenged by the fetal tissue. DSCs can be regarded as tissue-specific fibroblasts, because they share phenotypic and functional similarities with fibroblasts found in immunologically active tissues, particularly regarding immune functions. Thus, both types of cells can set up, support, and suppress immune responses. Additionally, both fibroblasts and DSCs are involved in the formation of ectopic foci, with fibroblasts contributing to the development of TLSs, while EnSCs (i.e., the endometrial counterparts of DSCs) are involved in the appearance of endometriosis. A careful review of the relationships between uterine SCs and fibroblasts, along with the identification of shared functions and molecules important for these functions, may offer valuable insights into the physiology of SC interactions with the immune system (see Outstanding questions). In addition, these analyses may lead to the identification of putative therapeutic targets to treat diseases associated with these cells, during pregnancy or non-pregnancy.

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Declaration of interests

The authors declare no conflicts of interest.

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Outstanding questions

Is each DSC differentiation stage associated with a different state of functionality? Although ScRNAseq makes it possible to identify different types of DSCs, their differentiation stages, and gene expression profiles from which functionality might be inferred, the relationship between different stages of differentiation and the functions of DSCs remains to be clearly established.

Does decidualization increase the immunoregulatory activity of DSCs? Decidualization increases the expression and secretion of DSC molecules with immunoregulatory activity which seems to support this possibility. However, most functional studies do not distinguish between non-decidualized and decidualized DSCs; therefore, further research is warranted.

Are decidualized DSCs more suitable than non-decidualized DSCs for the treatment of certain immune-related diseases? If decidualization increases the immunoregulatory activity of DSCs, this might be the case. Given that DSCs can promote a type 2 immune response and that decidualization can favor this response, decidualized DSCs might have a therapeutic effect in Th1-mediated diseases. To date, DSCs have been observed to have a therapeutic effect on graft-versus-host disease in humans; it remains to be determined whether this effect is enhanced by treatment with decidualized DSCs.

Like FRCs, are DSCs involved in tissue remodeling? The presence of decidual ILC3 cells, which interact with DSCs and increase ICAM-1 and VCAM-1 expression in a manner similar to the LTo–LTi interaction in the SLO primordium, seems to suggest this, but more research is needed.

Are there common molecules expressed by DSCs and fibroblasts of immunologically active tissues that might serve as targets in the treatment of diseases associated with these cell types? Could blocking these molecules prevent the formation of TLSs and thus help ameliorate the clinical course of certain autoimmune diseases? Could blockade prevent the formation of ectopic foci in endometriosis?

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