Reproductive BioMedicine Online (2010) **20**, 305–313

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REVIEW

Assessment of presence and characteristics of multipotent stromal cells in human endometrium and decidua

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Stanimir Kyurkchiev, MD, DSc has been working on problems of reproductive immunology such as tissue-specific antigens of human placenta, identification of gamete-specific antigens and immune mechanisms in recurrent spontaneous abortions. Since 2005, his research has been focused on the presence and characteristics of stem cells in the human reproductive tract and he has published several papers on this topic in peer-reviewed journals. Currently he is a scientific consultant in the Dr Shterev Reproductive Health Centre in Bulgaria.

Abstract This review discusses the presence and characteristics of multipotent stromal cells in human endometrium and decidua. A number of research groups have reported the isolation and characterization of multipotent stromal cells from the basal layer of the endometrium, and in a single case just from the menstrual blood, i.e. the superficial functional layer. Similarly, multipotent predecidual stromal cells are isolated from early decidua and characterized accordingly. Multipotent endometrial stromal cells and multipotent decidual stromal cells are shown to express the basic features of adult stem cells, which are clonogenicity, self-renewal, a potential to differentiate into adipogenic, osteogenic, chrondrogenic, endothelial-like cells and a specific set of surface molecules (CD73, CD90 and CD105). So far, it is not clear whether the same population of multipotent stromal cells is isolated from the basal endometrium or early decidua because it has been shown that in some cases the differentiation potential of endometrial stromal cells is more restricted in comparison to the decidual stromal cells. It is reasonable to assume that it is one cell population under different control by hormonal, paracrine and autocrine factors. Thus far, the functions of these cells have not been convincingly revealed.

KEYWORDS: decidua, differentiation potential, human endometrium, immunomodulatory proteins, multipotent stromal cells

Introduction

Endometrium is the inner mucous layer lining the mammalian uterus. This layer has numerous functions ranging from seemingly mechanical prevention of adhesions of the opposed myometrium walls to securing a suitable milieu for implantation of the embryo by secreting growth factors and cytokines, and morphological and physiological changes such as those under hormonal control. The endometrium is the basis for the development

1472-6483/$ - see front matter
doi:10.1016/j.rbmo.2009.12.011

of the placenta, which supplies the fetus with oxygen and nutrients.

The endometrium consists of a layer of columnar epithelium, resting on stroma which is a layer of connective tissue. Simple tubular uterine glands reach from the endometrial surface to the base of the stroma, which also carries a rich blood supply from spiral arteries. In a woman of reproductive age, these two layers of endometrium can be easily distinguished. These two layers occur only in the endometrium lining the cavity of the uterus, not in the mucosa of the Fallopian tubes.

Monthly regeneration of endometrium in mammalian species with menstrual cycles (e.g. human, great apes) and in mammals with oestrous cycles (e.g. mouse, cows) is accomplished by proliferation of cells followed by their further differentiation as epithelial glandular cells or stromal cells. So, it is quite reasonable to search for the cytological basis and the mechanisms for the repeated regenerations that occur over 350 times during the reproductive life of the female.

The possibility that cells with the characteristics of stem cells are actively engaged in the process of periodical regeneration of the endometrium has been discussed in some excellent reviews (Cervelló and Simón, 2009; Gargett, 2007; Hongling Du and Taylor, 2009). Still, there are a number of questions about the existence and characteristics of putative stromal stem cells in human endometrium that need further discussion. This review will predominantly discuss the presence of multipotent adult stem cells in human endometrium and deciduas, bearing in mind that some very interesting papers have been published concerning the mouse endometrium (Cervelló et al., 2007; Chan and Gargett, 2006).

Multipotent mesenchymal stromal cells in human endometrium

Human endometrium is characterized by an exclusively intensive and cyclic regeneration in the course of the menstrual cycle. During the reproductive period of the woman, the endometrium undergoes hormone-driven dynamic changes. It is known that following menstruation the endometrial thickness is about 0.5—1 mm while at the end of the menstrual cycle the thickness is about 10—12 mm. These changes are due to cell proliferation and differentiation, which are under strict hormonal control.

It has been described that the human endometrium consists of luminal and glandular epithelial cells, stromal fibroblasts, vascular smooth muscle cells and endothelial cells forming basal and functional layer. The glandular epithelial layer is formed by epithelial cells, which are considered a highly differentiated state, while the stroma consists of stromal cells originating from the mesoderm (Gargett, 2007). Different types and numbers of leukocytes depending on the cycle phase are present in the endometrium as well.

The characteristics of the endometrium are suggestive for the presence of an adult stem/progenitor cell population, which is responsible for the expressed regenerative capacity of this tissue. The idea that stem cells are involved in all regenerative processes occurring in the endometrium is a rather old one and it is based on evidence from histological studies of human and primate endometrium (Padykula, 1991; Prianishnikov, 1978). As early as 1978, Prianishnikov proposed a model based on the hypothesis that endometrial epithelium comprises of populations of stem cells, capable of long-term self maintenance and producing differentiated epithelial cells, which can be divided according to the model, into three types: (i) oestradiol-sensitive cells; (ii) oestradiol- and progesterone-sensitive cells; and (iii) progesterone-sensitive cells.

Cell subpopulations with the features of stem cells have been detected in a number of mammalian species including humans using a fluorescent stain (Hoechst 33342) and these minor populations are termed side-population (SP) cells which are distinguished by a high level of dye efflux after staining with Hoechst 3342 (Goodell et al., 1996). Kato et al. (2007) isolated endometrial cells from different phases of the cycle. Single-cell suspensions from endometrial cells were passed through a 32 μm filter and divided into two fractions — the upper fraction (retained by the sieve) consisting predominantly of glandular cells and the lower fraction (passed through the filter) consisting predominantly of stromal cells. SP cells are detected in both fractions with certain predominance in the lower fraction. SP cells from both fractions share some common features as they are small and round and do not express CD9 and CD13 on their surface. However the cells from the two fractions differ in their differentiation potential. SP cells from the upper fraction grow slowly on feeder cells and form colonies that can be cultured successfully for more than 9 months and differentiate as glandular-like cells, their phenotype is CD9+ C13D—. Lower fraction SP cells when cultured on collagen-coated surface differentiated as stromal-like cells with a surface expression of CD13+ and C9D—. It should be pointed out that the highest percentage of SP cells (3.91 ± 0.43%) was detected in endometrial cells isolated from menstrual blood.

Clonogenicity is considered to be a sign of the pluripotent character of a given cell population. Chan et al. (2004) reported the presence of small clonogenic subpopulations in single cell suspensions isolated from the basal layer of human endometrium. Using cell-cloning methods, it was shown that 0.22% of the epithelial and 1.25% of the stromal cells can form single colonies when seeded at concentrations of 500 cells/cm^2 for epithelial cells or 300 cells/cm^2 for stromal cells. A significantly higher cloning efficiency was recorded for the stromal cells (1.25 ± 0.18%) as compared with the epithelial cells (0.22 ± 0.07%). Experiments to characterize further the clonogenic endometrial stromal cells have not shown any statistically significant differences in relation to the cycle phase (Schwab et al., 2005). Further studies conducted by the same research group headed by Gargett have shown that CD146 and platelet-derived growth factor receptor β (PDGF-Rβ) might be surface markers of the putative endometrial stromal stem cells. Immunocytochemical experiments demonstrated that specific membrane staining for CD146 was observed not only on endothelial cells of the arterioles but on perivascular stromal cells as well as PDGF-Rβ positive cells localized perivascularly. In both cases, the intensity of staining did not show any cyclic changes (Schwab and Gargett, 2007; Schwab et al., 2008). Although in the course of these experiments the cells were cultured no longer than 15 days, the authors have speculated that these cells might be regarded as epithelial and stromal stem cells.

Generally, the most important feature of the stem cells is their potential for differentiation in different cell lineages. In line with this statement, Schwab and Gargett (2007) used flow cytometry sorting to isolate CD146+ PDFG-Rβ+ endometrial stromal cells and tested their potential to differentiate. When the double-positive cells were cultured in the presence of the corresponding inducing factors it was shown that they could differentiate as osteogenic, adipogenic, myogenic and chondrogenic cells. In each experimental series, the differentiation was proved by morphological criteria, specific staining methods and reverse-transcription PCR (RT-PCR) analysis of the expression of specific marker genes.

Similar results were reported by Dimitrov et al. (2008) about the clonogenic stromal cells in human endometrium. In that study the human endometrial stromal cells were cultured *in vitro* for more than 15 passages and they were shown to grow as fibroblast-like elongated cells forming a monolayer. Flow cytometry analysis of the cultured cells showed that they were positive for CD29, CD73 and CD90, which are considered to be the markers of cells of mesenchymal origin. The cells were negative for the haematopoietic cell markers (CD45, CD34, CD14, CD3, CD19, CD16/56 and HLA-DR). Further, it was shown that the third- and fourth-passage cells had 15% clonogenic efficiency and could be induced to differentiate into adipogenic cells containing typical lipid-rich vacuoles. These results demonstrate that the human endometrium contains a low number of cells with the characteristics of endometrial stromal stem/progenitor cells, which seem to belong to the family of the mesenchymal stem cells.

Additional evidence about the multipotent nature of endometrial stromal cells was provided by Wolff et al. (2007) who compared the capacity of stromal cells isolated from endometrium, myometrium, uterine tube and fibroids to grow *in vitro* and to differentiate. Their results showed that stromal cells from all samples could be cultured *in vitro* and form a monolayer but only the endometrial stromal cell could be induced to differentiate as chondrogenic cells. The efficient differentiation was confirmed by the demonstrated expression of sulphated glycosaminoglycans and type-II collagen typical of human articular cartilage.

Further progress with studies on the presence of mesenchymal-like cells in human endometrium came with the studies of Gargett et al. (2009) that combined the cloning approach with potential to differentiate. In these experiments cell cultures were initiated at cloning cell densities (10 cells/cm^2) and the cloned cultures were induced to differentiate. Such a combined approach aims to demonstrate the differentiation potential of a single cell progeny and can provide really valuable information. The important findings are that single stromal or epithelial cells can form cellular colonies that can be further serially cloned in a number of consecutive clonings thus demonstrating self-renewal properties which is fundamental for the stem cells. The surface phenotype of the stromal cells was shown to correspond to that of mesenchymal cells (CD29+, CD44+, CD73+, CD90+, CD105+, CD140B+ and CD146+) and no markers of the haematopoietic lineage were detected. Further on, it was shown that cloned stromal cells can be induced to differentiate into muscle cells, adipocytes, chondrocytes, and osteoblasts and the specific characteristics of the corresponding lineages have been analysed by RT-PCR, immunochemistry and flow cytometry analysis (Gargett et al., 2009).

The group of Gargett has repeatedly isolated endometrial stem/progenitor cells from the basal layer of the endometrium and localized these cells around the vessels in the basal layer. Contrary to that, Meng et al. (2007) isolated a stem-like cell population from human menstrual blood which is supposed to contain cell components from the shed superficial functional layer. These cells were cultured for more than 68 doublings and preserved their specific phenotype, CD9+ CD29+ CD41a+ CD44+ CD73+ CD90+ and CD105+. It should be mentioned that these cells could be induced to differentiate into at least nine cell lineages: cardiomyocytic, respiratory epithelial, neurocytic, endothelial, pancreatic, hepatic, adipocytic and osteogenic. This very broad differentiation potential is rather surprising because cells defined as stromal cells can transdifferentiate to cell lineages originating from different germinal layers. So, although the data of Kato et al. (2007) show that the highest numbers of SP cells (putative stem cells) are detected in menstrual blood and seem to substantiate the findings reported by Ming et al. *(*2007), it will be extremely important that these results are confirmed by an independent laboratory. In most publications, multipotent stromal cells with a restricted differentiation potential have been described (Dimitrov et al., 2008; Gargett et al., 2009; Meng et al., 2007; Schwab and Gargett, 2007; Wolff et al., 2007) (**Table 1**).

Thus, several research groups independently have demonstrated that human endometrium contains, albeit rare, populations of cells which are very similar to mesenchymal stem cells in their characteristics (Dimitrov et al., 2008; Gargett et al., 2009; Schwab and Gargett, 2007; Wolff et al., 2007). According to the opinion of the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy, human mesenchymal stem cells which are isolated from different tissues by various methods should be designated as multipotent mesenchymal stromal cells (MSC), which is the proper term for these cells. The definition of any cells as multipotent MSC must cover the following minimal criteria: (i) cells must be adherent to plastic surface when cultured in conventional culture medium in the presence of 10% fetal bovine serum; (ii) cells must express on their surface the mesenchymal marker proteins CD73, CD90 and CD105 and lack the expression of haematopoietic lineage markers (CD45, CD34, CD14, CD11b, CD19 and HLA-DR); (iii) cells must differentiate to osteoblasts, adipocytes and chondroblasts *in vitro* (Dominici et al., 2006).

So far, stromal cells isolated from human endometrium have been designated with different names such as endometrial stem cells (Cervelló and Simón, 2009), endometrial epithelial and stromal stem cells (Chan et al., 2004), endometrial stromal stem/progenitor cells (Dimitrov et al., 2008), endometrial stem/progenitor cells (Gargett, 2007) and endometrial regenerative cells (Meng et al., 2007). If the described cells are termed 'stem' cells, that would not be a true name because, as far as their differentiation potential is concerned, they are closer to the characteristics of the progenitor cells. This paper suggests that the stromal cells isolated from human endometrium adhering to solid phase in culture, having clonogenic activity and having the potential to differentiate to osteogenic, adipogenic, myogenic and chrondrogenic cells must designated as multipotent endometrial stromal cells (MESC).

Table 1 Comparison of differentiation potential of multipotent endometrial stromal cells (MESCs) as reported by different research groups.

Authors	*Source of isolation*	*Differentiation potential*
Gargett (2004)	Basal layer of endometrium	Clonogenicity
Schwab and Gargett (2007)	Basal layer of endometrium	Clonogenicity Adipogenic Osteogenic Myogenic Chondrogenic
Wolff et al. (2007)	Basal layer of endometrium	Clonogenicity Chondrogenic
Meng et al. (2007)[a]	Menstrual blood	Adipogenic Osteogenic Myocytic Chondrogenic Cardiomyocytic Respiratory epithelial Neurocytic Endothelial Pancreatic Hepatic
Dimitrov et al. (2008)	Basal layer of endometrium	Clonogenicity Adipogenic

[a]Clonogenicity not tested.

The decidua

What is the decidua? Is it a specifically transformed endometrium or is it a specific organ formed from cellular elements different from the endometrial constituents? The process of decidua formation was most appropriately termed 'an enigmatic transformation' by Dunn et al. (2003) in a very comprehensive review on the molecules engaged in this process.

The classical view is that a highly specialized but temporary organ called the decidua needs to be formed in mammalian species in order to secure the successful implantation of the conceptus and pregnancy. The decidua in the mammalian species develops after the invasion of the trophoblast which triggers the differentiation of the endometrial stromal cells to form the decidua. Contrary to this, in humans the formation of decidua is a stringently regulated process which is independent of the presence of a conceptus. In women, differentiation of endometrial stromal cells into decidual cells takes place during the mid- to late secretory phase of the menstrual cycle under the influence of progesterone and the presence of a conceptus is not required.

The mouse model

The mouse model studies on the origin of decidual stromal cells (DSC) have provided quite conflicting results. Some studies reported that decidual stromal cells appearing in pregnancy or pseudo-pregnancy originate from the residing endometrial stromal cells. Bell (1990) formulated the hypothesis according to which the decidua contained both bone-marrow derived cells and resident stromal cells. Bone-marrow derived cells are the cells comprising the metrial glands and are derived from infiltrating cells while the cells of decidual matrix arise from the resident stromal cells. Wegmann (1985), by using a sensitive isoenzyme marker founded solid evidence that only infiltrating cells in murine deciduas are from bone marrow.

Contrary to that Kearns and Lala (1982), Lysiak and Lala (1992) have used pseudo-pregnant bone marrow chimeric mice to study the origin of the decidual stromal cells and provided strong evidence that that 'at least a subpopulation of decidual cells of normal murine pregnancy are ultimately derived from progenitors that arise in the bone marrow.' However, since the frequency of the donor-derived decidual cells varied along the implantation sites within the same animal, the authors suggested that the recruitment of pre-decidual stem cells of bone marrow to the uterus was a random phenomenon. Further, a population of label-retaining cells in mouse endometrium that express markers c-kit and pou5f1, specific for undifferentiated cells, was described (Cervelló et al., 2007; Chan and Gargett, 2006). These cells are localized in the lower region of the stromal layer and are supposed to be real progenitor cells. So far, the studies reported have been directed to proving the existence of progenitor cells in the endometrium which have rather limited potential of differentiation as they have been shown to differentiate into epithelial or stromal endometrial cells.

Multipotent mesenchymal stromal cells in human decidua

In humans there are many questions about the origin of the decidual stromal cells which are not clear but the basic question is whether the fibroblast-like cells *in situ* or

isolated and cultured *in vitro* are homogeneous in their characteristics and origin.

Human decidua consists of stromal cells, glandular cells, and leukocytes (natural killer (NK) cells, macrophages, T cells and scarce B cells). Basic cellular changes specific to the decidualization include: (i) an influx of uterine NK cells characterized by high expression of CD56 and actively secreting various cytokines (King, 2000); and (ii) changes in the morphology and secretory activity of the present fibroblast-like stromal cells which are transformed into rounded cells secreting prolactin, insulin-like growth factor binding protein-1 (IGFBP-1, Richards et al., 1995) and vascular endothelial growth factor (VEGF) (Sugino et al., 2002).

The uterine NK cells which originate from the bone marrow and constitute about 70% of the decidual cellular components are recruited via circulation to the endometrium. Uterine NK cells have some characteristics to distinguish them from the peripheral blood NK cells as almost all of them are classified as CD56+bright, which are quite different from the peripheral blood where the CD16+ NK cells are the predominant subpopulation. Still, the origin of the leukocytes is quite clear because the cells express all the phenotypic markers typical for the haematopoietic lineage.

However, the question about the origin and the characteristics of the decidual stromal cells is still controversial in spite of the numerous cytological and morphological studies which began almost two centuries ago.

Richards et al. (1995) were the first to report that, parallel to the terminally differentiated decidual cells, the term decidua contains undifferentiated elongated fibroblast-like cells. These cells were shown to express human leukocyte antigens (HLA) A, B and C, which showed that they were of maternal origin, and expressed vimentin, which is a marker of the mesenchymal origin. It should be pointed out that the cells isolated and cultured in these experiments did not express cell surface antigens specific to haematopoietic lineage (CD45, CD14 and HLA-DR). The isolated cells could be induced to differentiate as decidual cells and to secrete prolactin and insulin-like growth factor binding protein (IGFBP)-1, which are specific products of decidualized cells. It is well known that endometrial stromal cells cultured in the presence of progesterone (medroxyprogesterone acetate, MPA) and oestradiol differentiate into prolactin-secreting decidual cells (Daly et al., 1983). However, the fibroblast-like decidual cells isolated from term decidua needed the addition of butyryl-cAMP to the steroid hormones in the culture in order to differentiate, as the presence of MPA and oestradiol in the culture was not enough to induce the decidualization of these cells. On the basis of the overall characteristics, Richards et al. (1995) designated these cells as pre-decidual stromal cells (pre-DSC) and suggested that they are stem-like cells present in human decidua.

Multifarious and detailed studies on the characterization of DSC have been performed by the research group headed by Olivare in the period 1997—2008. In general, these studies are directed to investigations on surface proteins expressed by DSC in attempts to define the origin of these cells and investigations to define the possible functions of the DSC.

In a series of experiments, this group has shown that DSC have an overall characteristic which is very similar to that of myofibroblasts. Oliver et al. (1999) reported that DSC isolated from 6—11-week human decidua and cultured in RPMI 1640 medium with 10% fetal bovine serum (FBS) expressed CD10+ and CD13+ but were negative for CD14, CD15 and CD45. The major finding in these experiments is that DSC express α-smooth muscle actin (α-SM-actin) and have ultrastructural features (cytoplasmic extensions, microvilli and pinocytic vesicles) which resemble myofibroblasts. Further, Kimatrai et al. (2003) confirmed these findings using RT-PCR specific for α-SM-actin primers and a functional test to study the cell contractility. Studies of the effect of different cytokines on the contractility activity of DSC have shown that pro-inflammatory cytokines such as transforming growth factor β1, PDGF-BB and interleukin (IL)-2 could induce or activate contractility of DSC. On the other hand, anti-inflammatory cytokine IL-10 tended to induce relaxation of DSC while IL-4 was had no effect (Kimatrai et al., 2005). All the data from these experiments definitely specify the cultured DSC as cells very similar to myofibroblasts with mesenchymal (non-haematopoietic) origin. It is speculated that their contractile activity might be related to the success of pregnancy under the control of cytokines and/or steroid hormones.

However, some contradictions arise from the results reported by the same research group that DSC are closely related to antigen-presenting cells with bone marrow origin. Olivare et al. (1997) isolated DSC from early decidua (6—11 gestational weeks) and cultured the cells in Roswell Park Memorial Institute (RPMI) medium supplemented with 10% FBS. Spindle-like cells were observed to adhere to the plastic and grow *in vitro* to form a uniform monolayer and these cells were analysed for the expression of surface markers by flow cytometry, immunochemistry and functional tests. The major findings emerging from these experiments under these conditions of cell culture are as follows: (i) DSC were positive for CD10, CD13, HLA-DR, CD80 and CD86 and negative for CD3, CD14, CD15 and CD45; (ii) DSC cultured in the presence of progesterone alone changed their morphology and expressed desmin and prolactin as demonstrated by immunoperoxidase technique; (iii) cultured DSC were able to stimulate the proliferation of allogeneic T lymphocytes; (iv) CD80 and CD86-positive cells were found around the decidual vessels by immunoperoxidase staining of cryostat sections of decidua. On the basis of these findings, the authors assumed that the cells that were isolated, cultured and characterized are pre-DSC that have not yet been decidualized. The overall conclusion of the authors is that the pre-DSC are most probably local specialized antigen-presenting cells which are involved in the defence against infections or under pathological conditions they might be a part of the immune mechanisms responsible for the elimination of the conceptus. Inflammatory cytokine would prime these functions of pre-DSC while progesterone would stimulate their decidualization and thus would protect the pregnancy. Further, Garcia-Pachecco et al. (2001), in an attempt to find some reconciling evidence, tested the effect of different culture media on the expression of surface markers on DSC. First of all they analysed by flow cytometry freshly isolated DSC and found that a significant proportion of the cells expressed CD34+, which is a known marker of the haematopoietic cell lineage, and STRO-1+, a marker of bone marrow stromal precursors. However, DSC cultured in RPMI 1640 medium supplemented with 20% FBS tend to lose the expression of these specific markers and after 8—10 weeks in culture CD34 could not

be detected on the surface of the cells. Cultured in low serum concentration medium (fibroblast basal medium with 2% FBS) the DSC preserved stable expression of CD34, STRO-1, CD86, CD80 and HLA-DR but lack the expression of CD14, CD15 and CD45. So, the discrepancies between the results from the experiments of the same research group can be explained by the fact that initially the immunophenotyping was performed with cells cultured for longer periods in high serum concentration culture media.

The general conclusion of Oliveira's group is that the DSC they have been working with are related to stromal precursors and have characteristics quite similar to myofibroblasts and follicular dendritic cells.

In the experiments by Dimitrov et al. (2008), first-trimester decidua samples were used to isolate and culture DSC. About 1 week after the initiation of the cultures, easily detectable cell colonies could be observed composed of elongated spindle-like shaped cells with centrally located round nuclei. These cells showed rather high proliferative activity and after about 10—12 days of culture, a morphologically homogenous layer was formed. After a series of passages, the DSC preserved their morphology and proliferation activity and no signs of cell senescence were observed at the morphological level in the course of 6—7 months in culture. DSC at the fourth passage analysed by flow cytometry for the expression of specific cell lineage markers were found to be negative for the haematopoeitic markers such as CD45, CD34, CD14, CD19, CD 56/16 and CD3 and, contrary to that, the cells expressed CD29, CD73 and CD90. HLA-DR was detected in 1.6—6.3% of the cells analysed while most of the cells expressed HLA class I antigens, as detected by the corresponding specific antibody. It should be mentioned that the DSC studied in these experiments were found to be positive for the expression of CD146 antigen (82.3% positive cells) which is considered to be a marker of perivascular and endothelial cells (Bardin et al., 2001). The cells expressed vimentin and were negative for cytokeratin and von Willebrand factor, which add to the evidence proving that these cells were mesenchymal stromal cells.

DSC at the fourth to fifth passage were cultured in low serum medium containing oestradiol, medroxyprogesterone acetate and 8-bromo-cAMP and the first morphological changes were observed after 4—5 days as the cells were turning from spindle-like cells to larger more round cells. Later the cells formed clusters in which the cells boundaries were not clearly seen and the concentration of prolactin in the culture medium increased steeply after day 6 in culture from 1.71 ng/ml up to 18.97 ng/ml at day 19.

These results clearly demonstrated that the cells isolated from early decidua and cultured could be designated as pre-DSC of mesenchymal origin. As it has been suggested that the pre-DSC might have the behaviour of stem cells, the clonogenic capacity was assayed and the cloning efficiency was determined to be $2.3 \pm 0.11\%$ for 10 cells/cm^2, $3.06 \pm 0.21\%$ for 25 cells/cm^2, $4.1 \pm 0.32\%$ for 50 cells/cm^2 and $4.8 \pm 0.37\%$ for 100 cells/cm^2.

It is known that multipotent cells of mesenchymal origin can be differentiated into cells of osteogenic and adipogenic lineages. To test this assumption, pre-DSC at the fourth to fifth passage were cultured in the presence of osteogenic-inducing factors (dexamethasone, ascorbic acid-2-phosphate and β-glycerophosphate). After 18 days in culture the cells changed their basic morphology turning to broad, flattened cells which showed enhanced alkaline phosphatase activity. The extracellular matrix secreted by the differentiated pre-DSC was shown to contain mineral deposits stained in black after von Kossa staining which demonstrated that the metabolism of the cultured cells has been changed upon induction to differentiate. Further on, another sample of DSC at the fourth to fifth passage was transferred to culture media inducing adipogenic differentiation and analysed after 18 days in culture for the presence of vacuoles containing neutral lipids. Staining with Oil red showed that pre-DSC cultured under these conditions contained vacuoles coloured in red with different size.

To induce pre-DSC differentiation into endothelial-like cells *in vitro*, they were cultured in DMEM-LG supplemented with 20% fetal calf serum, 50 ng/ml VEGF (bovine pituitary extract) and 10 ng/ml -fibroblast growth factor on the Matrigel coated six-well dishes. During the first 12 h, cells spread randomly and started to form small and seldom interconnected clusters. After 24 h in culture, the clusters became larger with thin connections among them and formed polygonal vessel-like structures on the Matrigel. Two days after plating, interconnections between clusters became thicker and capillary-like tubes were clearly seen. Morphological changes were observed under light microscopy and photographed. To evaluate the endothelial differentiation of the pre-DSC, cells were lifted from the Matrigel by collagenase treatment and the retrieved cells were allowed to attach to gelatin coated coverslips for 4 h. Positive bright green fluorescence was observed within the cells after staining with antibodies against Von Willebrand factor. The overall characteristics of the pre-DSC cultured and studied convincingly categorizes them as members of the family of the mesenchymal stem cells. These cells express both phenotypic markers and differentiation potential of typical mesenchymal stem cells but besides that the cells express the marker of endothelial and perivascular cells (CD146+) and are capable of undergoing differentiation to decidual cells (Dimitrov et al., 2008).

The results from Dimitrov et al. (2008) differ to some extent with some of the findings of Oliveira's group and these discrepancies might be due to the different culture conditions and different reagents used (**Table 2**). However, these results do not exclude the possibility that pre-DSC are involved in providing optimal conditions for development of the conceptus by modifying the local maternal immune response. To test this hypothesis, the effect of progesterone on the expression of immunomodifying factors was investigated.

Apart from the possible function as an antigen-presenting cell, the pre-DSC might be involved in the known immunomodulatory phenomenon in pregnancy. Blanco et al. (2008) presented convincing data that DSC cultured in low serum medium express a rather low concentration of HLA-G which is known to have definite immunosuppressive properties. Cytokines such as IL-10 (anti-inflammatory cytokine) and interferon (pro-inflammatory cytokine), although with different activities, increased the expression of HLA-G while contrary to that IL-2, which is a pro-inflammatory cytokine associated with abortions, had no effect on the expression of HLA-G by pre-DSC. Pre-DSC decidualized *in vitro* by progesterone and cAMP preserved the expression of HLA-G.

Table 2 Comparison of the characteristics of decidual stromal cells as reported by the different research groups.

Authors	*Source of isolation*	*Surface*	*Phenotype markers*
Olivares et al. (1997)	Early decidua	CD80+, CD86+	FDC-like
Oliver et al. (1999)	Early decidua	CD14−, CD15−, CD 3−, CD45−, CD21L+, CD21+, CD23+, CD80+	FDC-like
Garcia-Pachecco et al. (2001)	Early decidua	CD10+, CD13+, CD21+, HLA−DR+, CD34+, STRO-1	FDC-like
Dimitrov et al. (2009)	Early decidua	CD3−, CD45−, CD34−, CD29+, CD73+, CD90+, CD105+	MSC-like

FDC = follicular dendritic cell; MSC = mesenchymal stem cell.

HLA-G is a non-classical HLA class Ib molecule and initially has been shown to be expressed on trophoblast cells in the human placenta (Kovats et al., 1990). Numerous papers report the detection of HLA-G in a number of tissues such as human thymic epithelia (Mallet et al., 1999), endothelial cells of trophoblastic vessels (Blaschitz et al., 1997), mononuclear phagocytes (Yang et al., 1996), glioma cells (Wiendl et al., 2002) and intestinal mucosa in coeliac disease (Torres et al., 2006). However, mechanisms controlling the expression of the HLA-G genes are largely unknown although some cytokines have been demonstrated to up-regulate their transcription by human trophoblasts (Moreau et al., 1999).

Progesterone is considered a multifaceted hormone as it has been shown that it induces some morphological changes of the endometrium, stimulates the activity of some specific enzymes (Maccarrone et al., 2003), inhibits the antibody production (Wira and Sandoe, 1980), and suppresses T-cell activation and cytotoxicity (Mori et al., 1977; Szekeres-Bartho et al., 1985). In detailed studies, Szekeres-Bartho and collaborators have proved that some immunomodulatory activities of progesterone are mediated via the induction of a specific protein designated as progesterone-induced blocking factor (PIBF), which has been studied in detail (Polgar et al., 2003; Szekeres-Bartho et al., 2001).

Immune tolerance to the semi-allogeneic conceptus and the surrounding trophoblastic is due to the involvement of numerous interacting factors and cells as the main events are happening locally at the decidua and trophoblast. Since the pre-DSC belong to the MSC family, it is quite reasonable to assume that these cells might express immunomodulatory proteins. This assumption was tested by investigating the effect of progesterone on the expression of immunomodulatory proteins by human MSC, isolated from different sources (Ivanova-Todorova et al., 2009a,b). Human MSC were isolated from human adipose tissue (AT-MSC), bone marrow (BM-MSC) and early decidua (pre-DSC) cultured in the presence of progesterone. The expression of HLA-G is followed-up at the protein level by flow cytometry analysis, confocal immunofluorescence, Western blot analysis and ELISA and at mRNA levels by two-step RT-PCR. A general finding in these experiments is that progesterone up-regulates the expression of HLA-G in human MSC isolated from different sources but there are some differences referring to the extent of the up-regulation. Thus, AT-MSC were shown to express higher amounts of cell surface antigen while pre-DSC have a higher amount of intracellular HLA-G and this finding is confirmed by immunofluorescence and Western blots. It can be speculated that the pre-DSC contain the protein which seems to be ready to be secreted or different isoforms of HLA-G.

Another immunomodulatory factor is PIBF, described initially by Szekeres-Bartho et al. (1985, 1989) as an active factor intimately involved in regulation of the immune response in pregnancy. Detailed investigations have shown that PIBF enhanced the secretion of IL-3, IL-4 and IL-10 by spleen cells of mice when cultured *in vitro*, while the production of INF-γ was not modified in comparison to controls (Szekeres-Bartho and Wegmann, 1996). Szekeres-Bartho et al. (1996,) have convincingly proved that PIBF is a major factor for a shift of T-helper-1 to T-helper-2 type immune response and suppression of NK-cell activity in successful pregnancy. Polgar et al. (1999) presented some evidence that the most probable source of PIBF is $\gamma\delta$CD8+ T cells, or that these cells in some way control its secretion.

In this study centre's laboratory, experiments were carried out to compare the possible expression of PIBF in pre-DSC cultured in the absence or in the presence of progesterone acetate (Ivanova-Todorova et al., 2009a,b). Confocal microscopy and flow cytometry analysis were applied with the used of a specific anti-PIBF monoclonal antibody (Mab 3A6) produced in previous studies (Ivanova-Todorova et al., 2008). There were two important facts revealed in this study: the first was that multipotent pre-DMSC express constitutively PIBF intracellularly and the second was that the expression was significantly decreased secondly, in the presence of progesterone in the culture medium. This finding was particularly well documented by confocal microscopy showing that while high homogeneous positive staining was observed in control cells, progesterone acetate-treated multipotent pre-DSC showed a low amount of granular perinuclear staining. It is hypothesized that progesterone induces the release of PIBF by the multipotent pre-DMSC and this might be one of the mechanisms for paracrine control at the feto−maternal interface. An important finding in these studies is that the human pre-DMSC which manifest a well-documented immunomodulatory activity are newly detected targets of progesterone. It can be speculated that these cells contribute to both tissue remodelling and maintenance of immune tolerance during pregnancy.

Conclusion

All the data presented from different research groups so far convincingly show that multipotent stromal cells with the basic characteristic of mesenchymal stem cells are present in human endometrium and human decidua. These cells are clonogenic, grow continuously *in vitro*, which is an illustration of their self-renewal, can be induced to differentiate into adipogenic cells, osteoblasts, myoblast, chondrocytes and endothelial-like cells. In most cases, their surface phenotype corresponds to the set of surface markers specific to the mesenchymal stem cells isolated from other tissue sources. This paper proposes that these cells should be designated as multipotent endometrial stromal cells. So far, it is not clear whether the same population of multipotent stromal cells is isolated from the basal endometrium or early decidua. Some papers describe rather restricted potential for differentiation of endometrial stromal cells compared with the decidual stromal cells (Dimitrov et al., 2008; Wolff et al., 2007). Nevertheless, it is reasonable to assume that it is one and the same population, which is under a different hormonal, paracrine and autocrine control by known or unknown factors.

The controversial questions about the possible origin of the multipotent stromal cells in human endometrium and decidua as well as their precise functions in the reproductive process need further detailed and highly sophisticated studies and should be objects of a separate overview.

Acknowledgements

This work was supported by grant Genomika-4/2006 from the Ministry of Education and Science, Sofia, Bulgaria. The work of M Mourdjeva, I Bochev, D Kyurkchiev, E Ivanova-Todorova, and E Stoyanova from the Department of Molecular Immunology in performing the experiments is highly appreciated.

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Declaration: The authors report no financial or commercial conflicts of interest.

Received 30 July 2009; refereed 7 September 2009; accepted 27 November 2009.