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Review Article

The relationship between amniotic epithelial cells and their microenvironment

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Abbreviations:
hAFC, human amniotic fluid cells
BM, basement membrane
ECM, extracellular matrix
hAEC, human amnion epithelial cells
hESC, human embryonic stem cells
hAM-MSC, human amnion mesenchymal stromal cells
IDO, indoleamine 2,3-dioxygenase

ABSTRACT

Human amniotic epithelial cells (hAEC) are characterized by a great ability to differentiate, and immunomodulatory properties toward mother's immune system cells. These features have been described as being able to change during pregnancy. Thanks to their unique properties: low immunogenicity and high effectiveness of transplantations, amniotic epithelial cells constitute a very attractive source of stem cells for practical purposes in regenerative medicine and transplantology. In this review, we focus on natural factors potentially determining hAEC immunophenotype during pregnancy. Recognition of the impact of specific factors on hAEC would help in effective isolation, creation of appropriate culture conditions and regulation of desired cell function. We also indicate immunomodulatory properties of hAEC themselves. Discovering relations of hAEC with the microenvironment seems to be crucial for their clinical application.

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LN, laminins
MSC, mesenchymal stem cells
PG, prostaglandins

Introduction

Perinatal tissues such as the placenta, play a special role as a source of stem cells due to the fact that, by nature, they are a reservoir of cells of fetal origin (Parolini et al., 2008). Human amnion preserves a significant number of cells with the characteristics of human embryonic stem cells (hESC) and similar potential to differentiation. Depending on structural layer and gestational age it is more or less efficient source of pluripotent and mesenchymal stem cells. Subpopulations of the cells expressing pluripotency markers, such as transcription factors (*i.e.* Sox-2, Nanog and Tert) or surface markers (*i.e.* TRA-1-60, TRA-1-81, SSEA-3 and SSEA-4), are significantly more numerous in early amnia as compared to term ones (Izumi et al., 2009). Among the advantages of amnion derived cells, a limited expression of the MHC antigens class I, as well as lack of MHC class II should be underlined. These properties are basic for a natural immunosuppression mechanism against the maternal defense system. Furthermore, they may be important for the safe amniotic cells therapeutic transplantation to enhance a process of tissue regeneration (Toda et al., 2007) or in a treatment of such diseases as stroke, liver disease or lung fibrosis (Manuelpillai et al., 2011). Additionally, human amniotic cells have anti-inflammatory (Silini et al., 2013) and low tumorigenic properties (Miki et al., 2005).

Human amnion derived cells are phenotypically heterogeneous. Two main populations are, as follows: human amniotic epithelial cells (hAEC) and human amnion mesenchymal stromal cells (hAM-MSC). Control of the regulation of gene expressions in these cells by environmental factors seems to be particularly important to maintain their own properties as low-differentiated cells. hAEC are placed in the niche which is located between two different microenvironments: amniotic fluid and basement membrane (BM). It means that hAEC are exposed, on one hand – to the influence of amniotic fluid soluble components, on the other hand – to the insoluble compounds of BM, *e.g.* collagens, fibronectin, nidogen, and laminins (Niknejad et al., 2012; Lambshead et al., 2013). These components are able to modify the phenotype of amniotic stem cells. Many of these effects, for example, increasing or decreasing proliferation and stemness of pluripotent cells, as well as their ability to differentiation, result from ligands interaction with integrin receptors (Domogatskaya et al., 2008; Rodin et al., 2010).

In addition, hAM-MSC are cells important as a source of numerous extracellular matrix (ECM) components which can influence the hAEC phenotype. These cells are located in the mesenchymal tissue underlying the epithelial BM. Finally, hAEC themselves, are potentially a prominent source of ECM components. The result of the action of the cellular and extracellular factors is a set of unique features, specific to amniotic cells, such as immunomodulating properties against mother's immune cells (Insausti et al., 2014; Yamahara et al., 2014). Expression of hAEC genes might be also a part of autoregulatory mechanism maintaining amniotic stem cells stemness or regulating differentiation.

Identification of microenvironmental components, which can affect amniotic stem cells and potentially play a key role in the fate of these cells, seems to be crucial for the *in vitro* reconstitution of the natural amniotic microenvironment, as well as modulation of the stem cell phenotype. A control of cell-to-cell and cell-to-ECM relationships dependent on amniotic fluid/BM/ECM components might be crucial for the management of stem cell immunophenotype both, in culture and clinical applications *in vivo*.

Development of human amnion epithelial cells' niche

Amnion development begins early, at the stage of gastrulation. At this stage, pluripotent epiblast cells start to form three primary germ layers of the embryo, as well as other extraembryonic tissues, including yolk sac and extraembryonic mesenchyme. From the very beginning of placenta development, hAEC are influenced by three main streams of stimuli (Fig. 1). The first comes from amniotic fluid surrounding the fetus, the second – from BM, the third – from underlying mesenchymal layer of the amnion (Murphy and Atala, 2013).

Amniotic fluid provides soluble factors to hAEC. These factors can support hAEC potency and immunomodulatory properties preventing both inflammation and fetus rejection. Amniotic fluid changes its content during gestation. At the

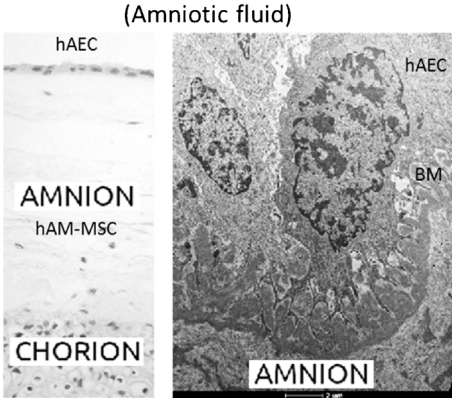

Fig. 1 – Topography of human amnion in H–E staining (left) and transmission electron microscopy (right). hAEC, human amnion epithelial cells; BM, basement membrane; hAM-MSC, human amnion mesenchymal stromal cells (Dept. of Cytophysiology, MUS).

beginning it is an isotonic liquid composed of water, proteins, carbohydrates, lipids, phospholipids, urea and electrolytes. Solutes are probably the first substances actively transported into amniotic space, with water crossing passively the amniotic membrane according to chemical potential gradient (Modena and Fieni, 2004; Murphy and Atala, 2013). In the second half of gestation fetal keratinization occurs and strongly influences amniotic fluid composition. It becomes hypotonic and its osmolality decreases during gestation. There are two main sources of amniotic fluid in the second half of gestation: urea inflow and fetal lung secretion. Transport across cell membrane, defined as water and solute exchange between fetal or maternal blood and amniotic fluid also plays a part in this process (Modena and Fieni, 2004).

Delicate and incomplete BM is detected in the first 6–8 weeks of pregnancy. As hAEC phenotype, also BM formation is strongly influenced by growth factors. Growth factors are both, secreted by hAEC and may affect amniotic cells (Grzywocz et al., 2012). Fibroblast growth factor (FGF) plays a critical role in BM synthesis. It induces transcription factor GATA 6 gene expression in epiblast cells. In turn, GATA 4 and GATA 6 participate in transformation of the cells into functional extra-embryonic endodermal cells. Then, GATA 6 activates production of all three subunits of laminin 111, collagen IV, nidogen and perlecan and in this way it initiates sub-endodermal BM deposition. Further, BM mediates epiblast polarization. Once GATA 4 or GATA 6 is activated, FGF signaling is not required for further BM synthesis. Also epiblast polarization, induced mainly by laminin 111, seems to be independent of direct FGF signaling (Li et al., 2004). These results indicate that BM laminins play an important role in cell differentiation, adhesion and proliferation processes and its synthesis is regulated by growth factors, for example FGF.

Some other growth factors, such as vascular endothelial growth factor (VEGF) and epidermal growth factor (EGF) can also influence amniotic cells phenotype and function. VEGF is associated with regulation of amniotic cell permeability. It plays a role in tissue neovascularization and proliferation of placental cells (Astern et al., 2013). EGF, which concentration in amniotic fluid changes during the course of pregnancy, regulates prostaglandin E2 (PGE2) synthesis in amniotic cells (Ribeiro et al., 2004). Moreover, it is able to activate tyrosine kinase, MAP kinase or PI 3 kinase through EGF receptor (EGFR) and in this way increases VEGF production (Kawano et al., 2005).

Mesenchymal layer of amnion is located below amniotic epithelium and it is composed of the compact and loose layer. The compact layer is a network of protein fibers composed of, among others, collagen type I, III, V, VI and fibronectin. The loose layer consists of fibroblasts surrounded by dispersed network of collagen type I, III and VI, nidogen, fibronectin and laminins. Amniotic mesenchymal stromal cells (hAM-MSC), including mesenchymal stem cells (MSC), are also placed in this layer and have been proven to produce both soluble and insoluble components of amnion ECM (Niknejad et al., 2008).

hAEC potency during amnion development

Microenvironment of hAEC changes in time. The studies with the use of placental perfusion system have shown the differences in cytokine production between preterm (32–36 weeks of gestation) and term human placentas. The experiments reveal different patterns of pro-inflammatory cytokines: interleukin 1β (IL-1β), interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α) release. Both, normal and lipopolysaccharide-induced term placentas are able to produce significantly higher concentration of TNF-α in the maternal part (Holcberg et al., 2007). Biosynthesis of IL-6 in this part is higher in term placentas compared with preterm ones. Interestingly, exposure to lipopolysaccharide (LPS, endotoxin) significantly decreases secretion of IL-6 in the maternal part in both, preterm and term placentas. Also interleukin 1β is expressed by preterm, as well as term placentas, particularly by epithelial cells of the amnion, as well as chorion cells, including syncytiotrophoblast and stromal cells of the villous tissue, and decidual cells (Holcberg et al., 2008). Concentration of IL-1β being released in maternal compartment is significantly higher in term placentas. At the same time, unchanged concentration of IL-1β was found in the fetal compartment. Interleukin 1 receptor antagonist (IL-1Ra) is expressed mainly by hAEC and no significant changes between preterm and term placentas were observed. Exposure to LPS significantly decreases the release of IL-1β in the maternal compartment of term placentas, whereas the synthesis of IL-1Ra is decreased in both, fetal and maternal parts. IL-1β was also demonstrated to stimulate a translocation of cytosolic phospholipase A2 (cPLA2) to the membrane fraction, which leads to production of PGE2 (Holcberg et al., 2008; Amash et al., 2009). Generally, the biosynthesis of cytokines in the maternal part is more intense than in the fetal one (Holcberg et al., 2007).

Another cytokine produced by hAEC is anti-inflammatory interleukin 10 (IL-10). IL-10 is also localized in chorion fibroblasts, cytotrophoblast, syncytiotrophoblast and macrophages. It plays a significant role in down-regulation of maternal cell-mediated response against the semiallogenic fetus. Huleihel et al. demonstrated that perfusion with LPS does not affect the expression of IL-10 in the placenta (Huleihel et al., 2003). Other factors, such as IL-1, IL-6 and TNF-α were described to down-regulate IL-10 production. On the other hand, up-regulation of its synthesis is caused by progesterone and prostaglandins, and was described as gestational age-dependent (Hanna et al., 2000; Huleihel et al., 2003). Moreover, IL-10 induces human leukocyte antigen G (HLA-G) expression that is a known immunomodulatory molecule (Castelli et al., 2014).

Cells react to signals originating from their microenvironment with phenotypic alterations and changes in gene expression. In the first trimester hAEC are slightly flattened and covered with short, sparse microvilli. During the course of pregnancy, hAEC become more cuboidal in shape, and the ultrastructure of the cells changes (Jones and Jauniaux, 1995). Both, proliferation and differentiation potency of hAEC, as well as their immunomodulatory properties change during amnion development. Preterm placentas are more efficient source of amniotic stem cells comparing to term ones. It was confirmed that amnion derived stem cells are phenotypically heterogeneous, with the dominance of the SSEA-4$^+$ cells (Bryzek et al., 2013). In human placenta at term, subpopulations of these cells are significantly more numerous (62–95%) as compared to

subpopulations of cells expressing other stem cell surface markers. Pluripotency markers, such as Sox-2, Nanog and Tert, are expressed at significantly higher levels in cells isolated from early amnia. Furthermore, more cells positive for TRA-1-60 and TRA-1-81 markers are present in early placentas between 17 and 19 weeks of gestation comparing to term ones. Expressions of Nanog and Sox-2 in hAEC examined by qRT-PCR are also higher in early pregnancy and reach levels characteristic for other stem cells. Surprisingly, expression of another stem cell marker, Oct-4, does not differ between cells of early and term amnion (Izumi et al., 2009). Additionally, cells obtained from early amnia exhibit higher expression ratios of adhesive molecules (CD29, CD49f and CD166). These cells tend to differentiate into adipocytes, whereas cells isolated from mid- to term gestation amnia exhibit osteogenic differentiation. In contrast to term hAEC, preterm hAEC cultured in small airway growth media exhibit greater cell proliferative capacity, while their potential for differentiation into lung lineages is limited (Lim et al., 2013a,b). Finally, gene methylation level is lower in cells from early amnia, underlining their more stem-like characteristics (Barboni et al., 2014).

In view of immunomodulatory properties, it was observed that MHC I expression increases progressively during gestation. HLA-G protein expression in hAEC is twice lowered in preterm amnion in comparison to fully developed term amnion (Lim et al., 2013a,b). hAEC ability to reduce lymphocyte proliferation seems to be independent of the stage of gestation. It has been confirmed both, in transwell system in which cells are separated with 0.4-μm pore size membranes, as well as in cell-to-cell observations (Barboni et al., 2014). On the other hand, it was found that hAEC isolated from preterm amnion are less potent to suppress macrophage migration into the injured lung, and term hAEC are more effective in reducing drug-induced lung injury (Lim et al., 2013a,b).

Moreover, in early gestation programmed death ligands (PDL) are present on amniotic stem cells and contribute to apoptosis of activated T and B lymphocytes, defending feto-placental cells against their attack. They take part in induction of immunological tolerance of mother's defense system toward semiallogenic fetus (Petroff et al., 2005). Expression of PDL may be regulated by proinflammatory cytokines (Banas et al., 2008). Nevertheless, in early stages of pregnancy interferon γ (IFNγ) stimulates expression of PD-L1 (B7-H1) and PD-L2 (B7-DC) ligands for receptors of programmed T and B cell death, on the surface of amniotic cells. At this time PD-L1 is also generated in syncytiotrophoblast. Moreover, in *in vitro* cultures PD-L1 synthesis is induced by IFNγ (Chang et al., 2006). Additionally, PD-L2 is expressed in syncytiotrophoblast during pregnancy (Petroff et al., 2005; Okazaki and Honjo, 2007).

Extracellular microenvironment as a source of factors determining hAEC fate and immunomodulatory properties

Both, epithelial and mesenchymal cells of the amnion, and mother's immune system cells exhibit secretory properties during development of semiallogenic fetus. As a result, amniotic fluid, BM and mesenchymal tissue of the amnion become sources of both, soluble and insoluble factors influencing hAEC immunophenotype and function during amnion development (Fig. 2).

Amniotic fluid

Most important components of amniotic fluid influencing hAEC are cytokines (Table 1). They are produced mainly by

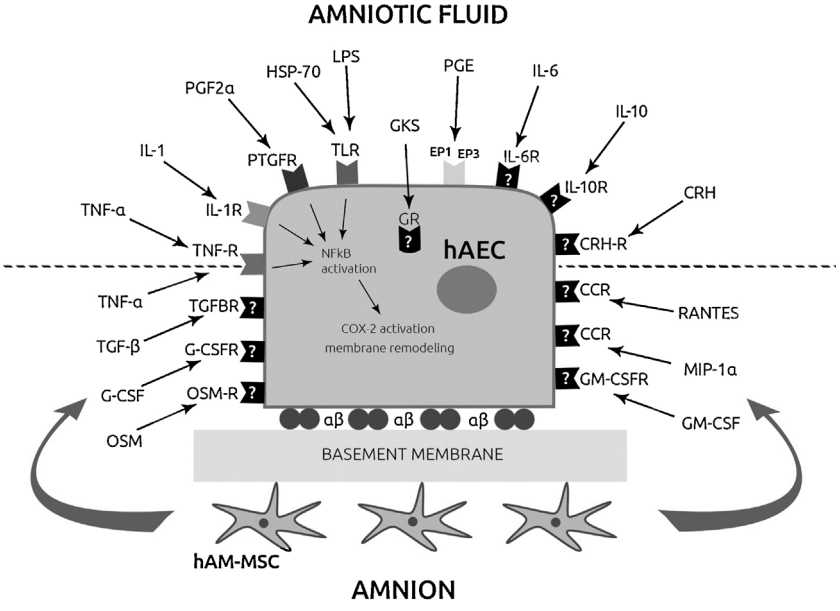

Fig. 2 – Soluble factors potentially influencing hAEC immunophenotype and function during amnion development originating from amniotic fluid and mesenchymal tissue. Most of them can influence cell phenotype activating specific receptors, but the presence of some receptors on hAEC has not been confirmed yet (?). Insoluble compounds, especially components of basement membrane being ligands for integrins (αβ) are also important.

Table 1 – Amniotic fluid components potentially influencing hAEC fate and immunomodulatory properties.

Amniotic fluid component	Confirmed placental source	Role	Ref.
IL-1β	hAEC, endothelial cells	Immune response, proinflammatory action, tight junction disruption, translocation of cytosolic phospholipase A2 (cPLA2) to the membrane fraction	Amash et al. (2009) Holcberg et al. (2007), Kobayashi et al. (2010)
IL-6	hAEC, hAM-MSC, endothelial cells	Tight junction disruption, translocation of claudin 3 and 4	Pratama et al. (2011)
TNF-α	macrophages, hAEC	AEC apoptosis, NFkB pathway activation	Holcberg et al. (2007), Kobayashi et al. (2010), Lim et al. (2013a,b)
IL-1 receptor antagonist	hAEC	Antiinflammatory	Holcberg et al. (2008)
IL-10	hAEC, hAM-MSC	Down-regulation of maternal immune system response against the semiallogenic fetus, induction of HLA-G expression	Hanna et al. (2000), Huleihel et al. (2003), Pratama et al. (2011)
Prostaglandins (PGE2, PGF2α)	hAEC, hAM-MSC	Modulation of immune response, activation of MMP2 and MMP9 genes in the decidua, initiation of labor	Amash et al. (2009), Christiaens et al. (2008)
Hyaluronic acid (HA)	Fetal lung and urinary tract cells	Regulatory role in both, physiological and inflammatory conditions; antiinflammatory action by binding CD44 receptor on T lymphocytes	Higa et al. (2005), Sezen et al. (2009a,b)
Heat shock proteins (HSP)	All cell types in response to injury	Chaperone properties, stimulation of immune response binding receptors on monocytes, dendritic cells, B cells, macrophages and NK cells.	Chaiworapongsa et al. (2008)
Gelsolin	Growing and apoptotic cells	Down-regulation of inflammatory cytokines and LPS concentration in amniotic fluid.	Sezen et al. (2009a)
Adenosine	Cells affected with infection, inflammation or ischemia	Down-regulation of TNF-α level in amniotic fluid	Perni et al. (2009)

immune cells activated during infections, but also by non-immune cells. They act in paracrine or autocrine way. Number of cytokines is associated with labor triggering or chorioamonitis (Christiaens et al., 2008). The most important factor increasing cytokine release to amniotic fluid is LPS. Its presence inside amniotic cavity is usually a result of bacterial infiltration. It has been proven in mice that the structure of amniotic membrane becomes altered following LPS administration because of tight junctions' disruption (Kobayashi et al., 2010). Endotoxin causes changes in the localization of tight junction protein – claudin-3, making the amnion discontinuous. In this context, some cytokine-dependent effects in AEC have been described. IL-6 causes significant decrease in expression of claudin-3 and claudin-4, whereas the decrease after TNF-α infusion is less evident. It was concluded that the effect of IL-6 results mainly from translocation of claudins from apical junctions to the lateral membrane. IL-1β injection causes fetal death within 12 h and PGE2-induced preterm labor a few hours after injection. In contrast to IL-6, TNF-α and LPS induce cell apoptosis in amniotic epithelium. It was supposed that their effects on AEC comprise activation of distinct signaling pathways, including NFkB, protein kinase A, protein kinase C and myosin light chain kinase. Moreover, changes in epithelium integrity after LPS/interleukin administration are similar to those in late pregnancy and might facilitate intra-amniotic infection increasing amniotic membrane permeability (Kobayashi et al., 2010).

Other important components of amniotic fluid are prostaglandins (PG). In pregnant women, the main source of PG is amnion (Table 1). They play a particular role in initiation of labor and its increased level associated with intraamniotic infection has been observed in preterm labor (Mogami et al., 2013). Experiments with the use of placental perfusion system revealed significantly higher level of PGE2 in maternal circulation comparing to fetal circulation. Proinflammatory cytokines like interleukin 1 (IL-1) and TNF-α were demonstrated to enhance the secretion of PGE2 and/or prostaglandin F2α (PGF2α) in human amniotic, chorionic and decidual cells through up-regulation of cyclooxygenase 2 (COX-2). Any significant changes in PGE2 level were found after infusion of LPS into the maternal compartment of the placenta. However, as human term placenta secrets PGE2, its autocrine role in the initiation of labor is strongly suggested (Amash et al., 2009). The main effect of PGF2α is activation of the matrix metalloproteinase 2 (MMP-2) and metalloproteinase 9 (MMP-9) genes (Christiaens et al., 2008).

PG synthesis is mediated by PG endoperoxide H synthase (PGHS). Two isoforms of the enzyme exists. PGHS-1 is constantly expressed in many tissues, including amnion. PGHS-2 is an inducible enzyme that is strongly activated at the time of parturition. Glucocorticoids have been found to increase the expression of PG-synthesizing enzymes in fetal membranes. It can be regulated by induction of PGHS-2 or indirectly by corticotropin-releasing hormone (CRH) (Pawelec et al., 2013). Also IL-1β and TNF-α are able to induce PG release. IL-6 was described as a factor stimulating PG synthesis in amnion and decidual cells, as well as regulating prostaglandin F receptor (PTGFR) expression level (Christiaens et al., 2008).

Some PG and cytokines are soluble components of amniotic fluid important for inhibition of immune response. It is known

that PGE2 is able to block T lymphocyte proliferation and production of proinflammatory and anti-inflammatory cytokines, as well as inhibition of maturation and presentation of antigens on dendritic cells (Yañez et al., 2010). Amniotic cells, including human amniotic fluid cells (hAFC) are also able to synthesize growth factors and their receptors, but the mechanisms of regulation and a significance of these processes is still not well recognized. It has been observed that hAFC can secrete hepatocyte growth factor (HGF), which induces caspase-dependent apoptosis of immune cells (Maraldi et al., 2015).

Factors, such as hyaluronic acid (HA), gelsolin, histone 2B and soluble TNF-α receptors are able to down-regulate pro-inflammatory response in amniotic cavity (Perni et al., 2009). In intact cultured hAFC, TNF-α production is inversely proportional to HA concentration. It was found that LPS can induce cells existing in amniotic cavity to release TNF-α. In turn, there is no relationship between intraamniotic HA concentration and IL-10 production, what could be important in the context of hAEC immunophenotype development. Possible mechanisms taking part in anti-inflammatory HA actions include inhibition of NFkB activation or competition with Toll-like receptor 44-CD44 complex (Sezen et al., 2009a,b). Moreover, endotoxin stimulation of cultured human chorioamniotic membrane results in increasing heat shock protein 70 (HSP70) mRNA expression (Menon et al., 2001). Probably, bacterial endotoxins and HSP70 are able to co-operate with toll-like receptor 2 (TLR-2) and 4 (TLR-4) to induce NFkB followed by production of pro-inflammatory cytokines (IL-1, IL-6, TNF-α) by macrophages and mononuclear cells. These changes lead to PG production and preterm delivery. The exact mechanism leading to increased amniotic fluid HSP70 concentration in spontaneous labor at term remains unknown. It has been suggested that amnion is the main source of HSP70 in case of fetal membrane infection (Chaiworapongsa et al., 2008).

Gelsolin was found in mid-trimester amniotic fluid to inhibit TNF-α induction (Sezen et al., 2009a). The mechanism of this action embraces inhibition of LPS-TLR-4 interaction and following inactivation of genes encoding inflammatory mediators. In culture, hAFC exhibit a strong negative association between the level of gelsolin and LPS-induced TNF-α production. At the same time, no correlation with IL-10 production has been observed. These observations led to conclusion that IL-10 and TNF-α probably function independently in response to LPS induction. It could be especially important in the aspect of hAEC immunophenotype regulation, as IL-10, produced also by Th1 and Th2 lymphocytes, dendritic cells or macrophages, has been shown to modulate HLA-G expression (Huleihel et al., 2003; Pratama et al., 2011; Kang et al., 2012).

It has been reported that incubation of mid-trimester amniotic fluid cells *ex vivo* with adenosine deaminase results in an increase in TNF-α level from 0.9 to 7.3 pg/ml. Inversely, no significant elevation in IL-6 and IL-10 concentration has been observed. These results are consistent with the mechanism of adenosine-related regulation of cytokine production. As sufficient level of adenosine is reached, it binds to specific receptors present on the surface of neutrophils, monocytes, macrophages and T lymphocytes and inhibits TLR-dependent cytokine secretion. There is a positive relationship between adenosine deaminase-stimulated TNF-α release and parity, opposite to gestational age, time of delivery, indication for amniocentesis or maternal age, where no correlation has been identified (Perni et al., 2009).

Basement membrane

ECM plays a particular role in either the maintenance of amniotic cells' potency and function, or their differentiation. Its composition differs depending on amnion layer (Table 2). Function of epithelial cells is strongly regulated by BM that is an equivalent of ECM for epithelial tissues. BM is composed of collagen type III, VII, XV, XVI, XVII and XVIII, collagen IV α1, α2, α5 and α6 chains, fibronectin, heparan sulfate, nidogen-1 and nidogen-2, fibulin-2, fibrillin-2, perlecan, agrin and laminins (Niknejad et al., 2008). It has been proven that denuded amniotic BM can support growth, stratification and differentiation, but not stemness of expanded limbal cells (Dietrich-Ntoukas et al., 2012).

Laminins are one of the most important noncollagenous components of BM which are secreted by adjacent epithelial cells. These proteins are composed of three subunits: α, β and γ. Each subunit contains different types of amino acid chains: α 1-5, β 1-3 and γ 1-3. Laminins critically contribute to regulation of gene expression and cell fate, especially cell differentiation through cell surface integrin receptors, as well as accompanying syndecan and dystroglycan complex (Takashima et al., 2008). COOH-terminal G domain of laminin α chain plays a major role in these interactions. Integrin-mediated signals have been reported to cooperate with growth factor signals (Yamada and Even-Ram, 2002).

BM of human amniotic epithelium contains a great variety of laminins: LN-211 (α2β1γ1), LN-221 (α2β2γ1), LN-311 (α3β1γ1), LN-321 (α3β2γ1), LN-332 (α3β3γ2), LN-511 (α5β1γ1) and LN-521 (α5β2γ1). Especially strong staining for α3 and α5 chains is observed in immunohistochemistry, whereas an expression of α1 and α4 subunits is not detected or observed at a very low level at term. Moreover, strong immunostaining for β1, β3, γ1 and γ2 chains has been shown. All these results have been confirmed with RT-PCR analysis (Takashima et al., 2008).

Despite laminin 111 is weakly expressed in the term amniotic membrane, it is required for epiblast differentiation in the early embryo and its expression is controlled by FGF. It has been described to induce epiblast cavitation by matrix-to-cell interactions dependent on specific receptors. Also exogenously added LN-111 is able to induce epiblast epithelialization. The direction of laminin-dependent differentiation is defined by the fact that hESC and their ectodermal derivatives can bind laminin, while primitive and visceral endoderm cannot (Li et al., 2004). Moreover, the expression pattern of laminin α1 chain dependent on the stage of placenta development has been studied. In the first trimester, an immunoreactivity of this molecule is primarily observed in trophoblastic BM in chorionic villi. Later, in the second trimester, placental laminin α1 chain is rarely seen in trophoblast' BM, while in the last trimester it is almost undetectable. Interestingly, the second-trimester placenta appears to be a source of laminin 121 (Champliaud et al., 2000).

Table 2 – Amnion ECM components influencing hAEC fate and immunomodulatory properties. The presence of some amniotic fluid components (*e.g.* prostaglandins, cytokines) secreted by amnion cells, in amnion mesenchyme is not excluded. WISH cells: human amnion epithelial cell line.

Location	Amnion ECM component	Confirmed source	Role	Ref.
hAEC basement membrane	Collagen type: IV, VII, XV, XVI, XVII and XVIII; Fibronectin; Heparan sulphate; Nidogen-1, -2; Fibulin-2; Fibrillin-2; Agrin	hAEC	Structural support, potential ligands for integrins	Dietrich-Ntoukas et al. (2012)
	βIG-H3 (TGFβ1)	hAEC	Membrane-associated growth factor; cell growth and differentiation	Alcaraz et al. (2013), Hopkinson et al. (2006)
	Perlecan	Undefined amniotic cells, WISH cells	Interaction with growth factors and cell adhesion molecules	Li et al. (2006), Murdoch et al. (1992)
	Laminins including: Laminin 332	hAEC	Promotion of cell scattering, adhesion and migration	Kariya et al. (2004)
	Laminin 511	hAEC	Immunomodulatory properties, inhibition of lymphocyte migration, T cell co-stimulator	Pouliot and Kusuma (2013)
Amnion mesenchyme	Metalloproteinases (MMP)	hAEC MMP-9), hAM-MSC (MMP-2, MMP-9)	Stimulation of inflammatory processes, degradation of ECM components, influence on chemotaxis of inflammatory cells	Chai et al. (2012) Hao et al. (2000), Niknejad et al. (2008)
	Metallopeptidase inhibitors (TIMP-1, -2, -3, -4)	hAEC, hAM-MSC	Inhibition of ECM components degradation	
	Fibronectin		Structural support for cells	Niknejad et al. (2008), Deshpande et al. (2013), Mogami et al. (2013)
	Nidogen	hAEC		
	Collagen type I, III, V, VI	hAM-MSC		

Laminin 211, as well as laminin 221 has been purified from human placenta, but their role is not defined yet (Nishiuchi et al., 2006). Human amnion, as well as human skin has been recognized as a source of laminin 311 and laminin 321. They seem to be important molecules in term of cell adhesion and migration, but their activity is less prominent as compared with laminin 332. It has been detected that LN-311 alone is not sufficient to stabilize epithelial cells binding to BM because it does not bind collagen type VII (Hirosaki et al., 2002).

Laminin 332 has been extensively studied *in vitro* and *in vivo* and it is found to promote cellular scattering, adhesion and migration (Champliaud et al., 1996). In its monomeric form, LN-332 functions as the primary bridge between collagen type VII in the stroma and integrin $\alpha_6\beta_4$ in the hemidesmosomes. In human amnion approximately half of the total amount of LN-332 is involved in formation of complexes with LN-311 and LN-321 through the interaction with their short arm. Such connection allows stable association of LN-332 with BM. LN-332, both monomeric, as well as associated with LN-311 and LN-321, plays an essential role in the epithelial-stromal interaction (Hirosaki et al., 2002).

Special type of LN-332, called laminin 5 variant (laminin 5B), is built with a full-sized α3 (α3B), β3 and γ2 chain. Mature α3B subunit is approximately twice as large as α3A chain and laminin 5B exhibits significantly higher cell adhesion and migration activities than laminin 5A. In human placenta, α3A subunit is predominantly expressed. It plays a regulatory role in proliferation of cells transfected with vectors containing laminin 5A and 5B genes and secreting laminin 5A and 5B in an autocrine manner. Both, laminin 5A and laminin 111 appears to stimulate cell growth stronger, as compared to laminin 5B (Kariya et al., 2004).

Laminin 511, contains α5 chain and exhibits immunomodulatory properties. It is synthesized at the early stage of embryogenesis and might have a significant influence on hAEC phenotype (Champliaud et al., 2000). It is able to inhibit migration of lymphocytes, acting simultaneously with other factors as a co-stimulator of T lymphocytes (Pouliot and Kusuma, 2013). LN-511 is a dominant isoform found in chorion and visualized in amniotic BM. Except LN-511, LN-521 was also isolated from amnion.

Laminin-stimulated activities in hAEC are mediated by cell-surface integrin receptors (Kariya et al., 2004). Integrins are heterodimeric transmembrane glycoproteins composed of α and β chain (Pfarrer et al., 2003). They can be divided into two major groups: those in which β_1 chain are associated with one of nine α chains and those where α_V subunit are bound with β_1, β_3, β_5, β_6 or β_8 chain. Such integrins as $\alpha_3\beta_1$, $\alpha_6\beta_1$, $\alpha_7\beta_1$ or $\alpha_6\beta_4$ are considered to be exclusive laminin receptors. Integrin $\alpha_6\beta_4$ is localized on the basal surface of hAEC cell membrane and exists within hemidesmosomes (Belkin and Stepp, 2000). Similarly, integrin $\alpha_6\beta_1$ attach epi- and endothelial cells to BM (Belkin and Stepp, 2000; Gonzalez et al., 2002). Extracellular (variable) domain of integrin receptors determines a ligand

(*i.e.* laminin, vitronectin, fibronectin or tenascin) specificity (Ahmed et al., 2004). Cytoplasmic domain plays an important role in intracellular signal transductions since it is able to interact with cytoskeleton and activate cell response (Belkin and Stepp, 2000).

Integrins are responsible for cell-to-cell and cell-to-matrix adhesion and signal transduction (Pfarrer et al., 2003). Relationships between laminins of BM and integrins located in hAEC cell membrane regulate such epithelial stem cells' properties as pluripotency, self-renewal, cell shape and migration. For example, LN-332 interacts with α_3 integrin subunit leading to enhanced proliferation (Domogatskaya et al., 2008). Integrin-mediated signaling plays a significant role also in BM assembly, cell differentiation, embryonic and extra-embryonic tissues development, as well as, tumor cell invasiveness. Integrin $\alpha_V\beta_6$, strongly expressed in various tumors, is also found in cells of human amniotic epithelium, but not in amnion mesenchymal layer. Its expression level in the chorion is lower than in amniotic epithelium (Belkin and Stepp, 2000). This integrin promotes cell proliferation and ECM degradation dependent on metalloproteinase MMP-9 in colon, as well as in ovarian and oral squamous carcinoma cells. In amnion ECM, metalloproteinases and their specific tissue inhibitors (TIMP-1, -2, -3, -4) regulate key inflammatory processes through degradation of ECM components. They influence also chemotaxis of inflammatory cells (Hao et al., 2000; Niknejad et al., 2008). This observation is interesting in the context of changes in the proteinase activities (MMP or plasminogen activator – uPA) in the amniotic fluid and gestational tissues, associated with a rupture of fetal membranes and following onset of labor. Normal vaginal delivery is correlated with enhanced pro-MMP-9 and pro-MMP-2 expression in fetal membranes, whereas only pro-MMP-2 is induced during cesarean section (Ahmed et al., 2004).

Integrin function can be influenced by proinflammatory cytokines (IL-1, IL-6 and TNF-α) and PGE2. TNF-α has been found to downregulate $\alpha_6\beta_1$ integrin expression on cultured umbilical vein endothelial cells through α_6 synthesis suppression. On the other hand, FGFβ increases the synthesis and surface expression of α_2, α_3 and β_6 subunit on cultured capillary endothelial cells and downregulates $\alpha_1\beta_1$ and $\alpha_V\beta_3$ integrins. In response to EGF signaling, activation of PKC and phosphorylation of β_4 integrin subunit take place and let to translocate $\alpha_6\beta_4$ from hemidesmosomes to cell protrusions (Belkin and Stepp, 2000). Inversely, integrin $\alpha_V\beta_6$ let to activate latent extracellular, anti-inflammatory, profibrotic cytokine – transforming growth factor β1 (TGFβ1) (Ahmed et al., 2004).

Amniotic mesenchymal tissue

The results of studies suggest, that both alteration of cytokine synthesis in hAM-MSC, as well as modifications in ECM composition may lead to changes in hAEC function and gene expression (Table 2). However, the particular mechanism, by which proinflammatory cytokines, LPS and ECM changes influence hAEC phenotype and function still remains unknown (Mogami et al., 2013). Cytokines produced by hAM-MSC, *i.e.*: TNF-α, oncostatin M (OSM), CCL5 chemokine (RANTES; regulated on activation, normal T cell expressed and secreted), granulocyte-macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), IL-6, IL-8, macrophage inflammatory protein (MIP-1α) and TGFβ could diffuse toward hAEC and influence their immunophenotype (Rossi et al., 2012). TNF-α (and LPS) is known as a factor increasing soluble fetal fibronectin (fFN) secretion by cultured hAEC. fFN is diffusely distributed in feto-maternal compartment, from amnion to decidua. It provides structural support for cells and takes part in adhesion of the fetal membranes to uterine tissues. In physiological conditions, fFN has been described in its fibrillary form, tightly bound to ECM. Its unbound form, secreted in response to injury, differs from the fibrillary form. In epithelial cells, endotoxin and inflammatory TNF-α increases the expression of both, matrix-bound and unbound fFN. fFN is able to regulate cell function through $\alpha_5\beta_1$ integrins. In amnion mesenchymal cells fFN induces activation of TLR4 (thanks to its specific extra domain A element), but not integrins. This interaction activates ERK1/2 pathway and protein-1, what in turn results in metalloproteinase 1 (MMP-1) and MMP-9 gene transcription. When treated with fFN, cultured amnion mesenchymal cells increase MMP-2 gene expression. Moreover, COX-2 mRNA expression and its enzymatic activity, as well as PGE2 biosynthesis are elevated after exposure of these cells to extra domain A of fetal fibronectin (EDA). EDA activates TLR4 in hAEC. In this reaction, TLR4 accessory protein MD-2 is required. MMP-1, MMP-2, MMP-9 or PGE2 synthesis are not altered in cultured hAEC (Mogami et al., 2013).

Another component of amniotic mesenchymal layer is fibrillin. Fibrillin-1 isoform is present throughout fetal development and postnatal life. In contrary, fibrillin-2 and fibrillin-3 are specific to fetal development. These molecules occur as non-striated fibrils associated with elastic fibers or BM. In the postnatal period the first 8-cysteine domain in fibrillin-2 is covered by microfibrils, but in the fetal life it is exposed (Charbonneau et al., 2010). The role of fibrillin in placental tissues is not defined. It is suggested that fibrillin plays a role in BM binding with underlying stroma. Some evidences indicate that microfibrils containing fibrillin play a role in cell adhesion, growth and differentiation (throughout EGF-like domains) (King and Blankenship, 1997).

Multipotent MSC are most important cellular components of the hAM-MSC population. They play a crucial role in ECM synthesis in the mesenchymal tissue and can modulate mother's immune system. In general, MSC originating from different tissues, *i.e.* bone, dental pulp, adipose tissue, umbilical cord blood or amnion exhibit similar properties in term of high regenerative potential and similar phenotype associated with expression of specific set of surface markers: CD73, CD90 and CD105 (Danisovic et al., 2007). In culture, MSC induce secretion of anti-inflammatory cytokines: interleukin 2 (IL-2), interleukin 4 (IL-4), IL-10, interleukin 7 (IL-7), interleukin 15 (IL-15), IFNγ and VEGF in mixed lymphocyte reaction (MLR). Also hAM-MSC secrete immunosuppressive factors crucial for creation the immune tolerance in mother's organism during pregnancy. Among different immunomodulatory, antiangiogenic and anti-inflammatory factors, IL-10, IL-6, TGFβ, VEGF, PGE2, chemokines, indoleamine 2,3-dioxygenase (IDO), HGF and intercellular adhesion molecule (ICAM) have been described in the amnion (Kang et al., 2012; Kyurkchiev et al., 2014).

IL-10 is a very important immunomodulatory factor. It is possible that hAM-MSC exhibit stronger immunosuppressive action comparing to adult MSC, because embryonic MSC produce larger quantity of IL-10 (Roelen et al., 2009). Moreover, these cells are able to induce secretion of IL-10 and Fas ligand (FasL) in $CD8^+CD28^-$ T lymphocytes in co-culture, enhancing their immunosuppressive properties (Liu et al., 2014). Secretion of IL-10 by hAM-MSC induces immune cells to produce greater amount of IL-10, thus empowering positive feedback-loop enhancing overall immunosuppressive effect (Yang et al., 2006). Existence of another, yet unidentified, soluble factors produced by hAM-MSC, able to induce IL-10 secretion in peripheral blood mononuclear cells (PBMC), tolerogenic macrophages and dendritic cells is supposed (Bassi et al., 2011; Kang et al., 2012; Eggenhofer et al., 2014; Kyurkchiev et al., 2014). It has been shown that IL-10 inhibits action of such proinflammatory cytokines as IL-1, IL-6, IL-8 and TNF-α (Couper et al., 2008). Presence of IL-10 in ECM inhibits maturation of dendritic cells. Similar effect is observed after activation of JAK1/STAT3 signaling pathway in immune cells (Liu et al., 2013).

TGFβ is constitutively synthesized and secreted by hAM-MSC. It was demonstrated to enhance immunomodulatory effects of hAM-MSC through the impact on T lymphocyte function. It has been proven that addition of antibodies neutralizing TGFβ and IL-10 to the co-culture of hAM-MSC with T lymphocytes enables lymphocytes to proliferate, what was not possible without these antibodies. It indicates that both, TGFβ and IL-10 play a crucial role in inhibition of lymphocyte proliferation by hAM-MSC. Also TGFβ released by bone MSC inhibits proliferation of $CD4^+$ and $CD8^+$ lymphocytes (Di Nicola et al., 2002). What is more, MSC isolated from dental pulp constrain PBMC by TGFβ secretion (Tomic et al., 2011). TGFβ impairs also $CD8^+$T lymphocyte function by down-regulation of expression such effector factors as perforins, FasL and IFNγ (Ahmadzadeh and Rosenberg, 2005). TGFβ, together with PGE2 produced by PBMC, induces generation of T regulatory cells (Treg cells) population, playing an important role in immune suppression (English et al., 2009).

IL-6, another important component of ECM, has somehow double nature, both stimulating and inhibiting the immune system. IL-6 is secreted spontaneously, but also after induction by proinflammatory factors, such as IFNγ, IL-1β and TNF-α (Kimura and Kishimoto, 2010). Surprisingly, hAM-MSC are both a source and a target for IL-6 (Kyurkchiev et al., 2014). Adult MSC of different origin are able to prevent neutrophile apoptosis by secretion of IL-6 (Ben-Ami et al., 2011). Additionally, experiments on adipose tissue MSC indicate that IL-6 plays a major role in formation and function of Treg cells ($CD8^+FOXp3^+$) (Nakagawa et al., 2010).

In addition to IL-10, TGFβ and IL-6, MSC, including hAM-MSC, produce VEGF. Its synthesis is enhanced in hypoxic conditions. Also TNF-α enhances VEGF expression in hAM-MSC through activation of STAT3 and p38 MAPK signaling pathways (Wang et al., 2009). VEGF plays an immunosuppressive role directly, inhibiting maturation of dendritic cells and impairing memory T lymphocytes function, or indirectly, stimulating dendritic cells to produce IDO (Dikov et al., 2005; Basu et al., 2010; Marti et al., 2014).

Immunomodulatory action of prostaglandin E2 is very complex and not fully discovered yet. In physiological conditions PGE2 is synthesized by hAM-MSC at a low level. This synthesis increases in hAM-MSC when are co-cultured *in vitro* with PBMC, monocytes and natural killer (NK) cells (Spaggiari et al., 2009). Moreover, secretion of this prostaglandin is induced by LPS, IFNγ, TNF-α or IL-1β (Chen et al., 2010). PGE2 regulates activation, proliferation and differentiation of different immune cells (Beyth et al., 2005). *In vitro*, PGE2, PGD2 and PGF2α were described to be responsible for immunosuppressive and anti-proliferative properties of conditioned medium obtained from hAM-MSC culture (Rossi et al., 2012). It is possible, that these factors cooperate with TNF-α, OSM, RANTES, GM-CSF, G-CSF, IL-6, IL-8, MIP-1α and TGFβ in a positive feedback loop. Not only lymphocytes, but also NK cells, macrophages and monocytes are the target for PGE2. It also influences the maturation of monocytes to immature dendritic cells (Van Elssen et al., 2011). It can be supposed that thanks to PGE2, hAM-MSC are able to control inflammatory processes and keep the homeostasis of immune system (Kyurkchiev et al., 2014). It is probable that PGE2 is involved in suppression of allogenic immune response throughout induction of IL-10 production in PBMC (Yañez et al., 2010).

Another important compounds of amnion stem cell niche are chemokines. They create a specific microenvironment and act as chemoattractants for immune cells (Kyurkchiev et al., 2014). Chemokines CCL2 and CXCL8, and previously described Il-6, are produced by hAM-MSC. They inhibit differentiation of monocytes into dendritic cells. On the other hand, hAM-MSC co-cultured with monocytes differentiating into dendritic cells have been described to prevent chemokines CXCL10, CXCL9 and CCL5, as well as pro-inflammatory cytokine TNF-α secretion (Magatti et al., 2009).

Additionally, hAEC produce specific proteins influencing macrophages function. One of them is macrophage inhibitory factor (MIF), restricting the migration of macrophages and NK cells action. Another molecule, called MIP-2, inhibits chemotactic activity of macrophages and neutrophiles (Li et al., 2005). Between all of these components of ECM, also HA can be found. It is released by amniotic cells and has an immunomodulatory characteristic. It reacts with CD44 molecule on T lymphocytes preventing their pro-inflammatory action (Higa et al., 2005). *In vitro* experiments reveal that addition of hAM-MSC to the culture of mitogen-activated lymphocytes results in three-fold increase in Treg cells number after 3 days co-culture. Similar effect was observed in co-culture of hAM-MSC with lymphocytes (MLR reaction) (Chang et al., 2006). MSC of different origin promote activation of Treg cells *in vivo*. It still remains unknown if this activation is direct (MSC-lymphocytes) or indirect process mediated by immature dendritic cells (Parolini et al., 2010).

Immunosuppressive properties of amniotic cells

Amniotic cells exhibit immunomodulatory properties and potentially may influence mother's immune cells indirectly – through secreted soluble molecules, and directly – by surface proteins (Li et al., 2009). This action comprises the expression of immunomodulatory cell-surface proteins and/or its

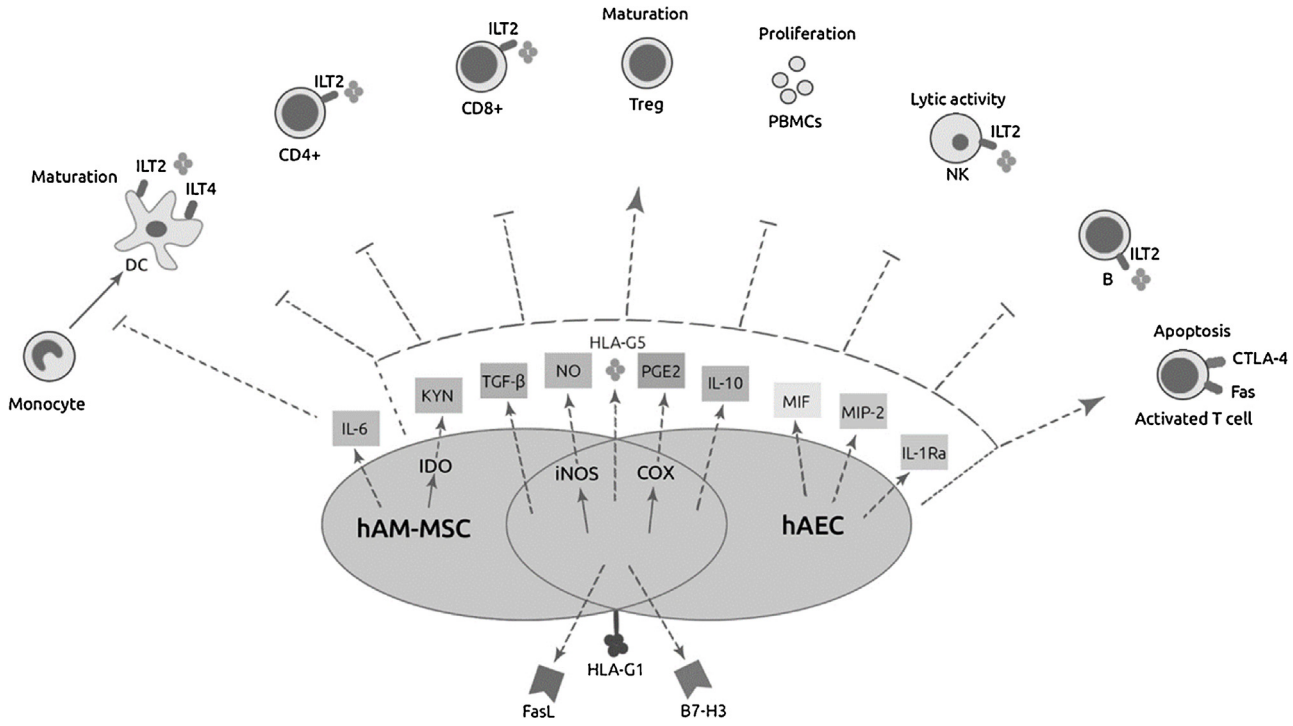

Fig. 3 – Functional relationship between amniotic and immune cells. Immunomodulatory factors are expressed on the cell-surface and/or secreted into ECM by hAEC, hAM-MSC or both cell types. Potential inductory (↓) and inhibitory (⊥) effects against immune cells are indicated.

secretion into ECM (Fig. 3). Similarities and differences in immunomodulatory actions of amnion cells are the effect of phenotypic and functional characteristics being a consequence of the cells' pluri- or multipotency. Amniotic cells are source of inhibitory and stimulatory substances regulating mother's immune system (Li et al., 2005). Some of these compounds are similar, but some of them are secreted specifically by epithelial or mesenchymal cells. These compounds exist both, in amniotic fluid and ECM of amnion mesenchymal tissue. Until present time, the precise mechanism of mother's immune system inhibition during pregnancy has not been fully defined. The key factors involved in immune tolerance are: non-classical major histocompatibility complex proteins, immunomodulatory proteins, ligands responsible for cell death induction, IDO enzyme and adhesive molecules (Tables 3 and 4).

Table 3 – Immunomodulatory molecules being expressed by hAEC. DC, dendritic cells.

Molecule	Location	Role	Ref.
FasL	Cell membrane	Induction of apoptosis in T and B lymphocytes	Li et al. (2005)
B7H3 (CD276)	Cell membrane, Extracellular	Co-stimulator of T lymphocytes proliferation, inhibition of activated T lymphocytes through CTLA-4 receptor	Chapoval et al. (2001), Sun et al. (2002)
HLA-G		Reacts with ILT receptor on DC, monocytes, macrophages, T and B lymphocytes; indirect role in Treg cells generation, protection of amniotic cells from NK cells, control of MLR reaction through interaction with ILT-4 receptor.	LeMaoult et al. (2005), Rajagopalan and Long (1999), Ristich et al. (2005), Riteau et al. (1999)
IL-10	Extracellular	Inhibition of proinflammatory cytokines: IL-1, IL-6, IL-8 and TNFα	Couper et al. (2008)
IL-1 receptor antagonist IL-1Ra)		Inhibition of proinflammatory activity of IL-1	Døllner et al. (2002)
MIF		Antymigratory effect on macrophages, inhibition of lytic activity of NK cells	Li et al. (2005)
MIP-2		Inhibition of macrophage and neutrophil chemotactic activity	
TGFβ		Inhibition of cell proliferation, including T cells, enhancement of ECM formation, induction of B lymphocytes apoptosis	

Table 4 – Immunomodulatory molecules being expressed by hAM-MSC. DC, dendritic cells.

	Role	Ref.
Extracellular molecules		
IL-10	Induction of immune tolerance, inhibition of macrophages, dendritic cells, Th1 lymphocytes, Treg and tumor cells function	Bassi et al. (2011), Døllner et al. (2002), Eggenhofer et al. (2014), Kyurkchiev et al. (2013)
IL-6	Either inhibition or activation of immune system: support for Treg cells generation, induction (together with TGFβ) of Th17 cell colony formation, inhibition of T-cell proliferation in MLR, cytokine secretion and cytotoxicity, stimulation of $CD4^+$ lymphocytes proliferation, support for IgG secretion by B-cells, inhibition of DC maturation, neutrophil protection from apoptosis	Nakagawa et al. (2010), Newman et al. (2009)
TGFβ	Inhibition of proliferation and differentiation of T lymphocytes and expression of effector molecules (perforins, FasL, and INFγ) on $CD8^+$ cells; Induction of Treg cells generation	Ahmadzadeh and Rosenberg (2005), English et al. (2009), Nasef et al. (2007)
Chemokines, including: CCL2, CXCL8 CXCL9, CXCL10, CXCL11 CCL2/MCP1	Chemoattractants of immune system reacting with neutrophils, monocytes, eosinophils, basophils, T and B lymphocytes, DC, NK cells, hematopoietic progenitory cells and endotheliocytes	Kyurkchiev et al. (2014), Meirelles et al. (2009)
	Inhibition of differentiation of monocytes toward DC	Magatti et al. (2009)
	Stimulation of T lymphocytes migration toward MSC	Ren et al. (2008)
	Induction of FasL, CCL2 and CCL5 (RANTES)-dependent apoptosis of lymphocytes	Boomsma and Geenen (2012)
Chemokine RANTES/CCL5	Stimulation of MSC migration toward regions of inflammation, ischemia, trauma and tumorigenesis	Zischek et al. (2009)
IDO	Proapoptotic factor; inhibition of B cells growth and T lymphocyte proliferation through rapid tryptophan degradation; inhibition of DC maturation; support Treg generation from naive cells; activation of Treg cells and promotion of their immunosuppressive properties	Chen et al. (2008), Hsu et al. (2013), Kyurkchiev et al. (2014), Liu et al. (2013), Maby-El Hajjami et al. (2009)
VEGF	Restriction of DC maturation, memory cells inhibition, induction of IDO synthesis by DC	Basu et al. (2010), Dikov et al. (2005), Marti et al. (2014)
PGE2	Regulation of proliferation, differentiation and activity of PBMC, NK cells, macrophages and monocytes	Beyth et al. (2005), Van Elssen et al. (2011)
Cell membrane molecules		
ICAM	Enables MSC migration, proliferation and differentiation	Fu and Li (2009), Xu et al. (2014)
HLA-DR, CD-45, CD14 CD86	Inhibition of resting allogeneic T lymphocytes proliferation	Magatti et al. (2009)
HLA-DR	CD3 lymphocytes proliferation through TcR receptor	

Major histocompatibility complex proteins

Amniotic cells, hAEC and hAM-MSC, show different ability to produce classical and non-classical (class Ib) MHC class I proteins. These molecules determine immunological neutrality or inhibit immunological reactions. HLA-G and human leukocyte antigen E (HLA-E) are not immunogenic both, in pregnant woman and her semiallogenic fetus, as well as in recipient after allogenic transplantation, because of low polymorphism. It has been observed in amniotic cells cultured *in vitro* that HLA-G expression is induced after interferon β (IFNβ) and IFNγ application (Lefebvre et al., 2000; Banas et al., 2008). HLA-G antigens are probably responsible for both, inhibition of monocyte *in vitro* differentiation into dendritic myeloid cells and their activity. Nevertheless, they do not exhibit inhibitory properties against dendritic cells of lymphoid origin to produce IL-10, what in turn activates Treg lymphocytes (LeMaoult et al., 2005).

HLA-G protein exists in seven different isoforms from which three (G1–G3) are surface proteins and four (G4–G7) are soluble. During the placenta development five of them have been detected in its tissues. Presence of HLA-G4 and HLA-G7 has not been documented (Hunt et al., 2005). Among hAEC, some stem cells exhibit only surface HLA-G expression and they do not secrete soluble isoforms. In general, hAEC are able to produce soluble HLA-G proteins and secrete them into amniotic fluid where they play an important immunosuppressive role, directly interacting with immune cells. hAM-MSC express HLA-G proteins on cell surface and secrete soluble forms into ECM (Banas et al., 2008; Tsuji et al., 2010; Wang and Ou-Yang, 2014). Metalloproteinases may take part in regulation of HLA-G protein secretion into ECM (La Rocca et al., 2012).

HLA-G proteins are able to interact with leukocyte Ig-like receptor B receptors (LILRB) also known as ILT. These later are known as major receptors for HLA-G on leukocytes, but

also on dendritic cells, macrophages, NK cells, as well as T and B lymphocytes. LILRB2 (also known as ILT-4) is probably a main receptor for HLA-G on monocytes/macrophages and dendritic cells, despite the fact that LILRB1 (also known as ILT-2) may also be present at these locations. Dual label flow cytometry confirmed the expression of ILT2 and ILT4 on CD14-positive decidual macrophages in the first trimester (Petroff et al., 2002). HLA-G regulates ILT-2 and ILT-4 expression on NK and T cells, protecting amniotic cells from immune cells action (Ristich et al., 2005). Thanks to the surface ILT-4 receptors expression, HLA-G proteins are also able to control MLR reaction (Riteau et al., 1999). ILT-4 receptors show the highest specificity to HLA-G dimers creating after its secretion into ECM (Rajagopalan and Long, 1999).

B7 proteins

B7-H3 immunomodulatory protein (CD276), representing B7 protein family, has been identified on cytotrophoblast, syncytiotrophoblast, fibroblasts and Hofbauer cells already in the first trimester of pregnancy. Its expression is dependent on the stage of fetus development. In term placenta it has been detected also on the surface of hAEC (Petroff et al., 2005). In culture, B7-H3 protein displays two different immunomodulatory strategies: as a co-stimulator of T lymphocytes proliferation and as an inhibitor of active T lymphocytes. Simultaneously, B7-H3 does not exhibit inhibitory properties toward inactivated lymphocytes (Sun et al., 2002). The protein can interact with CTLA-4 receptor of lymphocytes (Chapoval et al., 2001).

B7-H1 (PD-L1), a new member of B7 family protein, a surface marker, binds to its receptor, PD-1. It results in inhibition of antigen-induced T-cell activation. In placenta, B7-H1 is highly expressed on trophoblast cells (Petroff et al., 2005).

Proapoptotic ligands

The exact mechanism, by which hAEC mediate induction of apoptosis of lymphocytes, remains unknown. It is known, that hAEC are characterized by expression of TNF-related apoptosis-inducing ligand (TRAIL), TNF-α and FasL, the ligands specific for proapoptotic receptors (Li et al., 2005). Inducing apoptosis they take part in active inhibition of mother's lymphocytes during pregnancy (Hammer et al., 1999). FasL protein is responsible to a great extend for T and B lymphocytes apoptosis, including Jurkat cells. hAEC effectively mediate caspase-dependent process through the interactions between FasL and Fas-receptor in 50% of T lymphocytes (Li et al., 2005).

2,3-Indolamine-dioxygenase

IDO plays an important immunosuppressive role. It is an enzyme that catalyzes tryptophan turnover. Tryptophan is an amino acid essential to proper T lymphocyte functioning. Catalysis of this amino acid results in inhibition of T and B lymphocyte proliferation and development (Maby-El Hajjami et al., 2009). Kynurenine (Kyn) is a product of tryptophan metabolism which influences generation of Treg cells from naive T cells (Nguyen et al., 2010). Additionally, Kyn supports immunosuppressive properties of Treg cells and is toxic for T lymphocytes (Chen et al., 2008; Hsu et al., 2013). Except PGE2 and nitric oxide (NO), IDO activity is one of the factors inhibiting dendritic cells maturation (Liu et al., 2013).

In placental villi and decidua, IDO has been detected already in the first trimester of pregnancy (Chang et al., 2006). IDO is synthesized at a low constitutive level, but after induction with proinflammatory IFNγ its secretion increases (Liang et al., 2013). Also damage-associated molecular pattern molecules (DAMP) contribute to elevated IDO secretion (Lotfi et al., 2011). hAM-MSC exhibit IDO synthesis in culture in presence of T lymphocytes (Kang et al., 2012). hAFC, characterized with mesenchymal cells marker expression (CD29, CD44, CD105) and expression of pluripotency marker Stage-Specific Embryonic Antigen 4 (SSEA-4), as well as HLA-ABC co-operate with PBMC in IDO activation and IL-10 secretion. This relationship is thought to be the most important mechanism responsible for immunosuppressive properties of PBMC (Luo et al., 2014).

Adhesion molecules

Based on the studies on MSC and lymphocytes it can be suggested that cell-to-cell contact may play a particular immunomodulatory role of amnion cells. MSC-lymphocytes interactions result in induction of IL-10 and TGFβ, the cytokines being associated with HLA-G expression. These effects may be mediated by adhesion molecules, such as ICAM-1 and vascular cell adhesion molecule 1 (VCAM-1) (Tipnis et al., 2010). ICAM is expressed on the cell membrane of some MSC, including hAM-MSC, and is responsible for interaction with other immunocompetitive cells. ICAM expression is induced by proinflammatory cytokines (IL-1, IL-6 and TNF-α), as well as IFNγ produced by T lymphocytes. Soluble form of ICAM is secreted in pathological conditions, but also by normal umbilical and decidual MSC. Probably it takes a part in angiogenesis during placenta formation (Kim et al., 2012).

Final remarks

1. Numerous factors secreted by epithelial and mesenchymal cells of the amnion, as well as mother's immune system cells, may potentially influence hAEC phenotype and their immunomodulative properties. These factors become the components of amniotic fluid, basement membrane, and amnion mesenchymal tissue.
2. Among soluble agents present in amniotic fluid, are: growth factors (FGF, EGF, VEGF), prostaglandins (PGE2, PGF2α), proinflammatory cytokines (IL-1β, IL-6 and TNF-α) and anti-inflammatory interleukin IL-10. PGE2, HGF, cytokines and soluble TNF-α receptors can inhibit maternal immune response.
3. Some cytokines, *e.g.*: TNF-α, OSM, RANTES, GM-CSF, G-CSF, IL-6, IL-8, MIP-1α and TGFβ are found in amniotic mesenchymal tissue. These agents may diffuse toward hAEC and influence their immunophenotype. Immunomodulatory, antiangiogenic and anti-inflammatory factors: IL-10, IL-6, TGFβ, VEGF, PGE2, chemokines, IDO, HGF and ICAM act as chemoattractants for immune cells and play

an important role in immune suppression. Furthermore, metalloproteinases and their specific tissue inhibitors regulate key inflammatory processes through degradation of ECM components.

4. BM is composed of fibrillary proteins, glycoproteins and proteoglycans. Relations between BM insoluble components, especially laminins, and integrin receptors seems to be crucial for controlling hAEC fate.
5. Factors affecting hAEC and maternal immune cells form a very complex network of relations dependent on regulatory compounds, mainly LPS, HA, gelsolin, adenosine and HSP70. The latter cooperate with cell receptors, *e.g.* Toll-like receptors, resulting in a combined effect of various pathways (*e.g.*: NFkB, ERK1/2 and protein kinases).
6. hAEC may influence mother's immune cells indirectly – through secreted soluble molecules, and directly – by surface proteins. The key factors involved in immune tolerance are: non-classical HLA molecules, immunomodulatory proteins, ligands responsible for cell death induction, IDO and adhesive molecules.
7. Identification of amniotic microenvironmental components seems to be crucial for the reconstitution of the natural amniotic niche, as well as modulation of the stem cell phenotype.

Conflict of interest

None declared.

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