The similarities and differences between the suppressive profile of the endometrium/decidua and the cancer microenvironment

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Abstract

The phenomenon of immune tolerance in the endometrium determines the proper course of physiological reproductive processes as well as the development of pregnancy. In women with malignant tumors, this phenomenon may actually facilitate the development and progression of the tumor. In this study, we aimed to consider the similarities and differences in the microenvironment of the implanted ovum and the microenvironment of the developing cancer nest in order to understand the precise processes conditioning the development of the inhibitory microenvironment at the tumor site. It seems that once we have acquired a better understanding of the mechanisms that control the endometrial microenvironment during both the menstrual cycle and pregnancy, it will be possible to deal with the inhibitory microenvironment at the cancerous tumor site in clinical practice. Clinicians may be able to obtain useful information regarding the suppressive profile of the tumor microenvironment, and most likely in the near future, the characteristics of the Treg cell population in the microenvironment at the time of cancer treatment will influence the type of therapy applied.

Key words: immune tolerance, cancer microenvironment, endometrium, decidua, TAM, CAF

Introduction

It is well known that the endometrium is a special tissue able to regulate the immune cells that infiltrate it. The proper course of reproductive processes requires the presence of immune cytotoxic cells within the endometrium. Although these cells are able to attack, given their presence within the endometrium, they are also subject to selective suppression [1-3]. This phenomenon of the selective suppression of immune cells within the endometrium has been thoroughly described by Chao [4, 5]. Furthermore, it is well-known that the status of the endometrium (which involves the paracrine activity of endometrial cells and the presence within the endometrium of immune regulatory cells such as Treg cells) determines the final activity of the cytotoxic immune cells concentrated within the endometrium and deciduas [6-8]. Both molecular suppressing mechanisms depend on endometrial cells, and immune regulatory cells also participate in the development of the suppressive profile of the endometrium. The existence of this phenomenon has some clinical consequences, but as a physiological process it is under continuous control, and its intensity fluctuates in accordance with the phases of the menstrual cycle [9]. It begins a few days before ovulation in the late proliferative cycle phases and in the case of successful fertilization continues, enabling proper implantation and subsequent pregnancy development [10]. In the absence of fertilization, the start of the molecular processes linked with immune suppression within the endometrium leads to menstruation [11]. This process within the endometrium is subject to constant fluctuation. Arruvido and colleagues have described the changes in Treg cell infiltration into the endometrium that take place between the late proliferative and early secretory cycle phases [10], and the lack of such changes has been linked to recurrent pregnancy failure. The main characteristic of this physiological phenomenon is that it is reversible [12]. Reproductive success requires not only the suppression of the cytotoxic immune response, but
also local cooperation between maternal and fetal cells — that is, between maternal immune cells, endometrial cells, and trophoblast cells. Such local cooperation should be related to selective immune suppression, but when needed, the cytotoxic immune response must be stimulated and increase (as for instance, during the spontaneous beginning of labor) [3, 13, 14]. The trophoblast cells invading the endometrium and myometrium during pregnancy are very similar to the aggressive phenotype of cancer cells that evade its microenvironment. Since the 1990s when Clark compared pregnancy development to cancer development, many studies have found other molecular mechanisms both in the endometrium/decidua and in the cancer microenvironment [15-17]. Although these molecular mechanisms are similar, nevertheless the process of trophoblast cell evasion from maternal immune surveillance differs from that of the evasion of cancer cells from the host immune surveillance. In the case of pregnancy, this physiological process can be reversed. Proper pregnancy development is linked with the continuous control over the intensity of such selective immune suppression. If this control is disrupted, the pregnancy fails, as is observed, for example, in women with pre-eclampsia. Nevertheless, these same molecular suppressing mechanisms are responsible for generating a suppressive profile within a tumor microenvironment, such as the cancer microenvironment. The host loss of control over selective immune suppression is local at first and later general. The most important difference is that while within the normal endometrium this suppressive profile is subject to reversal, it is not reversible within the tumor microenvironment. In order to better understand how the generation of this profile is regulated we reviewed the available information on the generation of maternal immune tolerance against fetal antigens.

**The molecular background of maternal immune tolerance against fetal antigens**

This phenomenon of the selective suppression of cytotoxic immune cells is an on-going and progressive process, and different molecular mechanisms are incorporated into this phenomenon. It can be represented schematically as composed of at least four levels. At the first level, the cancer cells are invisible to immune cells; at the second, they actively kill immune cells; at the third, there are changes in the microenvironment of the immune cells that inhibit the maturation of immune cytotoxic cells, and at the fourth level, the suppressive mechanisms belonging to the maternal immune system are taken over [2, 8, 13, 17-21]. The selective suppression mechanism progressively increases, and the phenomenon of selective suppression begins to change from a local process at the time of implantation to a general mechanism during labor [22].

At the first level, the trophoblast cell evasion of the immune response occurs because the cells are able to present a state invisible to the cytotoxic immune response through the absence of classical MHC antigens such as HLA-A (leukocyte antigen-A) and HLA-B [2, 23, 24].

At the second level, the trophoblasts actively kill immune cytotoxic and NK cells. They express HLA-G antigens on cell membranes that are able to inhibit the NK cells and even the cytotoxic T lymphocytes by acting with the KIR receptors present on these cells [21, 25]. The presence of KIR receptors has been noted in many studies on natural killer cells, and most recently, Tilburgs and colleagues have described the presence of KIR receptors even on T lymphocytes within the deciduas [26]. Additionally, many membrane proteins that participate in immune regulation, such as Fas, RACS1, and B7H4, were found on trophoblast cell membranes. These proteins interact with receptors on cytotoxic lymphocytes and NK cells and inhibit the maturation of these cells and even directly stimulate apoptosis [27-38].

The trophoblast cells reconstruct the microenvironment. This process is linked with the generation of conditions that inhibit the proper maturation of cytotoxic immune cells. It is realized mainly through the expression of the indoleamine-2,3-dioxygenase (IDO) enzyme on the surface of trophoblast cell membranes [39]. This enzyme seems to be critical to the development of the tumor because of its role in tryptophan metabolism and in restricting cytotoxic T-lymphocyte maturation [40]. As the reconstruction of the microenvironment continues, the maternal immune response is redirected from cytotoxic action to the humoral immune response. This stage of the phenomenon of selective suppression is related to the concentration of Th2 interleukins (IL-10 and IL-4) [41].

Finally, trophoblast cells recruit Treg cells as well as regulatory immune cells and B7H4-positive macrophages to the decidua [6-8]. The presence of Treg cells in the endometrium and decidua is directly linked with the suppression of the cytotoxic immune response (this is the strongest of the suppressive mechanisms used by the host immune system). These T lymphocytes are typified by CD25 antigen expression. Since the antigen is the receptor for IL-2, these cells are concentrated in the place in which the cytotoxic action occurs (modulated by IL-2). Treg cells are able to secret chemokines and cytokines and finally to stop the immune response [42].
When the four stages have been realized, the phenomenon of selective suppression is at work in the decidua. To maintain this suppression, interaction between decidua and infiltrating immune cells is necessary. This is the reason why a strong, local reconstruction of the maternal immune response, enabling the intrauterine development of the fetus is not dangerous to the mother. When the phenomenon does not operate properly, a very dangerous clinical situation arises, for example, pre-eclampsia, placenta accreta or postpartum hemorrhages [3]. The endometrium/decidua is therefore necessary for providing the microenvironment of the implanted ovum and for the proper development of pregnancy.

**Cancer microenvironment**

The advancement of the disease requires the interaction between the cancer cells and the host immune system. This interaction will lead to either the elimination of the cancer cells or to the development of the phenomenon of cancer cell escape from the host immune control. Recently Liu et al. have demonstrated the progressive course of this phenomenon of cancer cell evasion of the immune surveillance [43, 44]. Initially, the immune system cells recognize the cancer cells and remove them effectively. In the next stage, cancer cells can survive in spite of immune recognition because the increased proliferation disrupts the homeostasis. During the third and final stage, the cancer cells are able to escape successfully from the host immune surveillance [44]. Siedlar has characterized this phenomenon as consisting of two phases. In the first phase, in tumors that are not advanced, cancer cells escape from the host immune surveillance, which is an antigen-specific process. The second, definitive phase, in tumors that are more advanced, is characterized by general immune system suppression [45]. This understanding of the tumor-immune interaction demonstrates the possibility that the tumor could take control of the host immune system. Initially, the process of tumor escape from the host immune surveillance amounts to a process of cheating the immune system. As the tumor grows, however, it takes control over the local immune system and the phenomenon develops into a generalized immune suppression. Selective suppression dominates at the beginning of cancer growth and in the advanced stage a reactivity in the immune response is observed [46-50]. This progressively increases with respect to the activation of the molecular mechanisms that are linked with selective suppression identical to these; this creates maternal immune tolerance against fetal antigens [15]. The recognition of the range of this phenomenon may be the key to both understanding this process and being able to influence it. Local control of the immune system response is mainly realized within the endometrium during pregnancy development, but as the tumor grows, such processes are not precisely controlled in the tumor microenvironment.

The tumor microenvironment makes up the stroma of the neoplasm and is the tissue that determines tumor growth, progression, and its ability to initiate metastases. The tumor microenvironment can also restrict the access of therapeutic agents to the neoplasm, alter the metabolism of such agents, and participate in developing resistance to chemotherapy. Because of the role that the tumor microenvironment plays in each stage of tumor development, the knowledge of the interactions between the tumor and its microenvironment would seem to be important for developing new treatment strategies to restore the normal mechanisms of control.

The tumor microenvironment is created by the tissue surrounding the tumor cells and is composed of cells, the extracellular matrix, and the proteins of the extracellular matrix. Many types of cells can be identified in the stroma, including endothelial cells and their precursors, pericytes, smooth muscle fibers, fibroblasts, tumor-associated fibroblasts, myofibroblasts, neutrophils, basophils, eosinophils, mast cells, T and B lymphocytes, and NK cells as well as antigen-presenting cells, such as macrophages and dendritic cells [46-58]. The tumor stroma may also play an important role in tumor development. Tumor cells are capable of penetrating the surrounding stroma, but at the same time it is the tumor stroma that provides the necessary blood supply and growth factors for the tumor cells that condition tumor growth. Some neoplasms use and modify the existing stroma for their growth while other tumors induce the development of new stroma [52]. In sum, the tumor stroma is not neoplastic tissue, but tissue which has been modified by the tumor for the purpose of its own growth and development.

Many processes, such as those related to the inflammation and immune responses that determine tumor growth, but do not provide the effective anti-tumor immune response actually take place within the tumor microenvironment. The tumor grows within the stroma which has been prepared to support this growth [59]. Moreover, it has been shown that the percentage of stromal cells reflects both the type of tumor growth and the prognosis. In ovarian cancer, the structure of the tumor microenvironment is related to poor prognostic
factors, such as the architecture of the vessels, the type of infiltration of the immune system cells, and the type of factors secreted by the fibroblasts, which interact with the extracellular matrix. The structure of the tumor microenvironment itself with its dominating cellular components has independent prognostic significance [46, 47, 59-61].

In some tumors, the stroma may constitute more than 90% of the tumor mass. Activated fibroblasts, the myofibroblasts of the tumor microenvironment, which are the chief cells constructing the tumor stroma, are called carcinoma-associated fibroblasts (CAF) because their number often increases significantly within malignant tumors and they have been shown to facilitate tumor progression [62]. Myofibroblasts have features in common with smooth muscle cells and fibroblasts; they induce the growth and differentiation of cells during embryogenesis, wound healing, and other processes of tissue remodeling [62]. Myofibroblasts may arise from resident tissue fibroblasts, pericytes, vascular smooth muscle cells, bone marrow precursor cells, and even cancer cells. The proliferation and activation of fibroblasts contribute to fibroblast accumulation in the microenvironment. The tumor itself stimulates the proliferation of fibroblasts by the secretion of PDGF, while TGF-β when secreted by macrophages can, in low concentrations, chemo-attract fibroblasts, and in higher concentrations may even stimulate their trans-differentiation into myofibroblasts [62]. Moreover, the tumor cells themselves express TGF-β [63]. Myofibroblasts appear just prior to tumor invasion and facilitate the degradation of the basal membrane and extracellular matrix by the secretion of serine proteases, matrix metalloproteinases, and urokinase plasminogen activator. Furthermore, myofibroblasts express IGF and HGF/SF (hepatocyte growth factor/scatter factor) which induce cell survival, cell migration, expression of pro-angiogenic factors (FGF-2, VEGF), interleukins (IL-1, IL-6, and IL-8), and other factors such as TNF-α. Myofibroblasts not only stimulate their own migration to the tumor, they also induce the survival, proliferation, and invasion of tumor cells and angiogenesis; together this increases the degree of invasiveness of the tumor [52-56]. The cancer-associated fibroblasts can be identified by α-SMA, smooth muscle actin, vimentin expression, or FSP-1, specific protein-1 expression. The recruitment of cancer-associated fibroblasts during the epithelial to mesenchymal transition (EMT) process activates the stromal response. It has been observed that after the exposure to TGF-β, proliferating endothelial cells can transdifferentiate in EMT process into fibroblast-like cells, having the expression of the mesenchymal marker FSP-1 [46, 47]. In normal ovarian tissue, the ovarian epithelial cells and stromal cells collectively inhibit inappropriate or pre-neoplastic epithelial proliferation. The transition into pre-neoplastic or neoplastic cells is realized by secretory communication with activated myofibroblasts. The progression toward ovarian cancer involves the secretory co-opting of ovarian CAFs through the exchange of secreted factors with CAFs to facilitate dissemination to the omentum. During this process, the CAFs can be recruited from cancer cells undergoing EMT. In this process they acquire the ability to migrate and to digest the extracellular matrix facilitating the dissemination to the omentum [59]. It has been demonstrated that the presence of activated CAFs marked by α-SMA and FAP expression is related in a statistically significant way to the presence of lymph node metastases and dissemination to the omentum as well as higher lymphatic vessel and microvessel density. Moreover, when CAFs have been isolated from the tumor, they have been observed to induce both the invasion and migration of ovarian cancer cells in vitro. This proves that CAFs are related to ovarian cancer progression and metastases. In addition, Jóźwicki and colleagues have recently shown that the more RCAS1 (binding cancer antigen expressed on SiSo cells)-positive fibroblasts detected in the ovarian cancer microenvironment (RCAS1+ CAF), the higher the resistance of patients with ovarian cancer to standard chemotherapy [59]. This may be related to the role of cancer-associated fibroblasts in modulating the immune response. These cells may suppress the infiltration of cytotoxic immune system cells into the tumor microenvironment in patients with ovarian cancer [59] and enhance cancer growth. The cells from the tumor microenvironment cooperate with cancer cells in evading immune surveillance, and in these processes they use the same molecular mechanisms that are used physiologically in the endometrium and deciduas [64-69].

Not only do the fibroblasts creating the tumor microenvironment affect its homeostasis, but recently, researchers have identified tumor-associated macrophages (TAMs) as a cellular population important for tumor growth. The role of TAMs in tumor development seems to be dual. On the one hand, these cells can stimulate tumor progression, but on the other hand, they can stimulate tumor rejection [54-57]. A positive correlation was determined between the number of TAMs and poor prognosis in many cases involving malignant tumors. Furthermore, monocytes/macrophages are present in the tumor microenvironment from the earliest stages of
tumor development in the surrounding hypotrophic and atypical cells [54-57]. The tumor and its microenvironment express the chemotactic agents for macrophages (for instance, IL-8), and macrophages secrete both growth factors and the pro-angiogenic factors that modify tumor growth (for example, EGF). Monocytes/macrophages that infiltrate both the tumor and the tumor microenvironment, when stimulated by the microenvironment factors, may acquire one of two phenotypes, either M1 or M2 [48, 67]. The M1 phenotype is associated with anti-microbial activity and inflammation. M1 macrophages are activated by pro-inflammatory cytokines and are typified by the secretion of TNF, IL-8, IL-12, and enhanced NO production. These macrophages participate in powering the inflammatory response. They also produce IL-15 by means of which they can influence the proliferation and activity of other immune system cells and so affect the profile of the microenvironment [22, 48, 51, 67, 70, 71]. Thus this kind of immune response supported by M1 macrophages is an antitumor response. In the tumor microenvironment, M2 macrophages predominate. The M2 phenotype is related to tissue remodeling and pro-angiogenic activity as well as the restriction of the host immune response against tumor cells. Tumor-associated macrophages (M2) are able to secrete TGF-α and PGE2 [45, 49, 70, 71]. The M2 phenotype is also related to marked IDO expression on the surface of the macrophage cell membrane. As described above, this enzyme seems to be critical to the development of the tumor because of its role in restricting cytotoxic T-lymphocyte maturation [46]. The phenotype of the dominant macrophages in the tumor microenvironment depends on the profile of cytokines in this particular microenvironment. Of all the factors determining this domination, IL-6 and IL-10 seem to be the most important. Molecular changes in the tumor microenvironment may affect the dominating profile, and in this way, the immune system response to the cancer cells may also be regulated in the microenvironment. Cancer cells can then inhibit the anti-tumor immune response by secreting IL-10. M2 macrophages also influence angiogenesis in a dual mode. While on the one hand, these macrophages secrete pro-angiogenic factors, on the other hand, they also secrete anti-angiogenic factors that disintegrate blood vessels. The pro-angiogenic function prevails in the interaction between tumor cells and macrophages, and the accumulation of M2 cells is related to VEGF and PDGF secretion [55]. The infiltration of macrophages to the hypoxemic tumor regions induces a pro-angiogenic program of activity in these cells. Among the factors expressed in macrophages under hypoxia, the following seem to be the most important: VEGF, TNF-α, bFGF, CXCL8 (IL-8), glycolytic enzymes the transcription of which is controlled by HIF-1 and HIF-2 factors [58]. Macrophages also influence lymphangiogenesis in the tumor microenvironment. Lymphangiogenesis is controlled by VEGF-C and VEGF-D interacting with VEGFR3. In uterine cervical cancer, the production of VEGF-C by macrophages plays an important role in lymphangiogenesis and tumor dissemination with metastasis through the lymphatic vessels. The local tumor development depends on controlled degradation of the extracellular matrix. Moreover, it has been shown that macrophages participate in the development of metastases. On animal models, the decreased number of macrophages was related to a reduced frequency of metastases [58]. Macrophages are typified by proteolytic activity that leads to basal membrane disintegration in pre-invasive lesions (in situ) and so enables the spread of tumor cells into the surrounding microenvironment. M2 macrophages secrete matrix metalloproteinases (including MMP-2 and MMP-9), degrading the basal membrane proteins and MMP activators, such as chemokines and other factors that can facilitate the degradation of the matrix and the invasion and migration of tumors cells (PDGF, IL-6, u-PA, and t-PA) [58]. Macrophages also secrete factors that encourage tumor cells to settle into the tissue (for example, EGF); the tumor cells in turn release chemotactic agents for macrophages, such as M-CSF (a macrophage colony-stimulating factor) [49, 58]. Many studies have proven that inflammatory cells and cytokines in the tumor and its microenvironment are able to stimulate tumor growth more than they are able to provide an effective anti-tumor immune response [49].

Antigen-presenting cells are important for the initiation and maintenance of a specific immune response related to tumor-associated antigens. The number of tumor-associated macrophages significantly surpasses the number of other antigen-presenting cells, such as dendritic cells, which are the most widely dispersed cell population in solid tumors [72-75]. Furthermore, it has been demonstrated in mice that tumor-associated macrophages interacting directly with tumor cells may promote tumor growth and metastases [48, 51, 56]. The B7H4 antigen is a recently discovered B7-family molecule member, co-stimulating T lymphocytes, negative regulators of T-cell response. It inhibits in vitro T-cell proliferation, cell cycle progression, and cytokine production. Kryczek et al. have demonstrated the presence of B7-
H4⁺-positive macrophages in the tumor microenvironment of patients with ovarian cancer and confirmed their strong suppressive activity in comparison to Treg lymphocytes [72, 73]. The tumor microenvironment has been shown to stimulate the expression of B7-H4⁺ on macrophages by IL-6 and IL-10, and the number of B7-H4⁺-positive macrophages in the tumor microenvironment has been correlated with survival rate in patients with ovarian cancer. Treg lymphocytes stimulate the production of IL-10 by APC (antigen-presenting cells), and IL-10 determines the dominating phenotype of macrophages in the tumor microenvironment, namely, the M2 phenotype [72-74]. On the other hand, through the secretion of TGF-beta and IL-10 these macrophages also influence the maturation of Treg cells. Macrophages expressing RCAS1 antigen (receptor-binding cancer antigen expressed on SiSo cells) or HLA-G antigen (human leukocyte antigen) [65, 66, 75-78] seem to play a role similar to that of B7-H4⁺-positive macrophages in the tumor microenvironment. Because the interaction of these antigens with ligands present on NK cells and cytotoxic T lymphocytes leads to the inhibition of the activity of these cells, the macrophages expressing RCAS1- and HLA-G-antigens in the tumor microenvironment of patients with ovarian cancer, endometrial cancer, and uterine-cervix cancer affect the suppression of an anti-tumor immune response. Recently, Galazka and colleagues demonstrated that the B7H4-positive TAM infiltration into the microenvironment of cervical cancer correlated with the local spread of the disease and was higher in advanced disease [64]. The observations described above point to the important role of M2 macrophages in the creation of a suppressive profile within the tumor microenvironment which in turn plays a key role in the tumor growth and progression.

**Conclusion**

In sum, the tumor microenvironment constitutes an example of less stable and organized tissue than the endometrium. The lack of precise control of the immune response against cancer cells within the cancer microenvironment, as occurs in the endometrium and decidua, enables immune cells to be used for promoting tumor growth. Cells such as TAM or CAF which are derived from the cancer microenvironment support tumor growth both directly and indirectly. On the one hand, they participate in both lymphangiogenesis and neoangiogenesis, and on the other hand, they also participate in evasion from immune surveillance. It seems that once we have acquired a better understanding of the mechanisms that control the endometrial microenvironment during both the menstrual cycle and pregnancy it will be possible to deal with the inhibitory microenvironment at the cancerous tumor site in clinical practice. Clinicians may be able to obtain important information regarding the suppressive profile of the tumor microenvironment, and most likely in the near future the characteristics of the Treg cell population in the microenvironment at the time of cancer treatment will influence the type of therapy applied.

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**References**

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