REVIEW

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Fetal-cell microchimerism, lymphopoiesis, and autoimmunity

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Abstract

During all human and murine pregnancies, fetal cells enter the maternal circulation and tissues and may persist there for decades. The immune consequences of this phenomenon have been explored for many years as a potential origin of autoimmunity or protection from cancer in women after pregnancy. The leading hypothesis, suggesting that semi-allogenic fetal T cells may trigger a graft-versus-host type of disease, has been supported by several studies showing an increased frequency of fetal-cell microchimerism (FMc) in women affected with systemic sclerosis. However, a large proportion of healthy women or women affected with non-immune disorders also display fetal T cells, challenging the direct pathogenic role of such cells. In addition, recent evidence showing the transfer of various fetal progenitor cells to the mother during gestation has shed new light on the interpretation of microchimerism in autoimmunity. This review discusses the functional capacity of fetal hematopoietic progenitors to form T and B cells in maternal hematopoietic tissues, where they undergo an educational process probably resulting in tolerance to maternal antigens. Therefore, hypotheses other than the transfer of fetal cells to the mother's circulation should be considered in explaining the observed association of FMc and autoimmune disorders.

Key words: microchimerism, pregnancy, fetal T cells, lymphopoiesis, auto-immunity, stem cells.

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Fetal-cell microchimerism (FMc) results from the exchange of fetal and maternal cells during pregnancy (Ariga et al. 2001). It has been widely described in human and animal models with hemochorial placentas (Jimenez et al. 2005). Fetal cells can persist in the maternal circulation for decades after delivery (Bianchi et al. 1996). Indeed, Bianchi et al. showed that fetal $CD34^+CD38^+$ cells were detectable as long as 27 years postpartum. The full biological significance of this physiological phenomenon is not yet understood, although an increasing body of literature links it to the ability to both "cause" and "cure" maternal diseases (Artlett 2002). In this view, several studies suggested that FMc may play a role in the pathogenesis of some immune disorders. It has been mainly associated with systemic sclerosis (SSc), but also some inflammatory disorders of pregnancy (Aractingi et al. 1998; Nelson et al. 1998). Alternatively, it has been proposed that fetal progenitor cells acquired through pregnancy (pregnancy-associated progenitor cells, PAPCs) persist in a maternal stem cell niche and are able to home secondarily to damaged tissues as part of a repair response (Khosrotehrani et al. 2004). The precise identity of these PAPCs has not been determined, but hematopoietic, mesenchymal, and placental stem cells, endothelial progenitors, hemangioblasts, or a mix of these have been proposed as potential candidates (Nguyen Huu et al. 2006).

IMMUNE REACTIONS TRIGGERED BY FETAL MICROCHIMERIC CELLS

Assuming that chimeric semi-allogenic fetal T cells may induce an allogenic response in maternal tissues, studies investigated the possibility that maternal autoimmune disorders may be associated with and/or induced by FMc. Since SSc displays clinical features similar to chronic graft-versus-host disease, and that it predomi-

nated in women, usually after the age of first pregnancies (Steen, 2007), this autoimmune disease has been thoroughly investigated. In agreement with this hypothesis, initial studies indeed displayed higher levels of fetal microchimeric cells in maternal peripheral blood of females with SSc than controls (Nelson et al. 1998). In addition, there is evidence that at least a subpopulation of the chimeric cells in the peripheral blood of 51% of women affected with SSc is T cells (Artlett et al. 2002; Lambert et al. 2002). Importantly, the presence of cells of fetal origin, including T cells, was also found in healthy women after pregnancy (31%), suggesting that microchimeric cells alone were not able to induce autoimmune conditions. Nevertheless, these results suggested an association between persisting FMc and the occurrence of scleroderma. However, more recently, other studies did not confirm an overrepresentation of FMc in the blood of women affected with autoimmune disorders (Gannage et al. 2002).

Similar to peripheral blood, fetal microchimeric cells can be found more frequently in the affected skin of women with scleroderma than in controls (58 versus 0%) (Artlett et al. 1998). Microchimeric cells were also present in uninvolved skin biopsies of women with SSc, raising the hypothesis that microchimeric cells in clinically unaffected SSc play a role in the later occurrence of sclerotic changes (Sawaya et al. 2004). This observation suggests that cytokines secreted by T cells and/or other cells of the immune system play an important role in the pathogenesis of SSc. Scaletti et al. was able to generate T-cell clones from peripheral blood and skin biopsies from women with SSc of recent onset and from controls, all having had at least one male child (Scaletti et al. 2002). There was an increased number of T-cell clones obtained from SSc patients, proliferating in response to autologous cells, compared with those obtained from the healthy women. However, only 18% of the "autoreactive" clones from the SSc samples and 9% of the clones from the healthy women displayed a Y chromosome, suggesting their fetal origin. The clones from the SSc patients produced higher levels of the profibrotic cytokine interleukin (IL)-4, whereas the normal clones did not. This observation suggests that male fetal cells are present in the circulation and/or skin of women with SSc that may be reactive against maternal major histocompatibility complex antigens and display a Th2-oriented profile. However, this study cannot demonstrate the causative role of microchimeric cells given the large presence of autoreactive maternal cells.

Despite these data, there was no study addressing the type of infiltrating fetal cells in SSc. Of note, McNallan (McNallan et al. 2007) investigated the role of fetal cells by immunophenotyping the chimeric cells in biopsies from 18 patients presenting a very different disease called localized scleroderma (morphea). The affected tissue contained a greater number of lymphocytic inflammatory cells: 38% of the total chimeric cells were $CD68^+$ (dendritic cells, monocytes, and macrophages), 29% Langerin/$S100^+$ (Langerhans cells, dendritic cells), 26% $CD8^+$ (cytotoxic T-lymphocytes), 20% $CD19/20^+$ (B lymphocytes), and 14% $CD4^+$ (T-helper lymphocytes). This study suggests that fetal chimeric cells in morphea were mainly displaying an antigen-presenting role. Therefore, another possibility is that the fetal chimeric cells could be the target of a host-versus--graft-like reaction; antigens from the chimeric cells would induce an immune response, consequently triggering an autoimmune-like reaction. However, this remains speculative. In addition to the study by McNallan et al. described above, the maternal immune system is directly responsive to fetal cells since the level of microchimeric cells seems dependent on the level of feto-maternal compatibility, at least in animal models (Bonney and Matzinger 1997; Khosrotehrani et al. 2005a; Tafuri et al. 1995).

An alternate hypothesis is that the microchimeric cells are not directly involved in the pathogenesis of autoimmune diseases, but that their presence in host tissues illustrates their intervention to repair maternal lesions. This is in accordance with the fact that several studies have not found any association with the presence of FMc. In SSc skin tissue, 29% of women affected with SSc compared with 35% of healthy women presented fetal cells (Gannage et al. 2002; Ohtsuka et al. 2001). In primary biliary cirrhosis, authors did not find any significant differences when they compared affected and healthy women (Corpechot et al. 2000; Fanning et al. 2000; Tanaka et al. 1999). Similarly, in primary Sjögren's syndrome, although fetal cells were reported in salivary glands, a significant association with FMc suggesting a role for these cells remains controversial (Aractingi et al. 2002; Kuroki et al. 2002). In systemic lupus erythematosus the level of FMc seems dependent on the affected tissue. In cutaneous lupus, no fetal cell could be detected (Khosrotehrani et al. 2005b). Similarly, in systemic lupus the frequency and number of microchimeric cells were comparable between cases and controls. However, in patients with lupus nephritis there was an increase in the number of fetal cells in the peripheral blood as well as the kidney (Kremer Hovinga et al. 2006; Kremer Hovinga et al. 2008; Mosca et al. 2003). The presence of fetal microchimeric cells in similar proportions in affected and healthy women suggests that these cells do not play a direct role in triggering maternal autoimmune disorders. Therefore it seems that a disease activity threshold is necessary for the significant detection of FMc, suggesting that their presence is a consequence and not a cause of the disease.

FMc: THE TRANSFER AND LONG-TERM ENGRAFTMENT OF FETAL STEM CELLS

Fetal cells have been retrieved within leukocyte, hepatocyte, epithelial (Khosrotehrani et al. 2004), and mesenchymal populations in human samples (O'Donoghue et al. 2004). The description of fetal cells adopting a large variety of phenotypes in affected

maternal tissues has lead to the idea that these cells originate from multipotent progenitors. In addition, in animal models with tissue injury, fetal leukocytes (Nguyen Huu et al. 2007), hepatocytes (Wang et al. 2004), various central nervous system cells (Tan et al. 2005), endothelial cells (Nguyen Huu et al. 2007; Nguyen Huu et al. 2009), and epithelial cells of the urinary tract (Wang et al. 2004) have been described.

MICROCHIMERISM AND CANCER

Recently, similar questions have emerged on the immune role of fetal cells in maternal cancer after the description of reduced frequencies of FMc in peripheral blood from women affected with breast cancer as well as other solid or hematological malignancies (Cirello et al. 2008; Gadi et al. 2008; Gilmore et al. 2008). The leading hypothesis presented by the investigators was that fetal cells were protective against cancer by a graft-versus-leukemia allogenic activity. Previous studies had described the presence of fetal cells in maternal benign thyroid adenoma and malignant cervical tumors (Cha et al. 2003; Srivatsa et al. 2001). In accordance with the above, we also extended the description of fetal cells in maternal tumors in human samples of breast cancer (Dubernard et al. 2008) and melanoma (Nguyen Huu et al. 2009) obtained during gestation as well as in murine models of skin carcinoma (Nguyen Huu et al. 2008), breast adenocarcinoma (Dubernard et al. 2009) and melanoma (Nguyen Huu et al. 2009). In all these human and animal studies we were able to show consistently that fetal cells were more frequent in maternal cancer tissues than in non-malignant controls. Besides, the phenotype of the fetal cells was not consistent with an immune effector activity. In most if not all cases, fetal cells infiltrating maternal tumors did not express CD45, the common leukocyte antigen. Instead, fetal cells mostly expressed cytokeratin, vimentin, or endothelial markers, suggesting their participation in the tumor stroma. The presence and the level of fetal-cell infiltration in the tumor were associated with higher-grade breast tumors and a trend towards increased rates of metastasis in melanoma (Dubernard et al. 2009; Nguyen Huu et al. 2009). Therefore, the above results do not support a protective immune role for fetal microchimeric cells in maternal tumors. Fetal progenitor cells possibly home to maternal tumors to participate in tissue remodeling, as previously described in maternal lesional tissues. This specific homing may explain their reduced number in maternal peripheral blood.

FETAL STEM-CELL ACTIVITY IN MATERNAL TISSUES

The presence of stem cells of fetal origin in maternal tissues has been reported; many authors have shown the transfer of fetal $CD34^+$ cells to the mother (Bianchi et al. 1996; Guetta et al. 2003; Jimenez et al. 2005) or the capacity of fetal cells to form hematopoietic colonies (Osada et al. 2001; Valerio et al. 1997). All these studies suggest the transfer of hematopoietic progenitors to the mother. In addition to hematopoietic progenitors, fetal mesenchymal stem cells able to display the various differentiation pathways of MSCs were detected in maternal bone marrow decades after gestation (O'Donoghue et al. 2004).

More recently we confirmed the functional capacity of fetal cells transferred during gestation to participate in maternal lymphopoiesis *in vivo* and to repopulate an empty thymus (Khosrotehrani et al. 2008). We reported for the first time the capacity of fetal lymphoid progenitor cells acquired during pregnancy to develop in the maternal thymus and bone marrow into functional T and B cells in wild-type as well as immunodeficient hosts. This was shown by the observation of an immature double-positive population of $CD4^+CD8^+$ TCRβ-low and IL-7-receptor--negative cells of fetal origin in the thymus of $RAG^{-/-}$ mice deficient in T- and B-cell development This clearly shows the functional activity of fetal lymphoid progenitors acquired during gestation that may develop in the maternal thymus by going through the double-positive and single-positive stages of T-cell development. In additional experiments in which fetuses were transgenic for a T cell receptor α and β chain specific to an ovalbumin peptide presented by an MHC class II molecule (OT2 transgenic mice) (Blanas et al. 1996), fetal thymocytes underwent a positive selection according to the recognition of MHC class II molecules and gave rise to CD4 single-positive thymocytes, as expected. In the periphery, fetal cells that developed in the maternal thymus were functional in response to allogenic as well as antigen-specific stimulation in two different transgenic models and responded by secreting interferon γ. Similar findings were obtained in fetal B cells that may develop in maternal bone marrow or spleen and produce immunoglobulins. These results suggest for the first time that fetal microchimeric T cells found in healthy women and in women affected with various autoimmune disorders may result from the transfer of lymphoid progenitors during gestation and their later development in the maternal thymus. This phenomenon implies that such T cells have undergone their educational program in terms of positive and negative selection in the maternal thymic environment, which does not allow anti-maternal allogenic responses. In fact, alloreactive T cells of fetal origin developing in the maternal thymus are most probably deleted, and some level of histocompatibility might be necessary for positive selection and the development of microchimerism, as previously described (Nelson et al. 1998). This may explain the high frequency of women with microchimeric fetal T cells without any sign of an immune disorder.

CONCLUSIONS

Fetal-cell microchimerism is a frequent phenomenon occurring during all human and mouse pregnancies

which allows the transfer of fetal cells with different phenotypes in maternal tissues. These fetal cells may have the capacity to trigger an immune reaction in peculiar situations, but it appears that in most cases they derive from long-lived fetal progenitors acquired during gestation that have the same properties as their maternal counterparts. The latest results showing the development of fetal T cells in the maternal thymus clearly allows us to understand why these semi-allogenic fetal cells do not systematically trigger a graft-versus-host--type reaction in healthy women, in whom they are frequently found.

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