Chimerism in health, transplantation and autoimmunity

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Male chimeric cells in female reproductive organs

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Submitted

Abstract

Background

During pregnancy, fetus-derived chimeric cells cross the placenta, reach the maternal circulation and can survive for decades. Chimeric cells have been found more often in women with autoimmune diseases, including Systemic Lupus Erythematosus (SLE) than in healthy women. We investigated the occurrence of chimeric cells in uteri of women with SLE and uteri of healthy controls.

Methods

We studied tissue specimens of uteri from 7 women with SLE and 11 controls, and one uterus with invasive placenta. In situ hybridization of the Y chromosome was performed to identify male chimeric cells.

Results

Male cells were found in 5 of 7 uteri (71%) of the patients with SLE, which was significantly more often than in the control group (2 of 11 uteri; 18%, $p \leq 0.049$). Significantly more male cells were seen in the uterus removed directly after pregnancy.

Conclusion

Chimeric cells are present significantly more often in uteri from women with SLE than in uteri from healthy women. Women with SLE are prone to miscarriages, and the majority of pregnancy losses in SLE is associated with (pre)eclampsia, in which reduced invasion of the extravillous trophoblast is one of the key features. In preeclampsia, more fetal DNA is present in the circulation of the mother during pregnancy. Placental pathology may be responsible for the higher number of male cells in uteri of women with SLE. This is supported by our finding of the highest number of fetal cells in the uterus with invasive placenta removed directly after pregnancy.

Introduction

During pregnancy, fetal cells cross the maternal-fetal interface and reach the maternal circulation and can ultimately be found in a variety of tissues.[1-3] The phenomenon in which cells from diverse genetic origin are present in one tissue, organ, or individual is defined as chimerism. Fetus-derived cells present in maternal tissues are therefore referred to as chimeric cells. They are most easily demonstrated by using in situ hybridization of the Y chromosome, by which male cells are identified in tissues from the mother. Besides pregnancy, other sources of chimeric cells include blood transfusion and organ transplantation.[4,5]

Recently, evidence surmounted that chimeric cells may play a role in the pathogenesis of various autoimmune diseases, such as Systemic Lupus Erythematosus (SLE).[6-8] There are a number of hypotheses on how chimeric cells could induce an autoimmune reaction leading to SLE.[9] SLE, as most autoimmune diseases, affects predominantly women during their fertile age,[10] and therefore, pregnancy as a source of chimeric cells is of particular importance. We previously demonstrated that in organs from women with SLE, chimeric cells are significantly more often present than in organs from healthy controls.[8,11] In line with the hypothesis that chimeric cells pass through the placenta into the maternal circulation, the question then rises whether in the uterus of a woman with SLE, significantly more chimeric cells are present than in the uterus of a healthy control. In the present study, we investigated the occurrence of chimeric Y chromosome-positive cells in the uteri of 7 women with SLE and 11 controls. Aberrant placentation may have implications for the influx of chimeric cells into the maternal circulation, but tissue samples for investigating this issue are scarce. For this study, we obtained one specimen of a uterus with invasive placentation.

Patients and methods

Patients and tissue specimens

Tissue specimens of uteri were obtained at autopsy of seven women diagnosed with SLE. The autopsies were performed at the Leiden University Medical Center (LUMC) between 1985 and 2001. Detailed clinical histories of these women are described elsewhere.[11]

Table 1. Clinical data and ISH results of patients with SLE and controls

Women	Age at death (yrs)	Age at diagnosis SLE (yrs)	Cause of death	Child status		Blood	Number of Y chromosome-positive cells
				Sons	Daughters		
Patients							
1	17	16	Acute pancreatitis	No	No	Unknown	1
2	28	27	Sepsis	No	No	No	0
3	34	33	Pulmonary hypertension	No	No	No	2
4	38	29	Lupus myocarditis	2 children		Yes	7
5	52	31	Sepsis	No	Yes	Yes	6
6	54	38	Mesenterial artery thrombosis	Yes	Yes	Yes	0
7	61	26	Miliary tuberculosis	No	No	Yes	1
Controls							
1	16	NA	Sepsis	No	No	Yes	0
2	27	NA	Myocardial infarction	No	No	No	1
3	30	NA	Sepsis	No	No	Yes	1
4	38	NA	Amniotic fluid embolus	Yes	Yes	Yes	0
5	38	NA	Myocardial infarction	No	No	Yes	0
6	43	NA	Sepsis	Yes	Yes	Yes	0
7	43	NA	Cerebral hemorrhage	No	No	Yes	0
8	51	NA	Non-Hodgkin lymphoma	Yes	Yes	Yes	0
9	52	NA	Melanoma	No	No	No	0
10	55	NA	Sepsis	Yes	Yes	No	0
11	57	NA	Neuro-endocrine carcinoma	Yes	No	No	0

ISH, in situ hybridization; NA, not applicable

As controls, tissue specimens were used from autopsies on women performed at the LUMC between 1999 and 2001. From this group, which was previously described elsewhere,[12] tissue specimens of the uteri of 11 women were included. Age at the time of death and the child status of the women in the control group was similar to those of the SLE patients (Table 1). Of the 11 controls, 4 had died from an infectious cause, 3 from a malignancy, 2 had died from a vascular or myocardial cause, one from a cerebral cause and one from an amniotic fluid embolus.

To investigate whether chimeric cells were present more frequently in uteri just after pregnancy, we obtained tissue specimens from women that underwent an emergency hysterectomy within a day after delivery because of severe postpartum hemorrhage. We identified 5 women that fulfilled these criteria, but only one of these women had delivered a son, the other four had given birth to singleton females. The uterus of the woman with a son was included, of which histological analysis showed a placenta increta.

Clinical data of patients and controls were retrieved from medical records (Table 1). Tissue slides of all uterus specimens were reviewed and there were no concomitant lesions. Only tissue slides that contained both endometrium and myometrium were included.

Information on the child status was obtained by review of gynecologic medical records, or if this information was lacking, by contacting the general practitioners of the patients. This was done with permission of the Medical Ethical Committee of the Leiden University Medical Center. Data on blood transfusions were obtained from the Department of Immunohematology and Blood Transfusion of the LUMC and were available from 1987 onwards.

In situ hybridization of the Y-chromosome

In order to detect chimeric cells, in situ hybridization of the Y chromosome was performed as described earlier.[12] Paraffin-embedded tissues were cut into 4-µm sections, and deposited onto Superfrost Plus glass slides (Menzel-Glaser, Braunschweig, Germany).

The sections were dried overnight at 37°C. A Y chromosome–specific DNA probe[13] was labeled with digoxigenin (DIG) according to the standard Nick-translation protocol. After labeling, the probe was precipitated, dried and dissolved in a hybridization mixture (50% deionized formamide, 0.05 M sodium phosphate buffer pH 7.0, 0.3 mol/L NaCl, 30 mmol/L Na citrate [2 × SSC] and 10% dextran sulphate). To prevent nonspecific binding of DNA, salmon sperm DNA, transfer RNA and Cot-1 DNA were added to the hybridization mixture.

Paraffin was removed by placing slides in xylene. Samples were rehydrated by serial passage through ethanol/water mixtures, followed by a distilled water rinse. The sections were pretreated with 0.01 M citrate buffer (pH 6.0) at 80°C for 80 minutes, rinsed in distilled water at 37°, and treated with 0.5% Pepsin (Sigma-Aldrich, St. Louis, MO, USA) in 0.01 M HCL at 37°C for 20 minutes. Slides were then dehydrated in an ethanol series and air-dried. Slides were covered with a 20-µL hybridization mixture containing 5 ng/µl labeled probe. DNA was denatured by placing the slides on a metal plate at 80°C for 10 minutes, followed by incubation at 37°C overnight.

The following day, sections were washed three times in 2 × SSC/0.1% Tween at 37°C, and three times in 0.1 × SSC at 60°C. To visualize the DIG-labeled probe, sections were incubated consecutively with a mouse-anti-DIG monoclonal antibody (Sigma-Aldrich), rabbit-anti-mouse immunoglobulin-HRP (Dako, Glostrup, Denmark), and swine-anti-rabbit immunoglobulin-HRP (Dako) at room temperature. Finally, sections were developed with Nova Red for ten minutes. Hematoxylin staining served as a background.

Male tissue samples served as positive controls. By PCR and sequencing we confirmed that the probe was specific for the Y chromosome [described in reference [12]]. As a negative control, a male tissue sample was used on which the complete in situ hybridization protocol was performed, but instead of hybridization mixture with the Y chromosome probe, only hybridization mixture was added. This negative control was consistently negative.

Scoring

All specimens were scored independently by two observers, who were blinded to the clinical information of the study subjects. A standardized, randomly chosen area was scored on all slides, which measured 58 mm^2. Strict criteria were used for scoring: a cell was only scored positive for the Y chromosome if there was a dot inside the nucleus that had a similar size and staining intensity as the dots that were found in the nuclei of male control samples.

Statistics

Categorical variables were compared with the use of the Fisher's Exact test and continuous variables were compared with the Student's t-test. P values of < 0.05 were considered to indicate statistical significance.

Results

Seven women with SLE (age range 17-61, mean 41 years) and 11 control women (age range 16-57, mean 41 years) were included in the study. In the patients with SLE, time from diagnosis to death ranged from 1 month to 20 years (mean 7 years).

Male cells were found in five of seven uteri (71%) of the patients with SLE (Table 1). This was significantly more often than the control group, in which Y chromosome-positive cells were identified in only 2 of 11 uteri (18%; $\chi^2=5.10$, $p\leq0.049$). In the positive samples, the number of Y chromosome-positive cells was higher in uteri of SLE patients than of those of controls. In the patient group, up to 7 male were found, compared to maximally one cell in the control group ($p<0.02$). One patient with SLE had 7 male cells, one patient six male cells, one patient two male cells and two patients had one male cell. All cells were solitary cells and located in the myometrium (Figure 1A). In the tissue slides from the uterus with invasive placentation, chimeric cells were of course present in the placental parts, and it could clearly be seen that they were scattered around the myometrial tissue in the vicinity of the placenta (Figure 1B). In the myometrium of this uterus, chimeric cells were abundantly more present than in the non-pregnant uteri of this study. Interestingly, in our normal control group, tissue blocks from the uterus of a

woman who had died from an amniotic fluid embolus just after giving birth to a son, were found to be negative for chimeric cells.

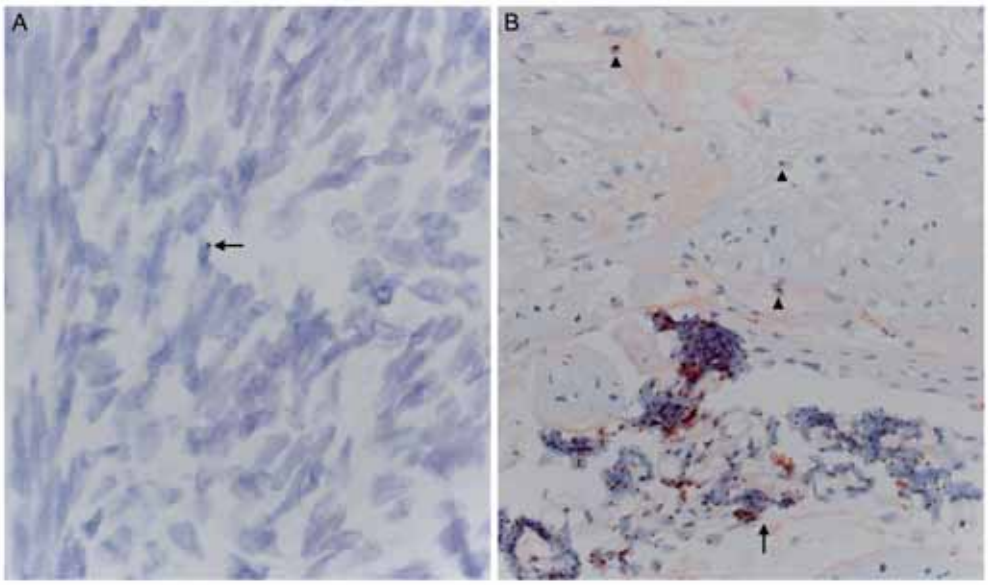
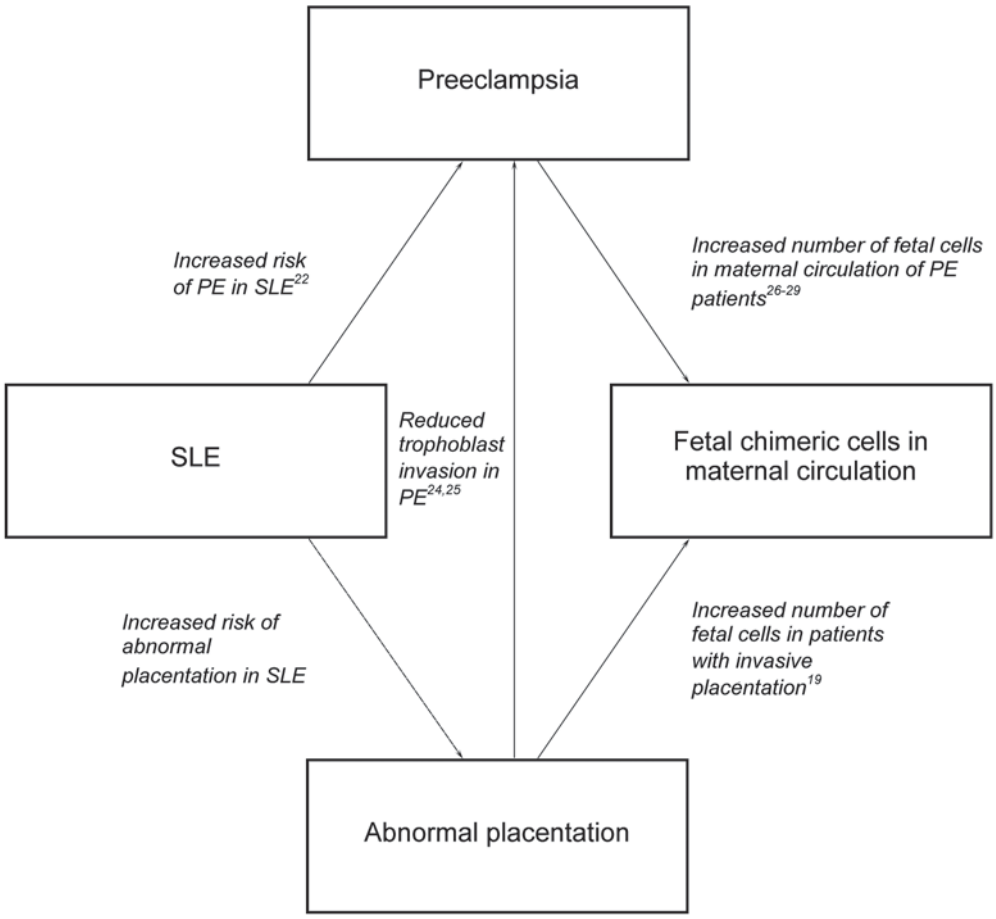


Figure 1. Chimeric cells in the uterus. Red-brown dots indicate presence of the Y chromosome, identified by in situ hybridization. (A) A solitary Y chromosome-positive cells (arrow) located in the myometrium of a healthy control. (B) High numbers of chimeric cells are present in the placental parts in the uterus of a woman with placenta increta (arrow). Furthermore, Y chromosome-positive cells were scattered around the myometrial tissue in the vicinity of the placenta (arrowheads).

Three of the seven SLE patients had children, four had no children. Detailed information about previous pregnancies was not available. Chimerism was found both in patients and in controls with and without children. We were able to obtain blood transfusion histories of 6 of 7 SLE patients and all 11 control patients. In the control group 7 women (64%) had received a blood transfusion, compared to 4 women (67%) in the patient group. Twenty-five percent of the chimeric organs of SLE patients were from women who had not received a blood transfusion, which was not different from the 40% found in the control group.

Figure 2. Hypothesis on the relationship between abnormal placentation, chimerism and SLE. The increased number of fetal cells in the maternal circulation in both patients with SLE and patients with preeclampsia is explained by abnormal placentation.



SLE, Systemic Lupus Erythematosus; PE, preeclampsia.

Discussion

Chimeric cells have been found significantly more often in women with autoimmune diseases than in healthy women,[7,8,14] and these cells are considered to be pregnancy derived. During pregnancy, extensive trafficking of fetal cells from the pregnant uterus to the maternal circulation takes place. The migration of fetal cells to various organ and

tissue sites has been demonstrated previously, both in animal models and in humans.[2,15] Importantly, it has been shown that fetus-derived chimeric cells may survive up to decades after pregnancy, which may be indicative of their stem-cell like nature.[16,17] Both in healthy controls and in patients with SLE, chimeric cells may be found in virtually any organ.[8,11,12,18] However, in patients with SLE, chimeric cells are more often present than in healthy controls,[8,11] and their presence may be related to tissue damage.[11]

In the present study we found that chimeric cells are more often present in uteri of women with SLE than in uteri of normal controls. We also investigated the occurrence of chimerism in the uterus of a woman who had given birth to a son only one day before an emergency hysterectomy was performed because of persistent postpartum hemorrhage. The hemorrhage was caused by a placenta increta, meaning that the myometrium was actually invaded by the placental villous tissue. In tissue sections, chimeric cells were found to be dispersed from the placenta to the myometrium, at various distances from the placenta. Fetal DNA in the maternal circulation is increased during pregnancy in women with placenta increta.[19] It is possible that abnormal placentation may give rise to a higher number of pregnancy-derived chimeric cells in the maternal circulation. Our case sample confirms that in women with placenta increta the chimeric cells enter the mother via the myometrium. Nevertheless, in women with normal placentation this may be different. For example, the woman who died from the amniotic fluid embolus and who showed no signs of placental pathology did not have any male cells in the uterus.

How do our findings relate to chimerism in women with SLE? Women with SLE are prone to miscarriages,[20,21] of which the exact cause is unknown, but antiphospholipid antibodies may play an important role in this phenomenon.[20,22,23] A majority of the pregnancy losses in SLE is associated with symptoms of (pre)eclampsia.[22] In preeclampsia, several placental abnormalities occur of which reduced invasion of extravillous trophoblast is most prominent.[24,25] Interestingly, in women suffering from preeclampsia, numbers of fetal cells in the maternal circulation are increased, and this elevation even predates the development of clinical symptoms.[26-29] Apparently, different forms of abnormal placentation can give rise to a higher number of pregnancy-derived chimeric cells. Abnormal placentation may be an important cause of the relatively

high occurrence of chimeric cells in uteri from women with SLE. How these findings relate to the pathogenesis of SLE is unknown and we can only speculate about that. If chimeric cells are pathogenic and can cause SLE, a high occurrence of chimerism may induce or worsen disease. An indication in favor of this theory is that, in contrast to some other autoimmune disease, SLE tends to worsen during pregnancy.[23,30] Still, the higher occurrence of chimerism in uteri of women with SLE could also be explained by the diminished capacities of the immune system to eliminate semi-allogenic cells. We summarize our hypothesis on the relationship between abnormal placentation, chimerism and SLE in Figure 2. Whether in the women with SLE from our study group abnormal placentation was present in previous pregnancies is unknown.

Abnormal placental development, particularly in the early stages of pregnancy, may not be detected by sonography and will thus be difficult to verify. Previous studies have demonstrated that especially at the time of pregnancy termination an influx of chimeric cells occurs.[31,32] Chimeric, male cells have also been detected in women who reported they had not been pregnant,[33] which has been attributed to unrecognized pregnancies, a frequently occurring event.[34] Our two controls with Y chromosome-positive cells in the uterus were women who did not have sons. Also in these women the chimeric cells are most likely to be derived from pregnancies which were terminated in an early phase.

In this study, we investigated the presence of Y chromosome-positive chimeric cells in uteri of women with SLE and of normal women, and we found that significantly more uteri of SLE patients contained chimeric cells than uteri of normal women. The exact sequence of events leading to increased chimerism in women with SLE is currently not known. However, our results indicate that abnormal placentation may influence the exchange of fetal cells into the maternal circulation. Whether this reflects pathogenic potential, or is simply the result from other factors allowing the chimeric cells to enter and persist in the maternal circulation, is currently unknown. Monitoring the presence of chimeric cells in early pregnancies of women with SLE may be a starting point for future studies on this subject.

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