Chimerism in health, transplantation and autoimmunity

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CHIMERISM OCCURS TWICE AS OFTEN IN LUPUS NEPHRITIS AS IN NORMAL KIDNEYS

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Abstract

Background

Systemic lupus erythematosus (SLE) is an immune-mediated disease that particularly affects the kidneys, causing lupus nephritis. In experimental mouse models, lupus nephritis can be mimicked by inducing a chimeric state through the injection of parental T cells in offspring. In humans, pregnancy-induced chimerism may play a role in the pathogenesis of autoimmune diseases such as SLE, but it is likely that only certain chimeric cells have pathogenic potential. In this study, we investigated whether the distribution of chimeric cells is different in the kidneys of women with SLE from that in normal kidneys, and we examined the phenotype of chimeric cells in women with SLE.

Methods

The presence of chimeric cells was investigated by in situ hybridization targeting the Y chromosome in 57 renal biopsies from 49 women with lupus nephritis. Fifty-one kidney autopsy specimens without histomorphologic lesions served as controls. Double-staining for the Y chromosome in combination with CD3 and CD34 markers was performed in 5 kidney specimens with lupus nephritis to identify the phenotype of the chimeric cells.

Results

Y chromosome-positive cells were found in 27 of 49 patients with lupus nephritis, and in 13 of 51 normal controls ($p < 0.01$). Both CD3+ and CD34+ chimeric cells were identified in lupus nephritis kidney specimens.

Conclusion

Chimeric cells are present significantly more often in kidneys with lupus nephritis than in normal kidneys, and some of these chimeric cells are T cells. This finding is interesting in light of experimental models demonstrating that lupus nephritis is initiated by chimeric T cells.

Introduction

Chimerism is defined as the presence of cells from one individual in another individual. Several sources of chimerism are known, of which pregnancy is the most common. During pregnancy, cells from the maternal circulation may enter the fetal circulation, and vice versa. Male fetal progenitor cells expressing CD34 can persist in the maternal circulation for as long as 27 years postpartum.[1] In addition to pregnancy, blood transfusions may be a cause of chimeric cells.[2]

Questions have arisen regarding whether chimerism can be harmful. Several studies have investigated the presence of chimeric cells in immune-mediated diseases, such as systemic sclerosis and Hashimoto's thyroiditis.[3,4] In systemic sclerosis it was demonstrated that chimerism is present more often in patients than in healthy controls, suggesting a pathogenic role for the chimeric cells.[4,5] The phenotype of the chimeric cells in immune-mediated diseases has not yet been thoroughly investigated, although some studies involving patients with systemic sclerosis demonstrated that some of the chimeric cells were T cells.[4,6,7] It is possible that only chimeric cells with a particular phenotype, for instance chimeric T cells, have pathogenic potential, whereas others, such as the CD34+ cells found in pregnancy, are harmless bystanders.

Systemic lupus erythematosus (SLE) is a severe immune-mediated disease that affects several organs. It occurs predominantly in women during their fertile years. Despite extensive research, the etiology remains unknown. Interestingly, in experimental mouse models, induction of a chimeric state through the injection of parental T cells in offspring can trigger an autoimmune-like reaction that resembles SLE.[8] This finding led to the hypothesis that chimeric cells may be involved in the pathogenesis of SLE in humans and would be present in affected organs.
Because SLE manifests preferentially in the kidney, we investigated whether chimeric cells are present more often in kidneys with lupus nephritis than in normal kidneys. Furthermore, we examined the phenotype of the chimeric cells in lupus nephritis and their relationship with clinical and histological parameters.

Patients and methods

Patients and biopsies

Fifty-seven renal biopsy samples from 49 women with SLE (age range at biopsy 11-66 years) were included. Multiple biopsies were available from 7 patients (2 samples obtained from 6 women, and 3 samples from 1 woman). The biopsy samples were evaluated for lupus nephritis according to the most recent modification of the WHO classification by the ISN/RPS (International Society of Nephrology/Renal Pathology Society).[9] Information on the childbirth status was derived from medical records or, if records were incomplete, by contacting the general practitioners of the patients (with permission of the Medical Ethical Committee of the Leiden University Medical Center (LUMC)).

As controls, renal tissue from autopsy specimens was used, obtained from women at the LUMC between 1999 and 2001. From this group, who were previously described elsewhere,[10] tissue blocks of kidneys were selected for processing. Upon review by light microscopy, 72 kidney specimens were entered in the control group, of which 21 were excluded because of features such as inflammatory infiltrates, interstitial fibrosis and/or tubular atrophy, hypoxia or diffuse acute tubular necrosis. The remaining 51 control renal specimens were histomorphologically normal, although a minor component of autolysis was sometimes present. To exclude the possibility that this latter feature influenced the results of in situ hybridization for the Y chromosome, we performed in situ hybridization for the X chromosome as a positive control according to the same method as described below, using an X chromosome-specific DNA probe; in all cases, satisfactory results were obtained, allowing us to rule out the influence of autolysis.

The age of the control group at the time of death ranged from 16 to 93 years. Among these women, deaths were attributable to the following causes: vascular or myocardial causes in 15 women, cerebral causes in 10 women, carcinomas in 12 women, infectious causes in 11 women and other causes (i.e. cachexia, amniotic fluid embolus, and liver failure due to cirrhosis) in 3 women. Thirty-one of the 51 control women gave birth to at least one son, and 20 women had no children.

Any history of blood transfusions, both in patients and in controls, was obtained from the Department of Immunohematology and Blood Transfusion (the IHB) of the LUMC, with records retrieved from 1987 onward.

The size of the investigated area of kidney biopsy tissue from both patients and controls was measured by digital image analysis, using the Image Tool program (University of Texas Health Science Center, San Antonio, USA).

In situ hybridization targeting the Y chromosome

Archived 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[11] 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 0.05 M citrate buffer (pH 6.0) at 80°C for 80 minutes, rinsed in distilled water at 37°, and treated with 0.5% Pepsin (Serva Electrophoresis GmbH, Heidelberg, Germany) 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 30-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, the 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, St. Louis, MO, USA), 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 Vector for ten minutes. Hematoxylin staining served as a background.

A tissue sample from a male subject served as a positive control for the in situ hybridization of the Y chromosome; this sample was characterized by red-brown dots, conforming a positive signal in 58% of the nuclei. By nested polymerase-chain-reaction (PCR) and sequencing we confirmed that the probe was specific for the Y chromosome [described in reference [10]]. As a negative technical control, a tissue sample from a male subject was used, on which the complete in situ hybridization protocol was performed, except that instead of the hybridization mixture with the Y chromosome probe, only the hybridization mixture was added. This negative control yielded consistently negative results.

Double staining

To identify the phenotype of the chimeric cells in lupus nephritis, we performed CD3 and CD34 immunohistochemical stainings in combination with in situ hybridization, and determined whether double-positive cells were present. Only five samples were selected for these analyses, because relatively large tissue blocks were available for the double staining procedures. After Nova Red development for in situ hybridization targeting the Y chromosome, slides were rinsed in distilled water and phosphate buffered saline (PBS), respectively, followed by an overnight incubation of either rabbit antibodies directed to CD3 (Neomarkers, Fremont, CA, USA) or mouse monoclonal antibodies directed to CD34 (Neomarkers). The following day slides were rinsed in PBS and incubated with mouse EnVision (Dako) for CD34 and rabbit EnVision (Dako) for CD3 for 30 min. Finally, the sections were developed with H_2O_2 and 3,3'-diaminebenzidinetetrahydrochloride (DAB) (Dako) as chromogen for ten minutes. Hematoxylin staining served as a background. A renal specimen of a male patient with pyelonephritis served as a positive control for the double staining procedures.

Scoring

All specimens were scored by two observers who were blinded to the clinical information of the study subjects. Strict criteria were applied in the scoring, in which a cell was scored positive for the Y chromosome if there was a dot inside the nucleus; this dot had to have a similar size and staining intensity as those found in the nuclei of male control samples.

Statistics

Categorical variables were compared with the use of the chi-square test and by calculating the phi value. Continuous variables were compared with the Student's t-test, and non-normally distributed continuous variables were compared with the Mann-Whitney U test. Correlations were determined by calculating point-biserial correlations. To determine the predictive value of age, WHO class, serum creatinine, proteinuria, and blood transfusion history for the presence of chimerism, we performed a logistic regression analysis.

Results

Y chromosome-positive chimeric cells were found in the first renal biopsy specimen of 25 (51%) of 49 patients with lupus nephritis, and in a total of 29 (51%) of 57 renal biopsy samples. The patients' clinical and laboratory data are given in Table 1.

Fifteen lupus nephritis renal tissue specimens contained one chimeric cell, nine specimens contained two chimeric cells, three specimens contained three chimeric cells, and two specimens contained five chimeric cells. In six women, two biopsy samples were assessed for chimeric cells: neither sample was positive for chimeric cells in three of the women, both samples were positive in one woman, while two women had a positive result in one sample and a negative result in the other. In one woman, three biopsy samples were assessed: the first had a negative result, whereas the two subsequent biopsy samples had positive results. Within the renal tissue specimens, chimeric cells were present in the glomeruli, tubules and interstitium (Figure 1A and B). Chimeric cells were not typically found in areas of active inflammation.

Table 1. Clinical data and results of women with lupus nephritis at time of biopsy

	Total group	Chimerism present	Chimerism absent
Number (%) of women	49	27 (55)	22 (45)
Number (%) of biopsies	57	29 (51)	28 (49)
Age at biopsy, mean years	31.0	30.9	31.1
WHO classes, number of samples			
WHO I	0	0	0
WHO II	6	2	4
WHO III	13	5	8
WHO IV	32	18	14
WHO V	3	2	1
WHO VI	1	0	1
unclassified	2	2	0
Serum creatinine			
median, µmoles/liter	140	165	116
range, µmoles/liter	58–489	58–489	65–198
Proteinuria			
mean, grams/24 hours	5.04	4.8	5.34
range, gram/24 hours	0.08–19	0.08–10.1	0.5–19
Antinuclear antibody positive, no./total no. (%)	45/49 (92)	22/24 (92)	23/25 (92)
Anti-dsDNA antibody positive, no/total no. (%)	28/38 (74)	13/19 (68)	15/19 (79)
Anti-cardiolipid antibody positive, no./total no. (%)	13/21 (62)	5/11 (46)	8/10 (80)
Blood transfusion received, no./total no. (%)	15/49 (31)	7/26 (27)	8/23 (35)

WHO, World Health Organization; anti-dsDNA, anti-double-stranded DNA

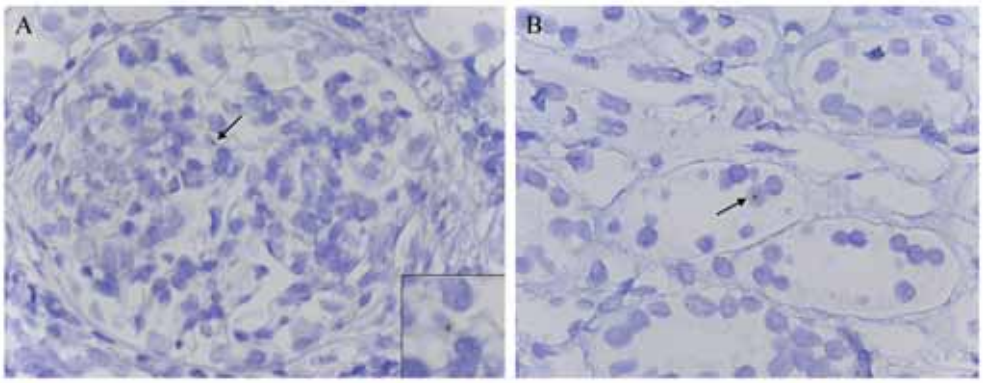

Figure 1. Chimeric cells in renal tissue of women with lupus nephritis. Red-brown dots indicate presence of the Y chromosome, identified by in situ hybridization. Y chromosome-positive cells (arrows) are present in glomeruli (A) (detail in inset) and tubules (B).

Information on history of childbirths was available for 41 (84%) of the 49 patients with lupus nephritis. Sixteen women had children, and 25 women had no children. Of the women with children, seven had at least one son and five had only daughters, whereas for four of the women the sex distribution of the children was not available. Chimerism was found both in patients with children and in those without (Figure 2).

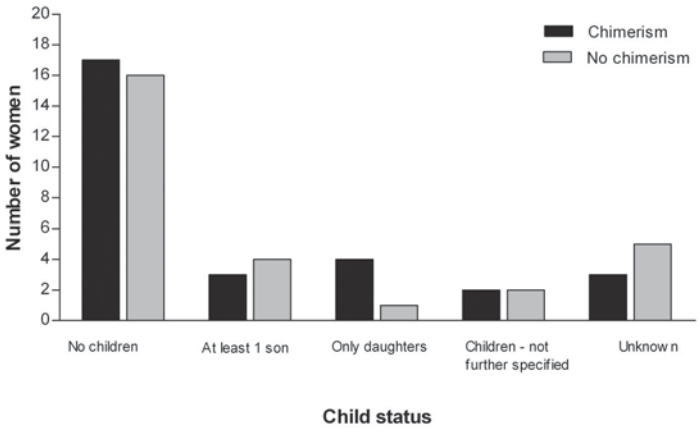

Figure 2. Occurrence of chimerism in relation to childbirth status of women with lupus nephritis at the time of biopsy. No significant differences were found between the groups with chimerism present (black bars) and chimerism absent (grey bars).

In the control group, 13 (25%) of 51 renal specimens contained Y chromosome-positive cells. Neither cause of death nor a history of childbirth was related to the occurrence of chimerism in the control subjects. Thus, when comparing the two groups, the results showed that chimerism occured twice as often in the kidneys of women with lupus nephritis as in the kidneys of normal control subjects ($\chi^2 = 6.91$, $p<0.01$).

The biopsy specimens from women with lupus nephritis covered a mean area of 8.3 mm^2, whereas the control specimens covered a mean area of 276 mm^2. Thus, approximately 33 times as much tissue area was evaluated in the normal control group as in the patient group, underlining the significant difference between the number of chimeric cells in normal kidneys and that in kidneys affected by lupus nephritis. Chimerism was not found more frequently in samples with larger surface areas ($p = 0.42$).

Renal biopsy samples positive for tissue chimerism were equally distributed over the WHO lupus nephritis classes. Age was not a predictor for the presence of chimerism, either in the controls (OR = 0.999, CI 0.963 - 1.036, p=0.961), or in the patients (OR = 0.998, CI 0.956 - 1.042, p=0.939). In this study, the clinical parameters of serum creatinine, proteinuria, and the presence of specific autoantibodies did not significantly differ between women in whom chimeric cells were found and women without chimeric cells. The level of serum creatinine and extent of proteinuria were not predictive of chimerism (data not shown).

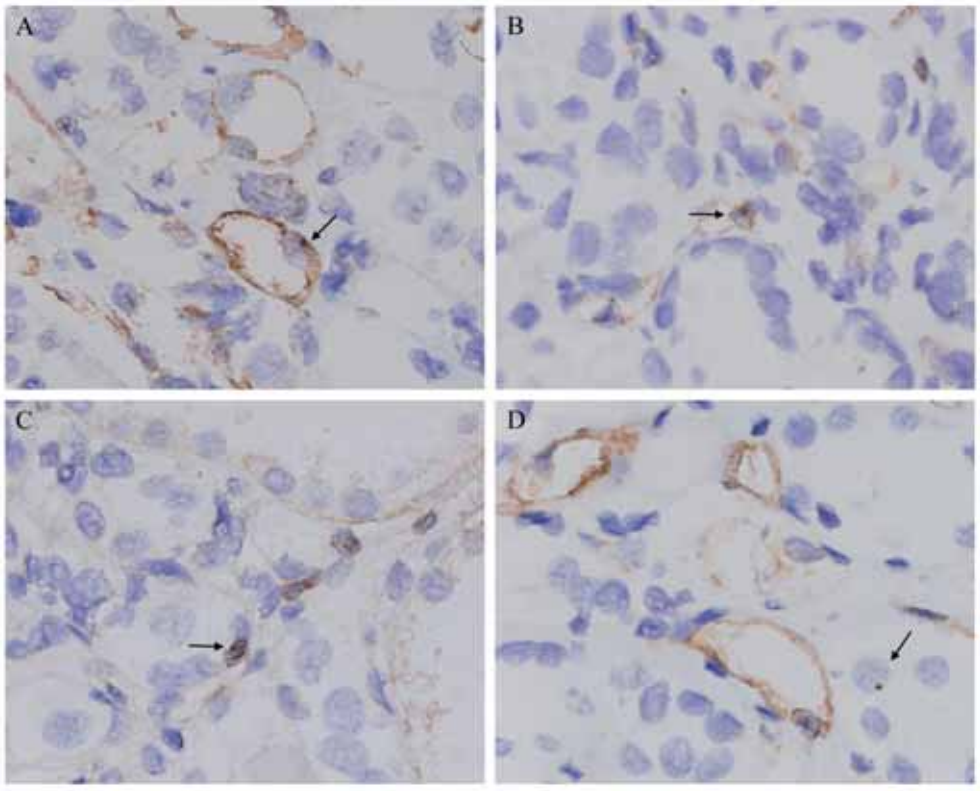

Figure 3. Cells that were both CD34+ and Y chromosome positive (A and B) and cells that were both CD3+ and Y chromosome positive (C) were identified (arrows) after double-staining by combining in situ hybridization of the Y chromosome and immunohistochemical staining for either CD34 or CD3 markers. Y chromosome-positive, CD34– cells (D) (arrow) and Y chromosome-positive, CD3– cells (not shown) were also found after double-staining

In five renal specimens with lupus nephritis, double-staining was performed. Both CD3+ and CD34+ chimeric cells were identified in these renal specimens from women with lupus nephritis (Figure 3A-C). In both staining procedures, a number of chimeric cells did not stain positive for these markers (Figure 3D). CD3+ cells comprised up to 63% of all chimeric cells in the CD3 double-staining procedure (meaning that up to 37% of chimeric cells were negative for CD3). CD34+ cells comprised up to 33% of all chimeric cells in the CD34 double-staining procedure (meaning that up to 67% of all chimeric cells were negative for CD34). In some instances, the CD34+ chimeric cells clearly lined a vascular wall, which could be an indication that they were endothelial cells. Some of these chimeric cells were solitary cells, possibly indicating a stem cell phenotype, although we cannot exclude that they were tangentially cut endothelial cells.

We were able to obtain the blood transfusion status of the SLE patients at time of biopsy for 49 renal samples from 43 women while for the control group, the blood transfusion status of all 51 women could be retrieved and was always determined at time of death, since the tissue specimens of the control group were obtained at autopsy. In the control group, 30 (59%) of the 51 women had received a blood transfusion, compared with 14 (33%) of the 43 women in the patient group. The occurrence of chimerism in the renal specimens investigated was comparable in patients with or without a history of blood transfusion (47% and 56%, respectively). There was no relationship between the occurrence of chimerism and blood transfusions in the patient group (Phi = -0.085, p= 0.551), nor was a relationship evident in the controls (Phi = 0.032, p= 0.818). Of the 17 patients with chimerism who had no children, two had received a blood transfusion. Of the four patients with chimerism who had only daughters, only one had received a blood transfusion.

Discussion

Our results show that in patients with lupus nephritis, chimerism occurs significantly more often than in healthy controls, and that both CD34+ and CD3+ chimeric cells are present. The finding that part of the chimeric cell population in lupus nephritis is

CD3+ is interesting, in light of previous experimental work demonstrating that chimeric T cells induce a graft-versus-host response leading to a lupus-like disease. Via and Shearer reported that in certain mice injected with chimeric T cells, a lupus-like disease developed.[8] Unaware of the existence of chimeric cells in humans, they concluded that it would be unlikely that in human SLE, chimeric cells would lead to B cell activation. It is now known that chimerism is a relatively common phenomenon in humans.

Recent studies that have investigated the presence of chimeric cells in patients with SLE have yielded inconsistent results. Four studies searched for male DNA in the blood of women with SLE,[12-15] and only one found a significant difference in comparison with a healthy control group.[12] Two studies searched for chimeric cells in tissue specimens of women with SLE; however, in these studies the phenotype of the chimeric cells was not examined.[16,17] We are the first to investigate a large number of tissue specimens of women with SLE, namely 57 kidney biopsy tissue specimens with lupus nephritis, and our findings demonstrate that chimeric cells occur twice as often in patients as in normal controls. Moreover, we are the first to investigate the phenotype of these chimeric cells in lupus nephritis, showing that they form a heterogeneous population of CD3+ and CD34+ cells. Although our results seem to indicate that the CD3+ and CD34+ cells are complementary subpopulations that together comprise the whole chimeric cell population, triple-staining procedures were not performed. Therefore, the possibility cannot be excluded that a small population of chimeric cells in lupus nephritis has yet another phenotype.

In previous studies, CD3+ chimeric cells were found in systemic sclerosis,[4,18] and it was suggested that these cells may have a pathogenic role in this disease by inducing a graft-versus-host-like reaction. In an elegant study by Scaletti et al.,[19] T cell clones were generated from three female patients with systemic sclerosis who had been pregnant with son. It was determined that these T cell clones showed a proliferative response to major histocompatibility complex (MHC) antigens on irradiated non-T cells from the same women. Eighteen percent of the reactive clones were chimeric. These chimeric cells, that were reactive T cells, produced significantly higher levels of interleukin-4 in response to the patient's MHC-antigens than did nonchimeric reactive T cells. These

data seem to suggest that chimeric T cells can be reactive against maternal antigens, and exhibit a Th2-oriented profile, which, in turn has been described to be associated with B cell activation. The authors therefore suggest that an alloreactive response is the basis of development of autoimmune diseases in which chimeric T cells are present.[19]

In the present study, the number of the chimeric cells found in the kidney biopsy specimens was relatively low, but it should be taken into account that the biopsy specimens were fairly small, resulting in fals-negative findings in some of the analyses for chimerism. Nevertheless, the question arises whether a low number of chimeric cells can play a role in the induction of lupus nephritis. We believe that the phenotype of chimeric cells may be of greater importance than their presence in numbers. In the experimental mouse model in which the injection of T cells led to a lupus-like disease, only 2 to 3% circulating chimeric T cells were found.[8]

We have focused on CD3+ chimeric cells being the effector cells in SLE, in parallel with experimental models demonstrating that chimeric T cells lead to a lupus-like disease. However, some of the chimeric cells that we detected in lupus nephritis were CD34+. Fetus-derived CD34+ chimeric cells have been found in the maternal circulation after pregnancy in healthy women, and they can stay there for decades.[1] Accordingly, these CD34+ cells have been described as harmless bystanders. It is therefore not surprising that we also found CD34+ cells in our patients with SLE, and that these CD34+ cells may be harmless bystanders in these patients, whereas CD3+ cells may be the ones that are pathogenic. There is as yet no evidence for the pathogenicity of these chimeric cells; it is possible their presence is a normal response to injury. Future studies need to be performed to investigate this into more detail.

It is assumed that chimeric cells are most likely derived from previous pregnancies. Our findingd show that childbirth status and the presence of chimerism were not related in our patient group, similar to our findings in normal healthy women. In fact, there were many patients with chimerism who did not have sons. Other causes of chimerism have been suggested, and they comprise early (unrecognized) miscarriage, induced abortion, (unrecognized) loss of a male twin in utero, cells from sexual intercourse, or

transfer, via the maternal circulation, from an older male sibling.[20] Studies investigating the frequency of unrecognized miscarriages have shown that the rate of pregnancy loss is 30% prior to implantation, and is also 30% after implantation but prior to the first missed period.[21] Without surveillance, data on unrecognized pregnancies are impossible to retrieve. Because we were unable to obtain data on miscarriages from the patients in our study, it is doubtful how much the data on the patients' childbirth status contributes to our understanding of the source of chimeric cells.

Apart from pregnancy, blood transfusions are also mentioned as a cause of chimerism. Information on history of blood transfusions in the patients in this study was available, and therefore patients with and without a history of blood transfusion could be clearly distinguished. We showed that there was no relationship between the occurrence of chimeric cells and a history of blood transfusions. Because chimerism was also found in women without a history of blood transfusions, our findings suggest that, at least in some women, blood transfusions were not a source for chimerism.

This study provides preliminary evidence that chimeric cells may have pathogenic significance in lupus nephritis. Future studies should be directed to unravelling the exact mechanism by which chimeric cells are involved in lupus nephritis. If the evidence indicates that chimeric cells play a role in the development of SLE, they may prove to be a relatively specific target for disease prevention and therapy.

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Reference List

1. Bianchi DW, Zickwolf GK, Weil GJ, et al. Male fetal progenitor cells persist in maternal blood for as long as 27 years postpartum. *Proc Natl Acad Sci U S A* 1996;93:705-708.
2. Lee TH, Paglieroni T, Ohto H, et al. Survival of donor leukocyte subpopulations in immunocompetent transfusion recipients: frequent long-term microchimerism in severe trauma patients. *Blood* 1999;93:3127-3139.
3. Klintschar M, Schwaiger P, Mannweiler S, et al. Evidence of fetal microchimerism in Hashimoto's thyroiditis. *J Clin Endocrinol Metab* 2001;86:2494-2498.
4. Artlett CM, Smith JB, Jimenez SA. Identification of fetal DNA and cells in skin lesions from women with systemic sclerosis. *N Engl J Med* 1998;338:1186-1191.
5. Nelson JL, Furst DE, Maloney S, et al. Microchimerism and HLA-compatible relationships of pregnancy in scleroderma. *Lancet* 1998;351:559-562.
6. Lambert NC, Evans PC, Hashizumi TL, et al. Cutting edge: persistent fetal microchimerism in T lymphocytes is associated with HLA-DQA1*0501: implications in autoimmunity. *J Immunol* 2000;164:5545-5548.
7. Artlett CM, Cox LA, Ramos RC, et al. Increased microchimeric CD4+ T lymphocytes in peripheral blood from women with systemic sclerosis. *Clin Immunol* 2002;103:303-308.
8. Via CS, Shearer GM. T-cell interactions in autoimmunity: insights from a murine model of graft-versus-host disease. *Immunol Today* 1988;9:207-213.
9. Weening JJ, D'Agati VD, Schwartz MM, et al. The classification of glomerulonephritis in systemic lupus erythematosus revisited. *J Am Soc Nephrol* 2004;15:241-250.
10. Koopmans M, Kremer Hovinga IC, Baelde HJ, et al. Chimerism in kidneys, livers and hearts of normal women: implications for transplantation studies. *Am J Transplant* 2005;5:1495-1502.
11. Lau YF. Detection of Y-specific repeat sequences in normal and variant human chromosomes using in situ hybridization with biotinylated probes. *Cytogenet Cell Genet* 1985;39:184-187.
12. Abbud FM, Pavarino-Bertelli EC, Alvarenga MP, et al. Systemic lupus erythematosus and microchimerism in autoimmunity. *Transplant Proc* 2002;34:2951-2952.
13. Gannage M, Amoura Z, Lantz O, et al. Feto-maternal microchimerism in connective tissue diseases. *Eur J Immunol* 2002;32:3405-3413.
14. Miyashita Y, Ono M, Ono M, et al. Y chromosome microchimerism in rheumatic autoimmune disease. *Ann Rheum Dis* 2000;59:655-656.

15. Mosca M, Curcio M, Lapi S, et al. Correlations of Y chromosome microchimerism with disease activity in patients with SLE: analysis of preliminary data. *Ann Rheum Dis* 2003;62:651-654.
16. Johnson KL, McAlindon TE, Mulcahy E, et al. Microchimerism in a female patient with systemic lupus erythematosus. *Arthritis Rheum* 2001;44:2107-2111.
17. Khosrotehrani K, Mery L, Aractingi S, et al. Absence of fetal cell microchimerism in cutaneous lesions of lupus erythematosus. *Ann Rheum Dis* 2005;64:159-160.
18. Evans PC, Lambert N, Maloney S, et al. Long-term fetal microchimerism in peripheral blood mononuclear cell subsets in healthy women and women with scleroderma. *Blood* 1999;93:2033-2037.
19. Scaletti C, Vultaggio A, Bonifacio S, et al. Th2-oriented profile of male offspring T cells present in women with systemic sclerosis and reactive with maternal major histocompatibility complex antigens. *Arthritis Rheum* 2002;46:445-450.
20. Lambert NC, Lo YM, Erickson TD, et al. Male microchimerism in healthy women and women with scleroderma: cells or circulating DNA? A quantitative answer. *Blood* 2002;100:2845-2851.
21. Macklon NS, Geraedts JP, Fauser BC. Conception to ongoing pregnancy: the 'black box' of early pregnancy loss. *Hum Reprod Update* 2002;8:333-343.