Systemic lupus erythematosus: pathogenesis, diagnosis, and treatment

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Increased Microchimerism in Peripheral Blood of Women with Systemic Lupus Erythematosus

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Abstract

Objectives

Past research suggests that microchimerism plays a role in systemic lupus erythematosus (SLE). In this study, we aimed to determine the presence and amount of microchimerism in peripheral blood of women with SLE as compared to control subjects. Additionally, we investigated the origin of chimeric cells and the relationship between microchimerism and disease onset, disease activity, and accumulated damage.

Methods

We performed a case-control study with 11 female SLE patients and 22 control subjects. Their children (both male and female) and, if possible, their mothers were also included. Quantitative PCR for insertion-deletion polymorphisms and null alleles was used to detect microchimerism in peripheral blood mononuclear cells and granulocytes.

Results

Microchimerism was detected more often in patients than control subjects (54.4% versus 13.6%, respectively; P=0.03). When present, microchimerism was fetal in origin in almost all cases, and the median total number of fetal chimeric cells was $5/10^6$ in patients and $2.5/10^6$ in control subjects (P=0.048). Maternal microchimerism was detected in one patient and one control subject. In 50% of patients with microchimerism, it originated from multiple relatives, whereas in control subjects, microchimerism was always derived from one relative. We found no relationship between microchimerism and clinical or laboratory parameters related to SLE.

Conclusions

SLE patients had microchimerism in peripheral blood more often and at higher levels than control subjects. In both patients and control subjects, microchimerism was predominantly fetal in origin. This study provides the first evidence that microchimerism in SLE can be derived from multiple relatives.

Introduction

Microchimerism (Mc) refers to the presence in an individual of a small number of genetically distinct cells of any type, originating from a different zygote. The most common (physiologic) source of Mc is pregnancy,[1] including both miscarriages and pregnancies resulting in live birth. [2-5] It can occur when fetal cells enter the maternal circulation, causing fetal Mc (FMc) in the mother. It may also develop in the opposite direction, with maternal cells crossing the placental barrier to the fetus, leading to maternal Mc (MMc).

The role of Mc in health and disease is unclear. Mc has been suggested to play a role in several autoimmune diseases, including systemic lupus erythematosus (SLE).[6-9] SLE primarily affects women and has a peak incidence in the reproductive years.[10] Studies in mice showed that, in selected parent-to-F1 combinations, injection of parental lymphocytes in their offspring led to a graft-versus-host response and a lupus-like disease.[11 12] These data suggest that pregnancy-acquired Mc may be of pathogenic significance in the development of SLE.

Women with SLE have a significantly higher prevalence of fetal Y chromosome-positive chimeric cells in tissue than healthy control subjects.[13-15] There is conflicting research as to there is an increased frequency of FMc in the peripheral blood of SLE patients as compared to control subjects.[16 17 18 19] Previous studies on FMc in SLE were limited to the detection of male Mc, thereby underestimating the total amount of Mc. Furthermore, because Mc was mostly studied in whole blood, the phenotype of the chimeric cells could not be determined. MMc in SLE in peripheral blood was studied by Kanold *et al.* and they did not find a difference between patients and control subjects.[20] However, their sensitivity of detecting chimeric cells was relatively low. None of these studies investigated FMc and MMc together.

The aim of our study was to determine the presence and amount of Mc in peripheral blood of SLE patients and compare it to healthy control subjects. We studied peripheral blood mononuclear cells (PBMCs) and granulocytes separately to determine if Mc was present in either subset independently, or in both. We used insertion-deletion polymorphisms (indels) or null alleles for the detection of Mc, enabling us to study the origin of the chimeric cells as either fetal, maternal, or both. We were also able to establish whether Mc was derived from one relative or from multiple relatives. To understand the role of Mc in SLE, we investigated the relationship between disease activity or accumulated damage since the onset of SLE, and the presence of Mc. Finally, the temporal relationship between the chimerism-causing pregnancy and disease onset in SLE patients was studied.

Materials and methods

Ethics statement

This study was approved by the Medical Ethics Committee of the Leiden University Medical Center (LUMC) (P09.047). Informed consent was obtained from all participants. Parents of minors gave written consent on their behalf.

Patients and control subjects

Participants included 11 female SLE patients and 22 female control subjects. From 2010 to 2015 SLE patients were recruited from four hospitals in the Netherlands: University Medical Center Groningen, Radboud University Medical Center Nijmegen, Bronovo Hospital The Hague and Meander Medical Center Amersfoort. All participants fulfilled at least four of the 1982 revised American College of Rheumatology Criteria for the classification of SLE.[21] SLE disease activity was determined using the SLE Disease Activity Index 2000 (SLEDAI-2K).[22] Accumulated damage since SLE onset was measured using the Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index (SDI).[23] The control group consisted of women with no history of autoimmune disease. For inclusion in the study, probands (SLE patients and control subjects) were required to have at least one child of at least 18 years old. Probands' children and mothers were invited to participate. Peripheral blood samples were gathered from the probands; either peripheral blood samples or buccal mouth swabs were collected from their children and mothers. All probands were asked to fill out a questionnaire including their age, ethnicity, reproductive history, history of blood transfusion, use of immunosuppressive medication and medical history.

Isolation of peripheral blood subsets

Peripheral venous blood samples were drawn in sodium-heparine solution vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ) and processed to isolate peripheral blood mononuclear cells (PBMCs) by Ficoll amidotrizoate (pharmacy LUMC) with density gradient centrifugation 1.077 g/mL. Erythrolysis (Qiagen, Hilden, Germany) was performed to remove the erythrocytes from the remaining granulocytes. Samples were stored in 10% dimethyl sulfoxide in fetal bovine serum at -180 °C until DNA extraction.

DNA extraction

DNA was extracted from PBMCs and granulocytes using the QIAamp DNA Blood Mini Kit (Qiagen), according to the manufacturer's instructions with a few modifications. We added

40 µL of proteinase K to 5 x 10^6 cells suspended in 200 µL phosphate-buffered saline. After adding 400 µL AL buffer, the suspension was incubated for 30 min (PBMCs) or overnight (granulocytes) at 56 °C. After adding 200 µl of ethanol, the mixture was applied to the Mini spin column. Buffers AW1 and AW2 were used to wash the column, after which 100 µl AE buffer was added and incubated at 70 °C for 10 minutes to elute the DNA. The eluate was reapplied for an optimal yield. DNA samples were stored at 4 °C until quantitative PCR (qPCR). DNA extraction from buccal sterile OmniSwabs (GE Healthcare Life Sciences, Little Chalfont, United Kingdom) was performed with the same kit according to the manufacturer's instruction.

Allele informativity and genotyping

A set of previously published indels and null allelles was used for the detection of FMc and MMc.[24-26] In order to detect both FMc and MMc in the proband, and to discriminate between the proband's children, informative alleles were required to distinguish between the different family members. Maternal DNA was available for six of 11 patients and eight of 22 control subjects. There was no fetal DNA available from any of the miscarriages. Genotyping by qPCR was performed with the same protocol described below, but with a DNA input of 20 ng. Of the published sets of null alleles and indels, 19 were informative in our study population: GSTM1, GSTT1, SRY, RhD (null alleles), and S01a, S01b, S03, S04a, S04b, S05b, S07b, S08b, S09b, S10a, S10b, S11a, S11b, Xq28 and R271 (indels). Primer sequences are listed in Table 1.

Chimerism detection by qPCR

FMc and MMc were detected and quantified by qPCR. In all assays iQ SYBR Green Supermix (Bio-Rad, Hercules, CA) was used, with 7.5 µM of each amplification primer. Amplification and melting conditions for all primers consisted of incubation at 96.5 °C for 10 min, followed by 44 cycles of 96.5 °C for 30 s and 60 °C for 1 min. The melting curve started at 65 °C for 5 s followed by 0.2 °C incremental increase, each lasting 5 s, to 95 °C. Amplification and melting data were collected by a Bio-Rad CFX96 detector and analyzed by Bio-Rad CFX Manager version 3.1.

Primer specificity was ensured by Sanger sequencing of the amplification product and comparing the sequences to known genomic DNA sequences. Sensitivity was determined by testing serial dilutions of DNA positive for the indel or null allele in a background of DNA negative for the respective indel or null allele. A sensitivity of one genome equivalent (gEq,

Table 1. Primers

Marker name	Position	5' Primer 3'
S01a	F	GGT ACC GGG TCT CCA CAT GA
S01b	F	GTA CCG GGT CTC CAC CAG G
S01a/b	R*	GGG AAA GTC ACT CAC CCA AGG
S03	F	CTT TTG CTT TCT GTT TCT TAA GGG C
S03	R	TCA ATC TTT GGG CAG GTT GAA
S04a/b	F*	CTG GTG CCC ACA GTT ACG CT
S04a	R	AAG GAT GCG TGA CTG CTA TGG
S04b	R	AGG ATG CGT GAC TGC TCC TC
S05b	F	AGT TAA AGT AGA CAC GGC CTC CC
S05b	R	CAT CCC CAC ATA CGG AAA AGA
S07b	F	GGT ATT GGC TTT AAA ATA CTC AAC C
S07b	R	CAG CTG CAA CAG TTA TCA ACG TT
S08b	F	GCT GGA TGC CTC ACT GAT GTT
S08b	R	TGG GAA GGA TGC ATA TGA TCT G
S09b	F	GGG CAC CCG TGT GAG TTT T
S09b	R	CAG CTT GTC TGC TTT CTG CTG
S10a	F	GCC ACA AGA GAC TCA G
S10b	F	TTA GAG CCA CAA GAG ACA ACC AG
S10a/b	R*	TGG CTT CCT TGA GGT GGA AT
S11a	F	TAG GAT TCA ACC CTG GAA GC
S11b	F	CCC TGG ATC GCC GTG AA
S11a/b	R*	CCA GCA TGC ACC TGA CTA ACA
GSTM1	F	GAA CTC CCT GAA AAG CTA AAG CT
GSTM1	R	GTT GGG CTC AAA TAT ACG GTG G
GSTT1	F	TCC TTA CTG GTC CTC ACA TCT C
GSTT1	R	TCC CAG CTC ACC GGA TCA T
RhD	F	GCC TGC ATT TGT ACG TGA GA
RhD	R	CAA AGA GTG GCA GAG AAA GGA
Xq28	F	TGG GTT CCA ACC AGC A
Xq28	R	ACT GAC AAT TAT CAC AGC TT
R271	F	AGA GGA TTG ACT CGG G
R271	R	GTT ACG TCT TAG ATG CCA G
SRY	F	TGG CGA TTA AGT CAA ATT CGC
SRY	R	CCC CCT AGT ACC CTG ACA ATG TAT T

F, forward; R, reverse, *common primer.

based on 6.6 pg DNA content per cell) in 100 000 gEq was reached for all primersets. Four aliquots, each containing 660 ng DNA (100 000 gEq), were tested in each subset (PBMC or granulocytes) for every proband. A standard curve for the specific assay was included to quantify the chimeric cells and validate the assay on each plate. It consisted of 100, 10, and 1 gEq spiked DNA per 100 000 gEq background DNA. Every sample was tested for the housekeeping gene GAPDH. Results were expressed as the gEq of chimeric cells per one million gEq (gEq/10^6). The qPCR plate included negative controls consisting of either a water control or background DNA not carrying the indel or null allele tested. Negative

controls were consistently negative across all experiments. If there was any doubt as to the specificity of the amplification product, the length of the PCR product was compared to that of the positive control using QIAxcel Advanced System (Qiagen) according to the manufacturer's protocol.

Anti-contamination procedures

Strict anti-contamination procedures were employed during blood work-up, DNA extraction, and qPCR preparation. Aerosol-resistant pipette tips and clean gloves were used in every stage and blood work-up was performed in a laminar flow cabinet. Before DNA extraction or preparation of the qPCR, the cabinet used was thoroughly cleaned with DNA decontamination reagent (Sigma-Aldrich, St. Louis, MO), and irradiated with UV light for one hour. All lab consumables were certified DNA free, and also irradiated with UV light for one hour. For the qPCR 8-well strips with individual lids were used.

Statistical analysis

For comparison of categorical data a Fisher's exact test was used (history of blood transfusion, presence of Mc). A Student's *t*-test was used to compare normally distributed data (age proband, age eldest child, age youngest child, SDI). For comparison of non-normally distributed numerical data a Mann-Whitney U test was used (number of pregnancies, number of children, number of chimeric cells, SLEDAI-2K). A *P*-value ≤0.05 was considered statistically significant. All analyses were performed using SPSS Statistics 20.0 (IBM, Armonk, NY).

Table 2. Baseline characteristics of SLE patients and controls

Parameter	SLE patients (n=11)	Controls (n=22)	*P*-value
Age proband (y)	56.6 ± 5.5	57.2 ± 5.5	0.79[a]
Age eldest child (y)	31.4 ± 5.2	28.9 ± 5.7	0.24[a]
Age youngest child (y)	27.5 ± 5.3	24.4 ± 4.9	0.11[a]
Number of children	2 (1)	2 (1)	0.60[b]
Number of pregnancies	3 (2)	2.5 (1)	0.37[b]
History of blood transfusion (%)	72.7	13.6	0.001[c]
SLEDAI-2K	0 (4)	-	n/a
SDI	2.2 ± 2.3	-	n/a

Results are shown as mean ±SD or as median (interquartile range), unless otherwise specified. *P*-values were assessed with [a] Student's *t*-test, [b] Mann-Whitney U test, or [c] Fisher's exact test. SLE, systemic lupus erythematosus; SLEDAI-2K, SLE Disease Activity Index 2000; SDI, Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index; y, years.

Table 3. Microchimerism in SLE patients and controls

Parameter	SLE patients (n=11)	Controls (n=22)	*P*-value
Mc present in PBMCs or granulocytes (%)	54.5	13.6	0.03[a]
Mc present in PBMCs (%)	36.4	9.1	0.15[a]
Mc present in granulocytes (%)	40.0 (n=10)	10.0 (n=20)	0.14[a]
Total number of fetal chimeric cells/proband, when Mc is present (gEq/10^6 gEq)	5 (8.1) (n=6)	2.5 (n/a) (n=3)	0.048[b]

Results are shown as mean ±SD or as median (interquartile range), unless otherwise specified. *P*-values were assessed with [a] Fisher's exact test or [b] Mann-Whitney U test. gEq, genome equivalents; Mc, microchimerism; n/a, not applicable because number of cases is too low to provide an interquartile range; PBMCs, peripheral blood mononuclear cells; SLE, systemic lupus erythematosus.

Table 4. Comparison of SLE patients and controls with and without microchimerism

Parameter	SLE patients			Controls		
	No Mc (n=5)	Mc (n=6)	*P*-value	No Mc (n=19)	Mc (n=3)	*P*-value
Age proband (y)	57.4 ± 5.2	56.0 ± 6.3	0.70[a]	57.6 ± 5.6	54.3 ± 4.3	0.33[a]
Number of children	2 (1)	2.5 (1.25)	0.84[b]	2 (1)	3 (n/a)	0.44[b]
Number of pregnancies	2 (1.5)	3.5 (2)	0.33[b]	2 (1)	3 (n/a)	0.71[b]
Blood transfusion (%)	60.0	83.3	0.55[c]	10.5	33.3	0.37[c]
SLEDAI-2K	0 (2.5)	2 (5.5)	0.37[b]	-	-	-
SDI	1.8 ± 2.0	2.5 ± 2.6	0.64[a]	-	-	-

Results are shown as mean ±SD or as median (interquartile range), unless otherwise specified. *P*-values were assessed with [a] Student's *t*-test, [b] Mann-Whitney U test, or [c] Fisher's exact test. Mc, microchimerism; n/a, not applicable because number of cases is too low to provide an interquartile range; SLE, systemic lupus erythematosus; SLEDAI-2K, SLE Disease Activity Index 2000; SDI, Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index; y, years.

Results

Baseline characteristics of patients and control subjects are shown in Table 2. SLE patients had Mc more often than control subjects (54.4% versus 13.6%, respectively; *P*=0.03). When Mc was present, the median total number of fetal chimeric cells per proband was higher in the patient group than in the control group (5 gEq/10^6 versus 2.5 gEq/10^6, respectively; *P*=0.048) (Table 3). When comparing patients and control subjects with and without Mc, there was no significant difference in age, number of children, number of pregnancies, history of blood transfusion, disease activity (SLEDAI-2K) or accumulated damage since onset of disease (SDI) (Table 4). No difference was found in the use of immunosuppressive medication between patients with and without Mc (data not shown).

With one exception, all patients and control subjects with detectable Mc had FMc (Table 5). Of the eight control subjects with maternal DNA available, one had detectable MMc. Of the

Table 5. Origin of microchimerism in patients and controls

	Relatives[a]	Chimerism in PBMCs		Chimerism in granulocytes		Blood transfusion in history	SLEDAI-2K	SDI
		Origin	Amount (gEq/10^6)	Origin	Amount (gEq/10^6)			
Patients								
1	Mother, daughter 1, miscarriage (n/a), daughter 2, son	Mother Daughter 1 or 2 Son	16.5 2.5 2.5	n/a	n/a	Yes	10	4
2	Mother, daughter 1, 2 and 3	Daughter 1	5	Daughter 3 Daughter 2 or mother Possibly daughter 1	7.5 2.5	Yes	4	0
3	Mother, daughter, son	Son -	2.5	Daughter, and possibly mother	2.5	Yes	0 (1st blood draw[b])	2
				-			0 (2nd blood draw[b])	2
4	Mother (n/a), miscarriages 1, 2 and 3 (n/a), daughter	-	-	Daughter	15	Yes	4	7
5	Mother (n/a), daughter 1 (deceased, n/a), miscarriage (n/a), daughter 2 (n/a), daughter 3	-	-	Daughter 3	5	Yes	0	1
6	Mother (n/a), son, daughter	Daughter	5	-	-	No	0	1
Controls								
1	Mother (n/a), daughter 1 and 2, son	Daughter 2	2.5	Daughter 2	2.5	Yes	-	-
2	Mother (n/a), daughter, son	-	-	Daughter	2.5	No	-	-
3	Mother, daughter 1, daughter 2, son	Mother	2.5	-		No	-	-

[a] chronologically from old to young based on year of birth/miscarriage

[b] due to technical problems with the material from the first blood draw, a second blood draw was done approximately 1 year later

gEq, genome equivalent; n/a, no DNA available; PBMCs, peripheral blood mononuclear cells; SLEDAI-2K, Systemic Lupus Erythematosus Disease Activity Index 2000; SDI, Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index.

six SLE patients with maternal DNA available, one had detectable MMc and three did not. Of two patients the possible MMc was indistinguishable from the FMc that was present, due to an overlap in indels and null alleles.

Additionally, we looked at whether the Mc originated from one relative or more. In all three control subjects with detectable Mc, it originated from one relative. In contrast, in at least three of the six SLE patients, the Mc originated from more than one relative, either from multiple children or from a child and mother (Table 5).

In patients with detectable Mc, we did not find a pattern in the temporal relationship between chimerism-causing pregnancies and the start of symptoms or diagnosis of SLE. One patient who experienced prior symptoms had an exacerbation of symptoms during her first pregnancy. This pregnancy resulted in a spontaneous miscarriage. Two patients experienced their first symptoms in their second pregnancy. In one of these patients, this pregnancy resulted in a spontaneous miscarriage. It could not be determined if this pregnancy resulted in long-lasting Mc. In the other patient, both her first and second pregnancy resulted in long-lasting Mc. Finally, three of six patients experienced their first symptoms one, seven and ten years after the birth of their youngest child of whom they carried chimeric cells.

Discussion

Our study demonstrates that female SLE patients are more likely to have detectable Mc in their peripheral blood than female control subjects. In almost all cases with detectable Mc, the origin of the chimeric cells was fetal. Additionally, MMc was detected in one patient and one control. The median total number of fetal chimeric cells in individuals with detectable Mc was higher in patients than in control subjects. Also, SLE patients often had chimeric cells originating from multiple relatives, in contrast to the control subjects, in whom the chimeric cells originated from only one relative.

Our results add support to two prior studies that found FMc more often in the peripheral blood of SLE patients than in control subjects,[16 17] contradicting other studies suggesting that there is no significant difference between the groups.[18 19] Differences in the blood compartment tested (PBMCs or whole blood), the specificities and sensitivities of the different techniques used, and the numbers of patients and control subjects included, may account for these conflicting results. In contrast to our study, previous studies did not demonstrate a difference between SLE patients and control subjects in the number

of chimeric cells present. However, a limitation of these studies was that they exclusively investigated the presence of the Y chromosome, limiting their findings to the detection of male Mc. Our approach allowed us to detect both male and female FMc, as well as MMc. If, in our present study, we had only investigated Mc using the Y chromosome in women with at least one son, we would not have found a statistically significant difference in the occurrence of Mc between patients and control subjects (data not shown). Only one study investigated the presence of MMc in SLE and found no difference between patients and control subjects, MMc occurring in 6% and 3%, respectively.[20] This low prevalence of MMc is in accordance with our results.

In literature, there are indications that within one individual some sources of Mc lead to persistent Mc, while others do not. In one case report about a woman with hepatitis C, the detected chimerism in the liver appeared to originate from only one of her five pregnancies.[27] After blood transfusions, it has been shown that, in the majority of cases with transfusion-associated Mc, there was evidence of only one or two non-recipient HLA-DR alleles, suggesting that the Mc commonly involves only one donor despite some patients receiving blood products from multiple donors.[28] However, in women with multiple children, it has not been systematically studied if there is a "favoured-child" with regard to the persistence of Mc, i.e. if FMc usually originates from one of the children, or from more children. Because we used indels and null alleles for the detection of Mc, we were able to show that at least half of the patients had persistent Mc from multiple relatives while all control subjects only had persistent Mc from one relative. The cause of this phenomenon is largely unknown. Studies in animals have demonstrated that syngenic or congenic matings resulted in more chimerism than allogenic matings, suggesting a role for HLA (mis)matches.[29 30] In humans, in certain autoimmune diseases mothers and children were shown to have fewer HLA disparities,[31 32] but these have not yet been significantly correlated to the presence of Mc.[31] Nevertheless, having a certain HLA allele (HLA DQA1*0501) appears to be associated with the presence of FMc.[33 34] Interestingly, HLA DQA1*0501 has been associated with SLE.[35]

The phenotype of a chimeric cell may affect its potential to lead to persistent Mc. We detected Mc in both PBMCs and granulocytes. Considering the relatively short half-life of granulocytes,[36] it is likely that the chimeric cells detected in this compartment are derived from stem cells. The existence of chimeric fetal progenitor cells was demonstrated in several studies (for review, see Seppanen *et al.*[37]). A higher prevalence of Mc in SLE patients than in control subjects can either mean that (a) more chimeric cells were acquired during pregnancy, (b) more chimeric cells persisted after pregnancy, (c) chimeric stem cells gave rise

to more chimeric cells due to an unknown trigger, or (d) a combination of aforementioned possibilities.

SLE patients were significantly more likely to have a prior history of blood transfusion than were control subjects. However, within the groups of SLE patients or control subjects, we did not find a difference in blood transfusion history between subjects with and subjects without detectable Mc. In literature, persistent chimerism was only described after blood transfusion following traumatic injury (for review, see Bloch *et al.*[38]), a condition that was not the indication for a blood transfusion in any of our subjects. Furthermore, a recent study in patients having received a blood transfusion in the peripartum period, like some of our subjects, did not show Mc at six weeks and six months after pregnancy.[39] Therefore, it is unlikely that the difference in blood transfusion history between patients and control subjects explains our results.

In our study there was no difference in disease activity (SLEDAI-2K) or accumulated damage (SDI) between patients with and without Mc. The former result is in line with previous research.[19] This finding may be a result of our small sample size. Future research will be required to further study the possible association. Additionally, many of the SLE patients who participated in our study were in clinical remission, which may have influenced results.

Our study had a few limitations. Because we did not have maternal DNA available for all subjects, we could not exclude a maternal source of the Mc in all cases. In cases where maternal DNA was available, it was not always possible to distinguish MMc from the detected FMc, due to an overlap in genetic markers. Furthermore, it was not possible to formally exclude all possible sources of Mc, such as unrecognized pregnancies or spontaneous abortions.

In summary, we detected Mc in peripheral blood more often and in higher numbers in female SLE patients than in female control subjects. The Mc detected was predominantly fetal in origin and was found in both PBMCs and granulocytes. This study provides the first evidence that SLE patients can have chimeric cells from more than one relative, while all of the chimeric control subjects had chimeric cells from only one relative. Any attempts to explain the phenomenon at this time are speculative. It may depend on the immune response evoked by specific chimeric cells, possibly relating to HLA, or on the activation status of the immune system of the recipient in general. Future studies addressing the immunological aspects of this phenomenon are necessary to improve our understanding of the process. The exact role of chimeric cells in SLE is still unknown, but our data substantiate the hypothesis that chimeric cells do play a role in SLE.

Acknowledgements

We thank J. Vork for her technical assistance.

Funding

This research was partly funded by Ars Donandi - Schokkenkamp Wegener Lonzieme foundation.

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